IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

STUDY OF SECONDARY METABOLITE CONSTITUENTS AND CURCUMIN CONTENTS OF CURCUMA LONGA

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ABSTRACT Historically, plants have shown great promise in the treatment of diseases and have been a source of inspiration for novel drug compounds. There has been considerable public and scientific interest in the use of phytochemicals derived from dietary components to combat human diseases. Turmeric (Curcuma longa L.) is the shining star among the cornucopia of traditional medicinal plants. Since 1970, when curcumin first attracted the interest of scientific medical researchers, studies have reported its suppressive effects on various medical conditions, including high serum cholesterol levels, free radical damage to tissues, diabetic cataracts, diabetic damage to pancreatic insulin-producing cells, diabetic wounds, rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, psoriasis, cancer, atherosclerosis, inherited peripheral neuropathies, liver damage caused by chemicals and drugs, and microbial infections. Curcumin also shows especially exciting prospects for the treatment of Alzheimer's disease, Parkinson's disease, and cystic fibrosis.

The main aim of this work is to conduct phytochemical screening for the presence of major secondary metabolites, physicochemical parameters, and curcumin content in turmeric. Using a combination of solvent extraction, chromatography, and spectroscopic techniques, we systematically separated and identified the total curcuminoids and turmerones from turmeric root. The extraction was optimized for maximum yield and purity. The isolated curcuminoids, primarily consisting of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, were quantified using High-Performance Liquid Chromatography (HPLC). Turmerones, including ar-turmerone, α-turmerone, and β-turmerone, were isolated and identified through Gas Chromatography-Mass Spectrometry (GC-MS). The results highlight the efficiency of the applied methods and provide a robust approach for the extraction of these valuable

bioactive compounds. This study not only contributes to the understanding of turmeric's phytochemical profile but also supports its potential therapeutic applications in pharmaceuticals and nutraceuticals.

INTRODUCTION

Turmeric is the rhizome or underground stem of ginger like plant. The plant is an herbaceous perineal, 60-90 cm high with a short stem tufted leaf. Its flowers are yellow, between 10-15 cm in length and they group together in dense spikes, which appear from the end of spring until the middle session. No fruits are known for this plant. The whole turmeric rhizome, with a rough, segmented skin. The rhizome is yellowish-brown with a dull orange interior that looks bright yellow when powdered. Rhizome measures 2.5-7.0 cm (in length), and 2.5 cm (in diameter) with small tuber branching off. Turmeric held a place of honor in Indian traditional ayurvedic medicine. In ayurvedic it was prescribed for the treatment of many medical problems ranging from constipation to skin diseases. It was used as digestive aid and treatment for fever, inflammation, wounds, infections, dysentery, arthritis, injuries, trauma, jaundice and other liver problems. In Unani turmeric is considered to be safest herb of choice for all blood disorders since it purifies, stimulates and builds blood. To most people in India, from housewives to Himalayan hermits, turmeric affectionately called the 'KITCHEN QUEEN', the main spice of kitchen. Long term use in turmeric, tulsi and trifala can be likened to a short term Pancha Karma treatment. Turmeric is relatively broad-spectrum antifungal. Turmeric exhibits antioxidant activity and protect from free radical damage. Curcumas also exhibits anti-tumour activities and prevent cancer. It inhibits the topoisomerase enzyme, which is required for cancer.

Biological classification: -

Kingdom: plantae

Division: - magnoliophyta

Subclass: - zingiberidae

Order: - Zingiberales

HISTORY OF TURMERIC

Marco polo (1280 AD) refers to turmeric as Indian saffron used for dying cloths. As far as documented evidence, it is used daily in India for at least 6000 years as medicine, beauty aids, cooking spice and a dye. Ostensibly it was used to worship the Sun during the solar period of India, a time when Lord Ram Chandra walked the Earth. It was mentioned in the Artharveda of India. Buddhist monks have used turmeric as a dye for their robes for at least 2000 years. It was listed in an Assyrian herbal circa 600 BC and was mentioned by Discorides in the herbal that was the western herbal rediscovered it 700 years ago

via Marco Polo, and it is used in the traditional lethal poison of pit vipers. In China, it was mentioned in the Pent-Sao of the 7th century. For at least 1000 years Chinese have used turmeric as medicine, especially for the spleen, stomach, and liver medicines. They use it to stimulate and purify and as an anti-biotic, anti-viral, and analgesic. As such it is used to stimulate and strengthen the blood and decrease blood pressure, to clean abdominal pain and stagnation in men, women, and children. They consider it one of the better herbals for women because it stimulates the uterus and clears menstrual stagnation. In the 1870's, chemists discovered turmeric orange-yellow root powder turned reddish brown when exposed to alkaline chemicals. This discovery led to the development of turmeric paper to test for alkalinity. European and American herbalists up until the rate 20th century, had little interest in turmeric. In one Western herbal from the early 20th century, Maude Greve's book A Modern Herbal, in which she gives a botanical description and the constituents of the herb as if the herb was of some importance, but then under medicinal actions and uses she says; "Turmeric is a wild aromatic stimulant seldom used in medicine except as a coloring. It was once a cure for jaundice. Its chief use is in the manufacture except as a coloring.it is used as an adulteration of mustard and a substitute for it and forms once of the ingredients of cattle condiments. Turmeric paper is used as a test for alkaloids and boronic acid". Daniel B. Mowrey tells the story. "Serious research on turmeric began in Germany, in the early 1920's. Sesqueterpenes in the essential oil of turmeric were isolated in 1926 and to them was ascribed the therapeutic activity.

MEDICINAL USES

- It is natural antiseptic
- Anti-inflammatory property: Inflammation is a necessary process for fighting against infections.
- Anti-oxidant property: Curcumin is an effective anti-oxidant and scavenger superoxide radicals, hydrogen peroxide and nitric oxide from activated macrophages. It inhibits the nitric oxide synthesis activity in macrophages.
- Effect of curcumin on lymphocytes: Muscular CD_4 (+) T- cells and B-cells increase in animals treated with curcumin suggesting that it modulates lymphocyte-mediated immune functions.
- Effect of curcumin on platelet aggregation: Curcumin inhibits platelet-activating factor (PAF).
- Natural analgesic.
- Speeds up wound healing.
- Improves digestion.
- Act as blood purifier.
- Helps alleviate coughing.

- Improves asthma.
- Anti-arthritis.
- Lowers cholesterol.
- Heals stomach ulcers.
- Improves skin conditions and act as skin tonic.
- Prevents progression of Alzheimer.
- Regulates blood sugar.
- Helps prevent cancer (breast, prostate, skin, colon, lymphoma, leukaemiaetc)

Natural vs. synthetic origin

JECFA specifications define only curcumin extracted from natural source materials. It can also be produced by chemical synthesis (Lampe and Milobedzka, 1913, Pabon, 1964, Merck Inex). Synthetic curcumin is not used as a food additive.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The roots of turmeric rhizomes that is Curcuma longa were collected from Kundapur, Bijapur and local areas of Karwar, in the month of February 2018. The identity of taxon called Curcuma longa by professor Dr.Shivanandh Bhat. Dept. of Botany. Govt. Arts & Science College

We have received Curcuma longa in large quantity, that was the reason we selected this species for the project.

PHYSICO-CHEMICAL ANALYSIS

Physicochemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed in Indian Pharmacopeia 1996 and WHO guidelines on quality control methods for medicinal plant material.

Proximate Analysis:

The physico-chemical parameters are helpful in judging the purity and quality of the drug. The percentage of active principles in the plant is determined only in the dry condition. Hence, the moisture lost percentage is very important to decide about the condition of the crude drug. The moisture should be kept minimum to prevent the drug from various kinds of decomposition. The total ash and the acid insoluble ash indicate the presence of any foreign matter, inorganic composition and purity of the drug. Their low value in the bark powder showed that the sample is free of any foreign matter.

Extractive Values

Determination of Alcohol Soluble Extractive Value: -

10 gm. of the air-dried coarse powder of Turmeric root were macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105 ^{0} C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs.

Determination of Water Soluble Extractive Value: -

Coarsely powdered drug (10 gm) was weighed accurately and macerated with 100 ml of water in a closed flask for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand. After 18 hours it was filtered rapidly. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^{0}C and weighed. The percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

Ash Values: -

Ash values are helpful in determining the quality and purity of crude drug,especially in the powdered form. It usually represents the inorganic salts naturallyoccurring in the drug and adhering to it. Hence, an ash determination furnishes a basisfor judging the identity and cleanliness of a drug. Procedure given in IndianPharmacopoeia was used to determine the different ash values such as total ash, acidinsoluble ash, and water-soluble ash etc.

Determination of total ash value: -

Exact 3 grams of air dried powdered drug was taken in a tared silica crucible.It was incinerated by gradually increasing the temperature to make it dull red hot untilfree from carbon. The crucible was cooled and weighed, repeated for constant value.Then the percentage of total ash was calculated with reference to the air-dried drug.

Determination of acid insoluble ash value: -

The ash obtained as directed under total ash value was boiled with 25 ml of2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper,washed with hot water, ignited and weighed, then the percentage of acid insoluble ashwas calculated with reference to the air-dried drug.

Determination of water soluble ash value: -

The total ash obtained was boiled with 25 ml of water for 5 minutes. Theinsoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed then the percentage of water soluble ash was calculated with reference to the air-dried drug.

Loss on drying: -

About 5g of drug accurately weighed in different petri dishes and kept in a hot air oven at 100^0 C for four hours. After cooling in a desiccator, the losses in weight are recorded in each case. This procedure is repeated till constant weight is obtained.

Loss of drying (%) =Loss in weight x 100/w

W=Weight of drug in gm

Loss on drying(%) = (0.260 / 5) x 100

= 5.2%

RESULT

Physico chemical parameters:

PARAMETERS	RESULT (% w/w)
Moisture content	5.2%
Total Ash Value	8.4%
Water soluble Ash	4.7%
Acid-insoluble Ash	1.01%
Water soluble Extractive value	10.56%
Alcohol soluble Extractive value	6.16%

Determination of Extractive values

These are useful for the evaluation of a crude drug. Gives an idea about the nature of the chemical constituents present in the crude drug. Useful for the estimation of constituents extracted with the solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists.

Preparations of the extracts

Cold Maceration

All the solvents and reagents used during the study were A.R. grade. Macerate 5g of the powdered crude drug of root of curcuma longawith each of 100ml of water, alcohol, chloroform, ethyl acetate, water separately in a closed flask for 24 hours, shaking frequently during first 6 hours and allowing to stand for 18 hours. Filter rapidly taking precaution.

against loss of alcohol, evaporate 25ml of filtrate to dryness in an evaporating dish to avoid the decomposition of natural metabolites, dry at 105 ^{o} C, and weigh. Calculate the percentage of water, alcohol, chloroform and petroleum ether soluble extractive value with reference to the air-dried drug.

RESULT :

Extractive values of different solvents:

S.No.	Solvents for Extraction	Weight of powdered rhizome, (g)	Weight of Extract, (g)	% yield
1	*n*-hexane	1.014	0.0160	1.58
2	Chloroform	1.015	0.0179	1.76
3	Ethyl acetate	1.011	0.0191	1.89
4	Acetone	1.017	0.0171	1.65
5	Ethanol	1.016	0.0229	2.24
6	Water	1.011	0.0114	1.03

Importance of Extractive values: -

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug. Thus, alcohol and water soluble extractive values were determined.

The Chemistry Of Natural Product: Plant Secondary Metabolites

Natural products are chemical compounds or substances isolated from living organism. The chemistry of the natural product includes their biosynthesis, extraction, identification, quantification, structural elucidation, physical and chemical properties and reactions They are produced by the pathway of primary or secondary metabolism. Metabolism is defined as series of enzyme catalyzed biochemical reaction or transformation occurring within the cells of an organism which are mainly required for its growth, development and for proper response to its environment. Metabolism can be in form of anabolism or catabolism. Metabolites are the intermediate or products of metabolism, the term metabolites are usually restricted to small molecules. A primary metabolite is directly involved in normal Growth, development and reproduction. Example carbohydrate, protein, fat and oil, alcohol etc. Secondary metabolites are not directly involved in growth, development and reproduction of an organism, but they have an ecological function. Plant secondary metabolite can be found in the leaves, stem, root or the bark of the plant depending on the type of secondary metabolite that is been produced. The most bioactive secondary metabolite are the Alkaloids, Tannins, Flavonoids and Phenolic compounds. Many of these secondary metabolites are indigenous plant use as food, spices and herbs.

Secondary metabolites differ from primary metabolite in having a restricted distribution in the plant kingdom. That is, particular secondary metabolite is found in only one plant species or related group of species, whereas primary metabolites are found throughout the plant kingdom. For many years these compounds were thought to be simply functionless end products of metabolism, or metabolic wastes. studies of these substances were pioneered by organic chemist of the nineteenth and early twentieth centuries who were interested in these substances because of their importance as medicinal drugs, poison,

flavour and industrial material. Only 5% to 15% of plant species have been chemically analysed so far. Unlike the primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long term impairment of the organism's survivability or aesthetics, or perhaps in no significant change at all. Secondary metabolite is often restricted to a narrow set of species within a phylogenetic group. Plant secondary metabolites are generic term used for more than 30,000 different substances which are exclusively produced by plants. the importance of these substances has only recently been discovered by scientists. Secondary metabolite carries out a number of protective functions in the human body, it can boost the immune system, protect the body from free radicals, kill pathogenic germs and much more keep the body fit.

TERPENOIDS

Terpenoids are the largest and most diverse family of natural products, ranging in structurefrom linear to polycyclic molecules and in size from the five-carbon hemiterpenes to naturalrubber, comprising thousands of isoprene units. All terpenoids are synthesized through thecondensation of isoprene units (C5) and are classified by the number of five-carbon unitspresent in the core structure (Mahmoud et al. 2002). Many flavour and aromatic molecules,such as menthol, linalool, geraniol and caryophyllene are formed by monoterpenes (C10),with two isoprene units, and sesquiterpenes (C15), with three isoprene units. Other bioactivecompounds, such as diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) show veryspecial properties.

PHENOLIC COMPOUNDS

Plants produce a large variety of secondary product that contains a phenol group- a hydroxyl functional group on an aromatic ring. These substances are classified as phenolic compounds. Plant phenolic is a chemically heterogeneous compound; some are soluble only in organic solvents, some are water-soluble, and others are insoluble polymers. Some simple phenolics are activated by ultraviolet light. Phenolic are wide spread in vascular plants and appear to function in different capacities. The derivatives of phenolic compounds include simple phenyl

propanoic, benzoic acid derivatives, anthocyanin, isoflavones, tannins, lignin, and flavonoid compounds beginning with phenyl alanines. Lignin is generally formed from three different phenyl propanoiod alcohols, namely, coniferyl, coumaryl, and sinapyl. Flavonoids are one of the largest classes of plant phenolics; the basic structure contains 15 carbons arranged in two aromatic rings connected by a three-carbon bridge. The basic function of flavonoids is for pigmentation and defense. The red,pink,purple, and blue colors observed in plant parts are a result of anthocyanin.the purple color of commelina comments was found to consist of a large complex of six anthocyanin molecules. For example, Arabidopsis thaliana mutants that lacks flavonoids are much more sensitive to UV-B radiation than wild-type individual and grow very poorly under normal conditions. Tannins were first used to describe compound that could convert raw material hides into leather in the process of tanning. Tannin are generally toxic that significantly reduce the growth and survivorship of many herbivores when added to their diets. tannins

can be seen in fruits like apple, black berries, tea and red wine. Tannins are mainly constituent of woody plants especially heart wood. Some derivatives of tannin include Gallic acid.

NITROGEN-CONTAINING COMPOUNDS

A large variety of secondary metabolites have nitrogen in their structure. these include the alkaloids, cyanogenic glucoside, glucosinate. Alkaloids are large family of more than 15,000 nitrogen containing secondary metabolites found in approximately 20% of the species of vascular plant. The nitrogen atom in these substances is usually part of the heterocyclic ring, a ring that contain both nitrogen and carbon atom. They show striking pharmacological effect on vertebrate animal. As their name would suggest, most alkaloid are alkaline, at pH value commonly. The first medically useful example of an alkaloid was morphine, isolated in 1805 from opium poppy papaversomniferum. The role of alkaloid in plant has been a subject of speculation for at least 100 years. Alkaloid were once thought to be nitrogenous wastes. most alkaloid are now belived to function as defence against especially mammals, because of the general toxicity and deterrence capacity. One group of alkaloids, the pyrooliziline alkaloid illustrates how herbivore can become adapted to tolerate plant defensive substance and even use them in their own defence.

Phytochemical Screening

Chemical tests were carried out qualitatively on each extract following standard procedures to identify the phytochemical constituents

Preparation of Extracts of Plant Material

Plant extracts of root powder were prepared using ethanol as extracting solvent. 100g of the dried and powdered plant material (rhizome) was extracted with 50 ml of ethanol at 65°C using Soxhlet extraction method. After filtering and evaporating to dryness, the crude ethanolic extract was obtained.

A**. Test for alkaloids**

Dragendroff's test: In a test tube containing 1 ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicated the presence of alkaloids.

Mayer's test: To 1 ml of the extract, 2 ml of Mayer's reagent was added, a dull white precipitate indicated the presence of alkaloids.

Wagner's test: To 1 ml of the extract, 2 ml of Wagner's reagent was added. Appearance of a reddish-brown precipitate indicated the presence of alkaloids.

Hager's test: Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

B. Test for flavonoids

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Lead acetate test: To the test solution, a few drops of lead acetate solution were added. Formation of yellow precipitate indicated the presence of flavonoids.

C. **Test for phenolic compounds**

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

D. Test for terpenoids

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour of interface after.

Test for carbohydrates: The extract was dissolved in 10ml of distilled water and filtered through Whatman filter no. 41 then filter it. The filtrate is used for carbohydrate test,

Molish test :2ml of solution in a test tube+ 1 drop of molish reagent + 2ml of conc. HCl from the sides of test tube, it forms the violet ring at the junction of two liquids indicates the presence of carbohydrates.**Test for Saponins:**About 0.2g of extract was shaken with 5ml of distilled water then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Test for proteins: Extract + 10ml of distilled water and filtered through whatsmann filter no. 41 and filtrate is subjected to test for proteins and amino acids,

Millons test: 2ml of filtrate + few drops of millons reagent are added. The white ppt indicates the presence of proteins.

Biuret test: an aliquot of 2ml filtrate was treated with drop of 2% copper sulphate solution + 1ml of ethanol followed by excess of KOH pallets. The pink colour in ethanol layer indicates presence of proteins.

Test for Glycosides:

Borntagr's test: Extract was boiled with dil. Sulfuric acid, filtrate it. Filtrate chloroform is shaken well. Organic layer was separated to which ammonia is added slowly. Presence of glycoside is denoted by pink to red colour in the ammoniacal layer.

Legal test: the test is employed for digitoxin contains glycoside. The extract was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. Pink to red colour indicates presence of glycoside.

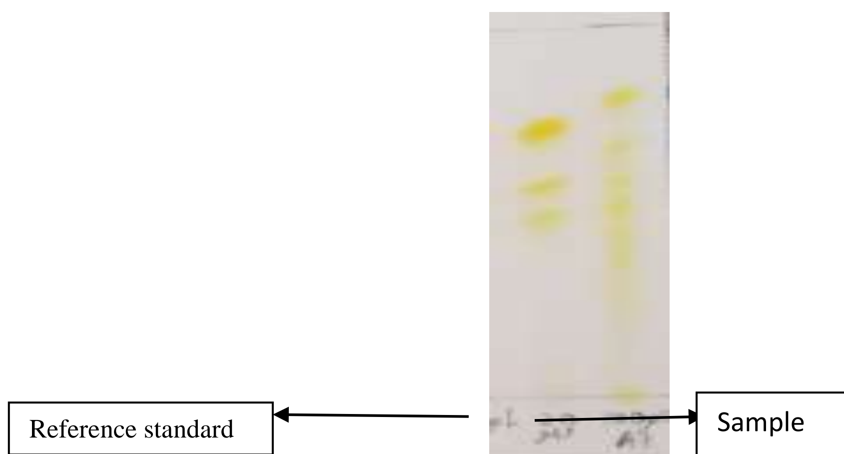
Secondary metabolites	Tests	Result
Alkaloids:	Dragendorff's test	+
Carbohydrates	Molish test	+
Proteins	Millons test	+
Glycosides	Borntagr's test	+
Terpenoids	Lieberman test	+
Flavonoids:	Alkaline test	+
Tannins	Ferric chloride test	-
Saponins	Frothing test	-
Phenols	Lead acetate test	+

ISOLATION OF CURCUMIN FROM TURMERIC RHIZOMES

Crude extract was dissolved in alcohol and was filtered. The filterate was concentrated. The concentrate hence obtained was dissolved in benzene. Sodium hydroxide (0.1% w/v) was added to benzene solution. Using a separating funnel curcumin was partitionedbetween the two layers. Sodium hydroxide layer was taken and curcumin was precipitated by adding dilute hydrochloric acid solution. The precipitate obtained was filtered using vacuum filtration unit and dried. The isolated curcumin was used for further evaluation.

EVALUATION OF ISOLATED CURCUMIN BY THIN LAYER CHROMATOGRAPHY (TLC)

TLC of the isolated curcumin was performed on precoated silica gel G plates (Stationary Phase) using mixture of Chloroform and Methanol in the ratio 9: 1 as solvent system (Mobile Phase).Curcumin was used as standard. Detection was done by Ultra Violet chamber. The Rf values for the separated spots were calculated and compared with Rf value of pure curcumin and values reported in the IP



Results of TLC study of curcumin.

Sample	Rf value
Standard curcumin Spot	0.467
Test sample	Spot 1 = 0.074 Spot 2 = 0.224 Spot 3 = 0.448

RESULTS AND DISCUSSION

Results of Preliminary Phytochemical Evaluation of Turmeric Rhizomes

Turmeric rhizomes collected from different districts were subjected to preliminary phytochemical analysis, revealing the presence of carbohydrates, proteins and amino acids, alkaloids, terpenoids, phenols, and flavonoids. Additionally, isolated curcumin powder exhibited a bright yellow color. Thin Layer Chromatography (TLC) results indicated a prominent spot with an Rf value matching that of standard curcumin. Two less intense spots observed in the test solution suggest the presence of other curcuminoids alongside curcumin.

Curcumin Content Analysis

The content of curcumin in turmeric is crucial for determining its color, quality, therapeutic efficacy, and consequently its economic value. Samples of turmeric rhizomes collected from various districts were analyzed to quantify their curcumin content.

Discussion

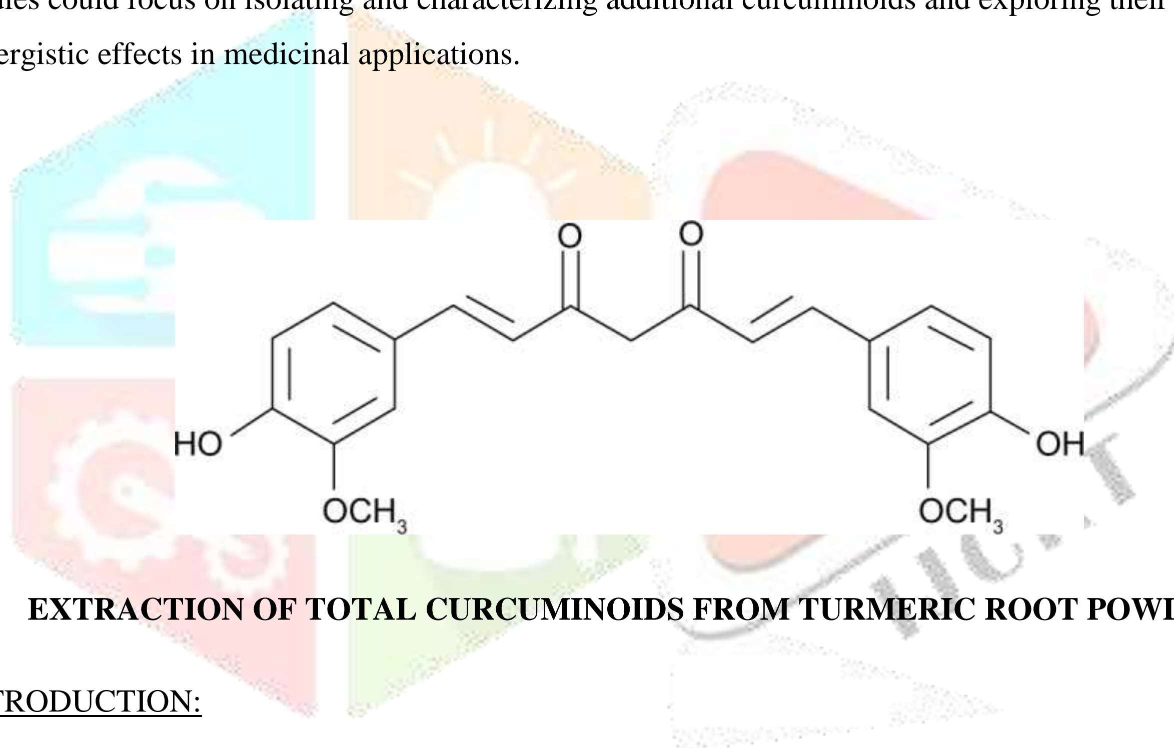
The presence of diverse phytochemicals such as carbohydrates, proteins, alkaloids, terpenoids, phenols, and flavonoids in turmeric rhizomes underscores its pharmacological potential. These compounds

contribute to turmeric's traditional medicinal uses and modern therapeutic applications. The bright yellow color of isolated curcumin powder confirms its purity and suitability for medicinal formulations.

Thin Layer Chromatography (TLC) is a reliable method for identifying and potentially quantifying curcuminoids in turmeric extracts. The observation of multiple spots in TLC suggests the presence of curcumin analogs or derivatives, which may contribute to turmeric's bioactivity beyond curcumin alone.

Quantifying curcumin content is essential for standardizing turmeric products in both economic and therapeutic contexts. Higher curcumin content correlates with increased therapeutic benefits, making it a crucial parameter for assessing turmeric's market value and medicinal efficacy.

In conclusion, the preliminary phytochemical analysis and curcumin content determination highlight the rich chemical diversity and therapeutic potential of turmeric rhizomes from different districts. Further studies could focus on isolating and characterizing additional curcuminoids and exploring their synergistic effects in medicinal applications.



EXTRACTION OF TOTAL CURCUMINOIDS FROM TURMERIC ROOT POWDER

INTRODUCTION:

Curcuma longa Linn. (C. domestica), commonly known as Haldi in India or Indian Saffron, belongs to the family Zingiberaceae. It is a perennial herb widely cultivated in tropical regions of Asia. The rhizomes of Curcuma longa, known as turmeric, are renowned for imparting color and flavor to food. Turmeric is often referred to as the "golden spice" and the "spice of life" due to its extensive culinary and medicinal uses without known adverse effects. Its historical medicinal use dates back to ancient civilizations, with evidence of cultivation by the Harappan civilization as early as 3000 B.C. Indigenous to South and Southeast Asia, turmeric has been a staple in Ayurvedic medicine for millennia.

Turmeric contains bioactive compounds known as curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These curcuminoids exhibit a wide range of biological activities, including anti-inflammatory, anticancer, antibacterial, antiviral, antioxidant, antiseptic,

cardioprotective, hepatoprotective, and digestive properties. The therapeutic potential of turmeric has been extensively studied and continues to be explored for modern medical applications.

This study focuses on the extraction of total curcuminoids from turmeric root powder using various solvents, with ethyl acetate yielding the highest extraction efficiency. Column chromatography was employed for the isolation and purification of curcuminoids, followed by their analysis for purity using High-Performance Liquid Chromatography (HPLC). Pre-testing of solvents for Thin Layer Chromatography (TLC) revealed that a chloroform

ratio of 95:5 provided optimal separation of curcuminoids, with Rf values of 0.75, 0.55, and 0.27 for Curcumin (C), Demethoxycurcumin (DMC), and Bisdemethoxycurcumin (BDMC), respectively.
This review explores the traditional uses of turmeric and its curcuminoids in Ayurvedic medicine, as well as their evolution to modern applications in medicine and nutrition.

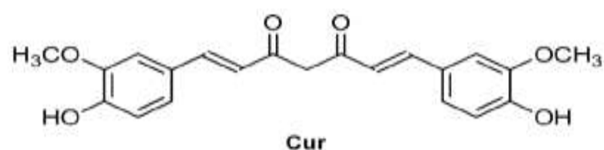
CHEMICAL CONSTITUENTS

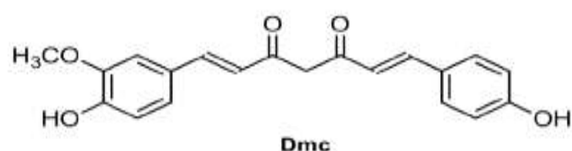
Chemical constituents of turmeric have been extensively investigated by many researchers. Currently, more than 235 compounds, primarily phenolic compounds and terpenoids, have been identified from turmeric, including diarylheptanoids and diarylpentanoids, phenylpropene and other phenolic compounds, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, sterols, and some alkaloidal compounds (Ravindran PN, Nirmal Babu K, Sivaraman K. Turmeric: The Genus Curcuma. CRC Press; 2007).

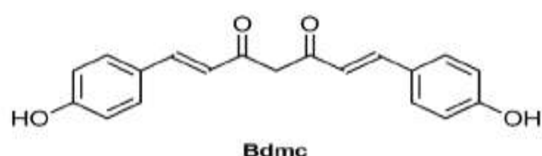
The active ingredient in turmeric is curcumin. Curcumin (diferuloyl methane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a yellow pigment isolated from the rhizomes of Curcuma longa, has been widely used as a food additive for many years. Curcumin demonstrates significant cytotoxic activity and induces apoptosis in various cancer cell lines by modulating the activities of numerous transcription factors, growth regulators, adhesion molecules, apoptotic genes, and cellular signaling molecules (Aggarwal BB, Surh YJ, Shishodia S, eds. The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease. Springer; 2007).

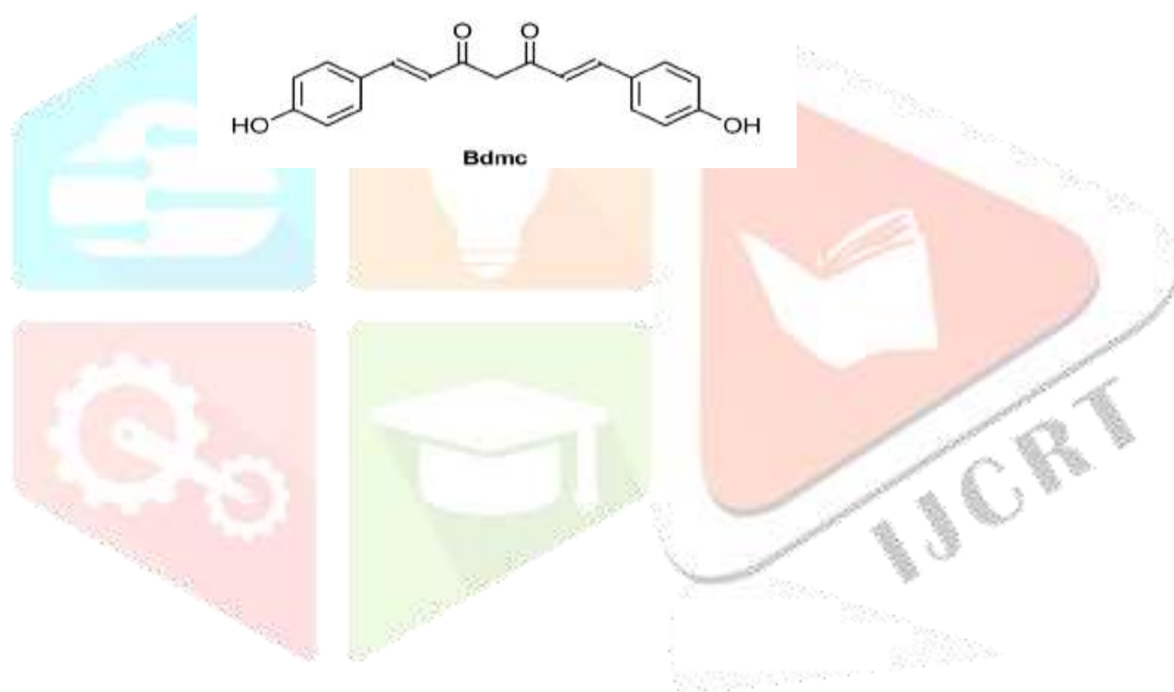
Curcumin is insoluble in water but soluble in methanol, ethanol, DCM, or DMSO. It has been extracted using methods such as maceration, digestion, and infusion. This project focuses on the efficient isolation and characterization of curcumin. Initially, curcumin was extracted using the Soxhlet extractor method. This material can be further purified by recrystallization and characterized using spectrometer analyses.

Ethyl acetate, with restrictions on the use of chlorinated solvents such as dichloroethane, has been found to be a suitable alternative due to its polarity, providing acceptable product quality and commercially viable yields (Chattopadhyay I, Biswas K, Bandyopadhyay U. Turmeric and Curcumin: Biological Actions and Medicinal Applications. CRC Press; 2007).









CHEMICAL PROPERTIES OF CURCUMIN

Boiling Point	660 °C
Density	0.954 g/cm^3
IUPAC Name	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione
Melting Point	183 °C
Molar Mass	368.385 g/mol
Molecular Formula	$C_{21}H_{20}O_6$
Synonyms	Curcumin; Diferuloylmethane
Density	0.9348 g/mol

EXTRACTION OF TOTAL CURCUMINOIDS

Extraction, as defined in pharmaceutical contexts, involves separating the medicinally active portions of plant or animal tissues from inert components using selective solvents in standardized procedures. The resulting products from plants are typically impure liquids, semisolids, or powders intended for oral or external use. These preparations include decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts, and powdered extracts, commonly referred to as Galenicals, named after the ancient Greek physician Galen (Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 2nd ed. Asiatic Society of Bangladesh; 2003).The standardized extraction procedures for crude drugs aim to isolate the therapeutically active components while eliminating inert material using a selective solvent known as a menstruum. The resulting extract may be used directly as a medicinal agent in forms such as tinctures and fluid extracts, further processed into dosage forms like tablets or capsules, or fractionated to isolate individual chemical entities such as ajmalicine, hyoscine, and vincristine, which are modern drugs. Thus, the standardization of extraction procedures significantly contributes to the quality of the final herbal drug.

Methods of Extraction of Medicinal Plants

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 or decantation after standing.

Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

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.

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, heatstable 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, e.g. 1:4 or 1:16; 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.

Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate.

Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

Hot Continuous Extraction (Soxhlet)

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 of the Soxhlet apparatus. The extracting solvent in flask is heated, and its vapors condense in condenser. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube , the liquid contents of chamber siphon into flask . 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.

Aqueous Alcoholic Extraction by Fermentation

Some medicinal preparations of Ayurveda (like *asava*and *arista*) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (*kasaya*), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel. In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are karpurasava, kanakasava, dasmularista. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts.

Counter-current Extraction

In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators toproduce a fine slurry. In this process, the material to be extracted is moved in one direction (generally in the form of a fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. Further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end.

Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation.

Although the process is useful in some cases, like extraction of rauwolfia root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.

Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt. The collection of the extracted analyte following SFE is another important step.

For extraction of Curcuminoids we used Soxhlet extraction method.

- Extraction of Total curcuminoids by curcuma longa root powder

Part used: Turmeric Root

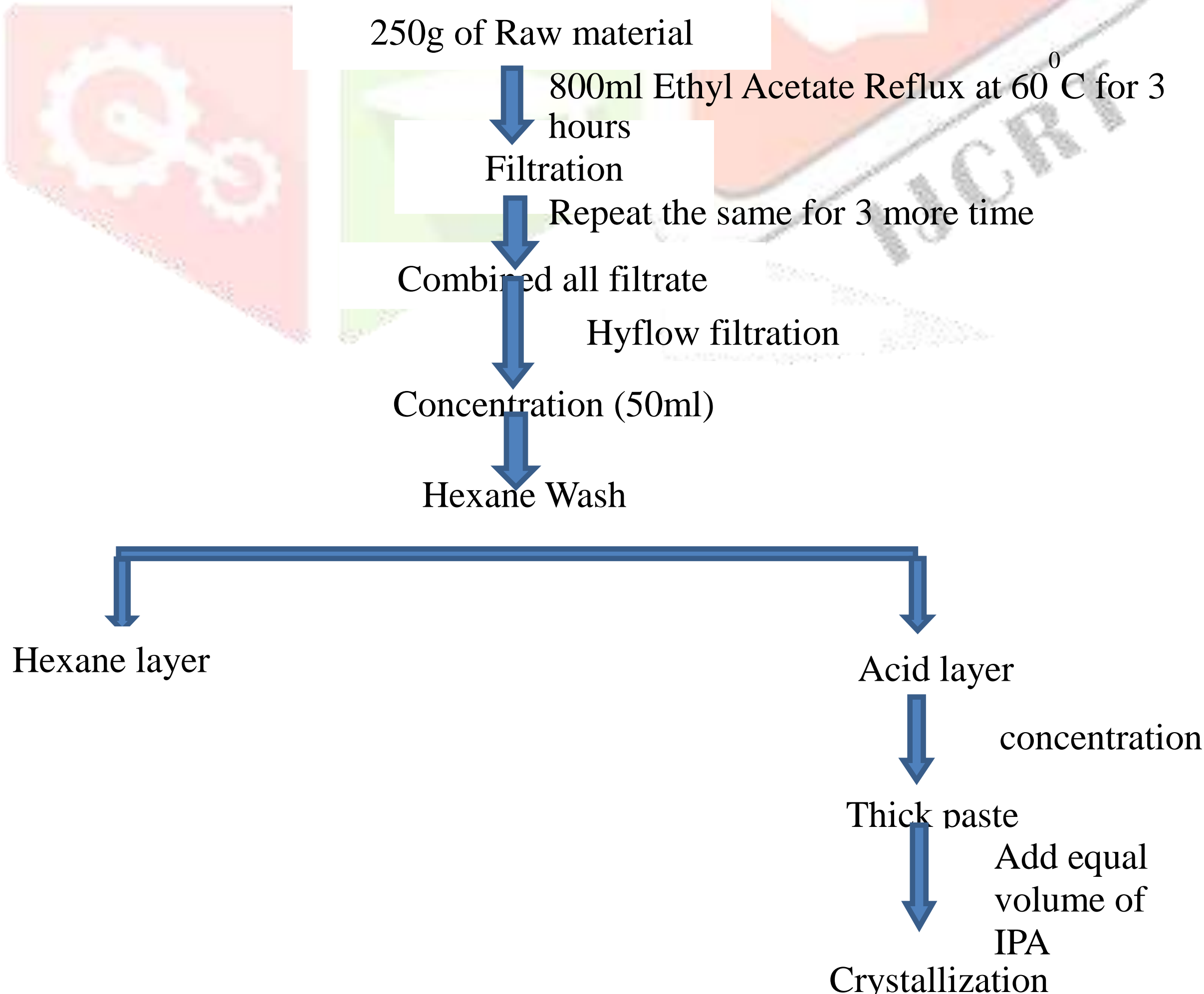
Apparatus and Equipment used:

- Round bottom flask, separating funnel, conical flask, beaker, Buchner funnel and filtration flask, glass plate, distillation adapters,Water condenser.
- Mixture, Weighing machine, , Laboratory stirrer, Rotary evaporator, Reactor, Vacuum tray drier, UV chamber, Water bath.

Chemicals used:

Ethyl Acetate, Hexane, Isopropyl alcohol

PROCESS FLOW CHART



Procedure:

Step 1: 250 g of dry turmeric root powder was taken in a 2L round-bottom flask and soaked with 800 ml of ethyl acetate, then refluxed for 3 to 4 hours at 60°C.

Step 2: The mixture underwent high-flow filtration and was concentrated to 50 ml.

Step 3: Take the insoluble part in 800 ml of ethyl acetate and reflux for 3 hours at 60°C.

Step 4: Repeat the reflux process two more times.

Step 5: Combine all four washes and filter through a celite bed. Collect the filtrate and concentrate to a final volume of 50 ml.

Step 6: Add hexane to the concentrated extract and mix well.

Step 7: Separate the hexane layer and collect it in a round-bottom flask. Concentrate the hexane layer to a thick paste to remove hexane vapor.

Step 8: 5.5 g of paste is obtained. Add an equal amount of isopropyl alcohol (IPA), mix well, and place in the refrigerator to crystallize for 1 hour.

Step 9: Filter the IPA layer to separate the solid layer, which is then dried using a vacuum tray dryer.

Step 10: Weigh and pack the dried sample.

Step 11: Perform TLC to analyze the sample.

Thin Layer Chromatography (TLC):

Thin Layer Chromatography (TLC) is a chromatographic technique used to separate non-volatile mixtures. It involves applying the sample onto a sheet of glass, plastic, or aluminum foil coated with a thin layer of adsorbent material, typically silica gel, alumina, or cellulose, known as the stationary phase. A solvent or solvent mixture, called the mobile phase, is drawn up the plate via capillary action. As different analytes ascend the TLC plate at different rates due to their varying affinities for the stationary and mobile phases, separation occurs.

The mobile phase is chosen based on its differing polarity compared to the stationary phase. For instance, with silica gel (a polar substance), non-polar mobile phases like heptanes are commonly used. Mixtures of solvents can also be employed to adjust the overall properties of the mobile phase.

After the TLC experiment, spots on the plate are visualized. This is often achieved by exposing the plate to ultraviolet (UV) light, causing compounds to fluoresce. Alternatively, chemical methods such as treating the plate with a reagent like anisaldehyde can form colored adducts with compounds, or using sulfuric acid can char organic compounds, leaving dark spots on the plate.

To quantify the results, the distance traveled by a substance of interest is divided by the total distance traveled by the mobile phase (ensuring it does not reach the end of the stationary phase). This ratio is known as the Retention Factor (Rf).

Preparation of mobile phase:

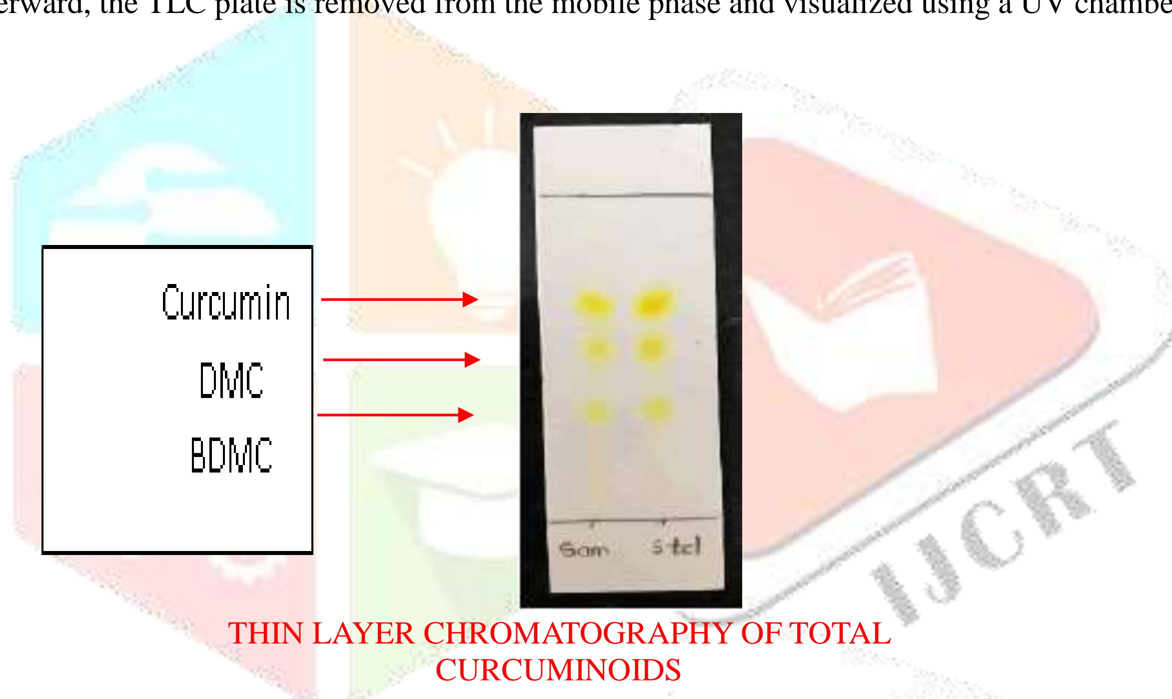
9ml Chloroform + 1ml Methanol

Detection

Ultra violet chamber

Procedure

Freshly prepared mobile phase is taken in a clean and dry 250 ml beaker, which is covered with foil. A small amount of standard Curcumin dissolved in a minimum amount of methanol is taken in a small test tube. In another test tube, the sample dissolved in a minimum amount of methanol is taken. A TLC plate of suitable dimensions is chosen. Two spots are marked on the plate, one for the standard and another for the sample. Then, two drops of the standard and sample solutions are applied to their respective spots using a capillary tube. The TLC plate is placed in the mobile phase, allowing adsorption to occur. Afterward, the TLC plate is removed from the mobile phase and visualized using a UV chamber.



THIN LAYER CHROMATOGRAPHY OF TOTAL CURCUMINOIDS

PURIFICATION AND SEPARATION OF INDIVIDUAL CURCUMINOIDS FROM SPENT TURMERIC OLEORESIN, A BY-PRODUCT FROM CURCUMIN PRODUCTION.

Turmeric is rich in curcuminoids and recognized for its broad spectrum of biological activities; curcuminoids vary in chemical structures, physicochemical characteristics as well as functional properties. This study focused on the separation of individual curcuminoids from spent turmeric oleoresin, considered an industrial waste, and its purification by column chromatography followed by purity analysis by HPLC. Chromatographic purification of spent turmeric oleoresin was done with silica gel.

INTRODUCTION OF CRTO: -

CRTO is nothing but curcumin-recovered turmeric oleoresin. The CRTO is called hazardous waste because we know that by-products or waste products of plant food processing represent a major disposal problem for the industry concerned, and also, the resins are not easily biodegradable in the environment. For the disposal of by-products, it covers lots of areas and also the transportation charges, which cause problematic situations for the industries. Because of all these reasons it leads to environmental pollution.

ADVANTAGES OF RECYCLING CRTO

1. Complete utilisation of Raw material-By recovering or recycling the waste product,the wastage of raw material is avoided.

2. Conversion of Hazardous waste in to useful for-Byisolating three curcuminoids from CRTO which is a hazardous waste, we can avoid environmental pollution.
3. We can avoid transportation charge-For transporting the waste product to the place where it going to be disposed, it requires more cost so, we can avoid the unnecessary cost which will help the industry economically.

4. In recent times, natural food and additives are in great demand due to consumers and health concern associated with the use of synthetic additives.CRTO cab also be used as food additives, which may be used because of their desirable functional properties.

5. CRTO oil and its fractions were tested for antioxidant activity and antioxidant capacity

6. Antibacterial, antifungal and antimutagenic activities of CRTO has been discovered.

7. Now a day's experiments are going on using CRTO as a fuel in boilers.

STEPS FOR CONVERTING CRTO IN POWDER FORM

1. After the partial separation of curcuminoids, the remaining oleoresin is CRTO. It has been seen that CRTO contains approximately 40%   oil. Since the aim of the experiment was to recover the total curcuminoids from CRTO, for that oil part has to be completely removed by Hexane.

The reasons for using hexane to remove oil are,

- Hexane has greater ability to extract oil when comparedOther solvent.
- With a B.P. 69^0 c, it is able to retain its liquid state at all atmospheric condition other than for extreme climates.
- Its responsible volatility aids easy removal from solids and oils using low energy.
- When compared to any other solvent hexane records the lowest skin irritation.

2. After the complete removal of oil, it took a form of sticky substance. But for the isolation of curcuminoids, sticky substance has to be converted in to powder form. In order to make it so, the sticky form of CRTO was blended with silica (200 mesh passed) with the drop wise addition of methanol and it was stirred using magnetic stirrer for three hours. During the stirring at one point the lump was broken in to small fragments and finally reduced to powder. The suspended powder was dried in an oven at 60^0 C. The obtained crude powder was weighed it was about 6 g in weight.

The next step was to separate the three curcuminoids from the CRTO (Powder form) by column chromatography method. Before that let us study in detail about the column purification method.

COLUMN PURIFICATION METHOD

Column Chromatography is a preparative technique used to purify compounds depending on their polarity and we also know that in column chromatography, a mixture of molecule is separated based on their differential partitioning between a mobile phase and stationary phase.

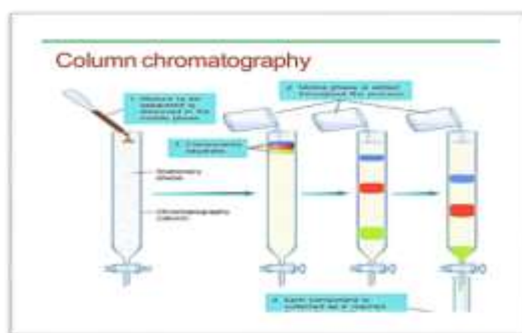
PRINCIPLE:

This is a solid-liquid technique in which, the stationary phase is a solid and mobile phase is liquid .The principle of column chromatography is based on differential adsorption of substance by adsorbent.

Column chromatography can be done using gravity to move the solvent or using compressed gas to push the solvent through the column at different rates, allowing them to be separated in to fractions. The technique can be used on scales from micrograms up to kilograms.

The main advantage of column chromatography is the relatively low cost and disposability of the stationary phase used in the process. The latter prevents Cross-contamination and stationary phase degradation due to recycling.

COLUMN PREPARATION



A column is prepared by packing a solid adsorbent in to a cylindrical glass or plastic tube. The size will depend on the amount of compound being isolated. The base of the tube contains a fitter, either a cotton or glass wool plug as shown in the above diagram; it helps to hold the solid phase in place. .A solvent reservoir or may be a part of the column be attached at the top of the column. Two methods are generally used to prepare a column i.e. the dry method and the wet method.

For dry method the column is first filled with dry stationary phase powder, followed by the addition of mobile phase which is flushed through the column until completely wet and from this point it is never allowed to run dry.

For the wet method, slurry is prepared of the eluent with stationary phase powder and then carefully pouredin to the column. The eluent slowly passed through the column.

The individual components are retained by the stationary phase differently and separate from each other. While they are remaining at different speeds through the column with eluent.

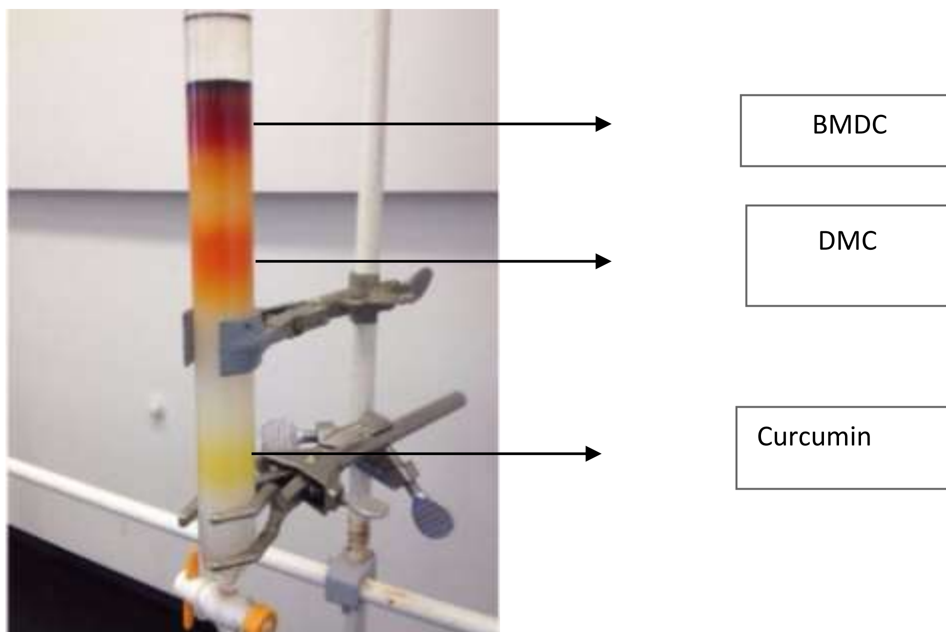
At the end of the column they elute once at a time during the entire chromatography process. The eluent is collected in series of fractions. Fractions can also be collected automatically by means of fraction collector. Finally, the crystallization of collected fraction is done.

COLUMN PURIFICATION METHOD FOR THE SEPARATION OF TOTAL CURCUMINOIDS FROM CRTO

In this method, mobile phase used is chloroform and silica is used as stationary phase.First rinse the column with chloroform and then carefully pour the silica in column. Silica is selected as an adsorbent because it is a powerful adsorbent and more polar compare to Alumina. Carefully add the solvent i.e. chloroform to the column without disturbing the silica. Add sand at the top of the column so that when we pour solvent in to the column, it disturbs the sand layer and leaves the silica layer. For the best results we want our silica layer to be level and not bumpy which is what would happen if we pour the solvent directly in to it.

After the addition of chloroform stirred very well to drive off air bubbles. Normally a separation will begin by using non-polar solvent or low polarity solvent, allowing the compounds to absorb the stationary phase. Then slowly switching the polarity of the solvent to desorb the compounds and allow them to travel with the mobile phase.

After all the set up pour the slurry of sample which was already prepared by adding chloroform carefully in to the column using a dropper. After the slurry application, start collecting the fractions.



In the first one to ten fractions we got curcumin which is confirmed by spotting it on TLC along with our standard. A thin layer chromatography shows how a mixture of compounds will behave when purified by column chromatography. Since the aim was to collect three curcuminoids separately, for that purpose the polarity of the system is increased gradually during the separation by increasing the proportion of the more polar solvent.

First the polarity of the system is increased by 5% i.e. 9.5ml chloroform +0.5 ml methanol. For 5%, spot were observed in eleven to twenty fraction demethoxycurcumin is separately obtain and it is collected. Finally, by increasing the polarity by 20% i.e. 8ml chloroform +2ml methanol, bisdemethoxycurcumin is collected.

Usually Fractions of certain volume are collected and then evaporated to small volume and spotted on TLC. The similar fractions were collected together for more purification or crystallisation.

CRYSTALLISATION:

silica gel column chromatography to separate the individual curcuminoids with chloroform: methanol at increasing polarity. 10ml each 40 fractions were collected and Concentrated separately . TLC profile done. Same Rf fractions collected & evaporated.

SILICA GEL COLUMN CHROMATOGRAPHY ELUTION PROFILE

Fraction Numbers	Total volume collected	Curcuminoids present	Weight of extract	Percentage of total curcuminoids
1-10	200ml	Curcumin	800mg	93.2
11-20	200ml	DMC	1000mg	90.4
21-30	200ml	BDMC	1100mg	88.9
31-40	200ml	BDMC	800mg	90.6

Parameters used:

1. Thin Layer Chromatography

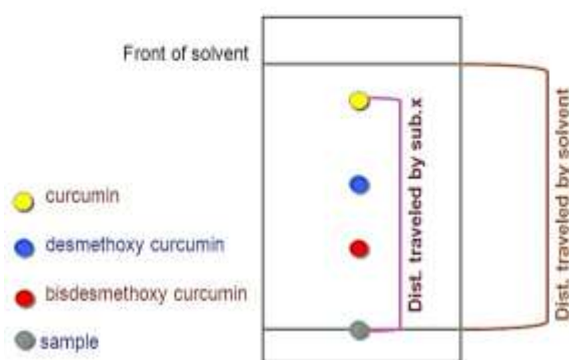
2. High Performance Liquid Chromatography

Thin Layer Chromatography (TLC):

Thin Layer Chromatography (TLC) is chromatography technique used to separate non-volatile mixtures. TLC performed on a sheet of glass, plastic or aluminium foil, which is coated with thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known is known as stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC place at different rates, separation is achieved. The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non-polar mobile phases such as heptane's are used. The mobile phase may be a mixture, allowing chemist to fine – tune the bulk properties of the mobile phase.

After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light on the sheet; the sheets are treated with a phosphor, and dark spots appear on sheet where compounds absorb the light impinging on a certain area. Chemical process can also be used to visualize spots anisaldehyde, for example, forms colored adducts with many compounds, and sulphuric acid will char most organic compounds, leaving a dark spot on the sheet.

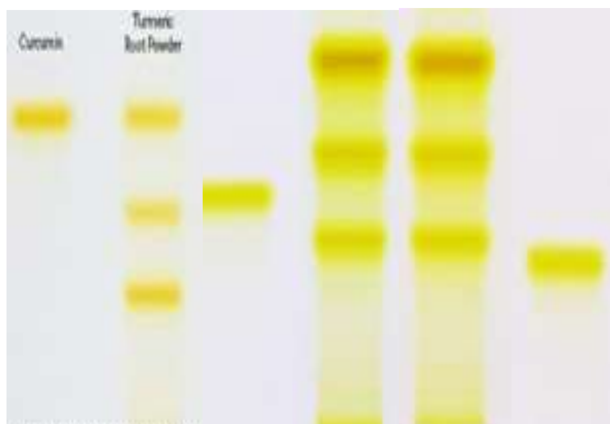
To quantity the results, the distance travelled by the substance being considered is divided by the total distance travelled by the mobile phase. (the mobile phase must not be allowed to reach the end of the stationary phase). The ratio is called the Retention factor or R_f.



Preparation of mobile phase:

9ml Chloroform + 1ml Methanol

Detection : U V light



Column fractions (1to 10)   Column fractions (11 to 20)   Column fractions(21 to 40)

RESULT:

Demethoxycurcumin and Bisdemethoxycurcumin in pure form commercially not available. A method developed for separation of three curcuminoids from spent turmeric oleoresin - which is now considered as an industrial waste in curcumin production industry . The new process developed is simple and economical to adopt.

EXTRACTION OF TURMERONE FROM CRTO

Turmerone is principle flavouring compound of turmeric (curcuma longa). Turmerones are the main constituents of turmeric rhizome essential oils. Curcumin, the yellow colouring pigment of turmeric is produced industrially from turmeric root. The mother liquor after isolation of curcumin from oleoresin known as curcuminremoved turmeric oleoresin (C R T O) was extracted with n-hexane,obtain turmeric oil. The turmeric oil was subjected to fractional distillationunder vacuum and Column purification. The chemical constituentsof turmeric oil, column fractions were determined by Thin Layer Chromatography (TLC) and Identified by GCMS. Aromatic turmerone, α- turmerone andβ- turmeronearemajor compounds present in fraction

INTRODUCTION

Turmeric is essential colouring spice of India. There is variety of chemical constituents occur widely in nature. Volatile constituent from plant plays an important role in various fields like fragrance, food, pharmaceutical, and**Turmerone Induces Neural Stem Cell Proliferation** etc. Active compound in turmeric are typically classified as non – volatile or volatile. Major non-volatile curcuminods are curcumin, demethoxycurcumin, bismethoxycurcumin. Curcuminoids which is about 4-6%, turmeric contains 2-4% essential oil and 2-3% of fixed oil and various volatile oils like turmerones. Turmeric

volatile oil is yellowish and commonly evolves aromatic flavours and is comprised of roughly 33% of ar-turmerones, 24% alpha turmerone, 23% beta termerone and others. Aromatic termerone is major components present in turmeric oil.

Curcumin Removed Turmeric Oleoresin Turmeric Oil

Turmerone's are applied in various fields i.e.its medicinal value has been known for long and it use as a cure for hypercholesterolemia,arthritis, indigestion and liver problems.

Aromatic turmerone is major component of curcuma longa. Synthetic Turmerone's act as neoplasm inhibitor and anti-carcinogenic, and anti-vinomactivity. Recently anti fungal and mosquitocidal activities are reported. Turmerone's act against anti inflammatory, neurologic diseases, anti microbial activity, reduces joint issues, leukaemia cells, breast cancer, tumour cells etc.

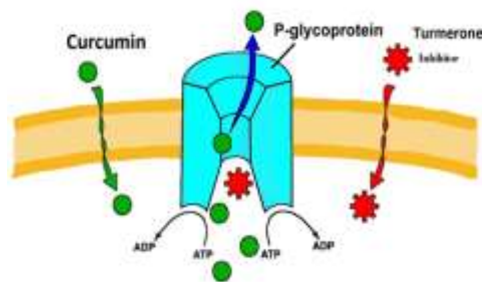
TURMERONE'S V/S CURCUMINOIDS

- Turmerones and curcuminoids are not the same.
- Curcuminoids are diarylheptanoids.Curcuminoids is believed to be much of turmeric medicinal effect.
- Turmerones are bioactive molecules in turmeric known to benefit health.

Currently, it is not possible to explain exactly that benefits are due to curcuminods or turmerones or combination of both.

Turmerones actions are completely independent of curcuminods. i.e.

- Protects cell from damage due to heavy metal or radiation exposure.
- Protects neuronal health against dementia and Alzheimer's diseases.
- Inhibit the replication of HIV
- Balance plasma liquid levels
- Impede the aggregation of blood platelets
- Treat multiple sclerosis(MS) symptoms
- Inhibit the per oxidation of lipo proteins



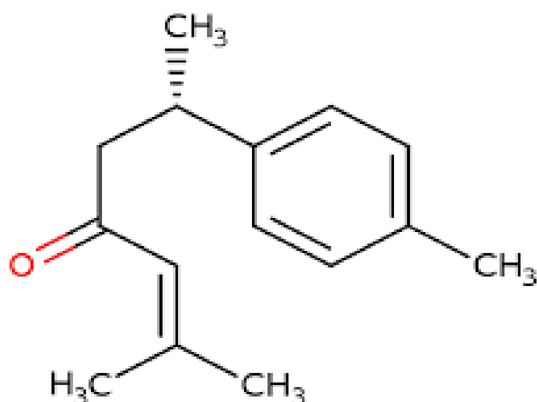
PHYTOCHEMISTRY OF TURMERONES

Turmeric oil is isolated by steam distillation from the rhizome of turmeric.The color and appearance of turmeric oil is pale yellow liquid and store at 4 °Caway from direct light. GC– MS indicate that the main components of turmeric oil are ar-turmerone (61.79%) and curlone (β- turmerone) (12.48%). Other major andminor ingredients in this essential oil are ar-curcumene (6.11%), phenol (3.45%),zingiberene (2.98%), α-sesquiphellandrene (2.81%), 1-ethyl- 4-isobutylbenzene(2.62%), β–bisabolene (1.48%), benzene (1.48%), benzaldehyde (1.44%), 1,2,3,5-tetramethyl-benzene (1.42%), silane (0.84%), and 4-methyl-carbanilonitrile(1.09%) some other constituents are ar-turmerol, caryophyllen oxide, d-3-carene,α-phellandrene.

Major components of turmeric oil are

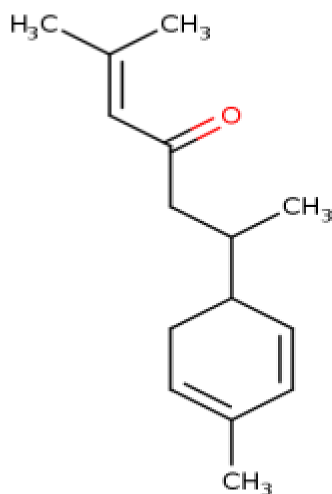
- Aromatic turmerones
- Alpha turmerones
- Beta turmerones

1. AROMATIC TURMERONE: -



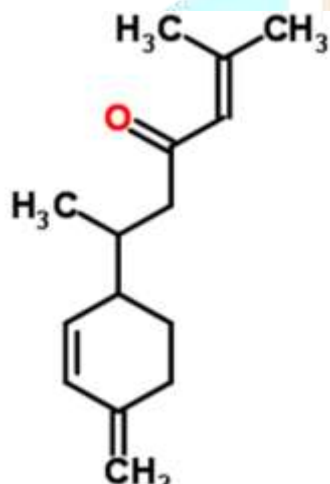
- Molecular formula: - C 15 H 20 O
- IUPAC Name: (6S)-2-Methyl-6-(4 methylphenyl) hept-2-en-4-one
- Molecular weight: - 216.324g/mol
- Boiling point: - 325-3260c
- Solubility: -Alcohol, Hexane

2. ALPHA TURMERONE



- Molecular formula: - C15H22O
- IUPAC Name : 2-methyl-6-(4-methylcyclohexa-2,4-dien-1-yl)hept-2-en-4-one
- Molecular weight:- 218.34g/mol
- Boiling point:- 318-320^{0}c
- Solubility:- Alcohol and Hexane

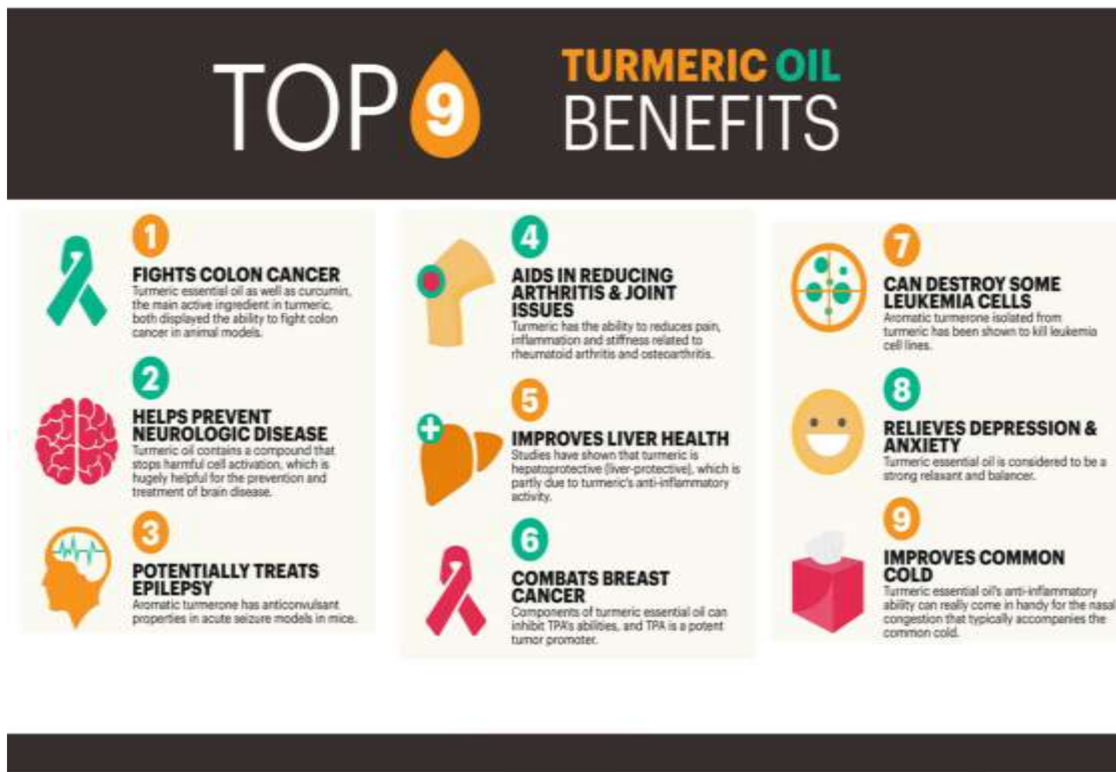
1. BETA TURMERONE



- Molecular formula: - $C_{15}H_{22}O$
- IUPAC Name: 2-methyl-6-(4-methylenecyclohex-2-en-1-yl) hept-2-en-4-one
- Molecular weight: - 218.34g/mol
- Boiling point: - 319-320°c
- Solubility: -Alcohol and Hexane

MEDICINAL USES AND BENEFITS

Turmeric essential oil has many health benefits and uses. Some of them include:



- **Lowers Inflammation**

  The alpha-curcumin found in turmeric essential oil comes in very high concentrationsand is the main anti-inflammatory substance in this oil. This chemical is derived from curcumin, which is the active ingredient in the turmeric spice that provides so many of its health benefits. This allows turmeric oil to so the inflammation associated with arthritis, gout, headaches, muscle pain, joint disorders, and various chronic diseases throughout the body, including gastrointestinal inflammation.

- **Prevents Cancer**

  Significant research has been conducted on both turmeric and turmeric oil, and the anti-mutagenic properties of this oil are well known. It can not only prevent the multiplication and reproduction of cancer cells within the body but also impair the energy-generating structures in cancer cells, causing apoptosis and functioning as a secondary treatment for a variety of cancer types.

- **Stimulates Circulation**
  Turmeric oil is known as a stimulant, as it not only boosts the metabolism but also promotes heart health by reducing platelet aggregation in the arteries and blood vessels. This can allow for better circulation of blood in the body.

- **Detoxifies the Body**
  One of the active ingredients in turmeric essential oil is ar-turmerone, which is directly linked to liver health and detoxification. It can also stimulate urination, as a diuretic substance, which further speeds the release of excess toxins, fats, and salts from the body.

- **Boosts Immune System**
  Few essential oils are as trusted when it comes to immune health and turmeric essential oil is widely used in the pharmaceutical industry, due to its antiseptic, anti-microbial, antibacterial, anti-fungal, and anti-parasitic qualities [6]. When ingested, even in small amounts, the powerful active ingredients can offer serious support to your body's natural defences.

- **Improves Digestion**
  Zingiberene makes up more than 30% of turmeric essential oil's components and is known to have a strong effect on the gastrointestinal system. It can not only promote flatulence and reduce bloating/cramping, but also optimize nutrient uptake and the digestive process.

- **Hair Care**
  Turmeric essential oil has long been used as a natural remedy for hair loss and male pattern baldness. It is believed to be the result of the many antioxidants present in this oil, which can stimulate the regrowth of healthy cells and ensure the functioning of hair follicles, while also eliminating infections and inflammation on the scalp.

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- **Skin Care**
  Topically applying turmeric essential oil to the skin, in moderate amounts, along with a carrier oil, can help make skin look and feel younger, thanks to the concentrated antioxidants in this oil such as curcumin , zingiberene, and other sesquiterpenes. This can improve skin elasticity, reduce the appearance of wrinkles, and protect the skin from infection and irritation.

- **Improves Allergic Response**
  The immune-boosting properties of turmeric essential oil can improve the body's ability to respond accurately to allergens. More specifically, this essential oil can reduce the severity of allergic reactions by regulating the immune response.

- **Boosts Cognitive Function**

  Many of the antioxidants found in high concentrations in turmeric oil can prevent plaque deposition in neural pathways, helping to avoid neurodegenerative diseases and dementia, as well as Alzheimer's disease. Inhaling this oil is particularly effective for a boost to memory, focus, and concentration.

- **Improves Heart Health**

  By reducing platelet aggregation, lowering blood pressure, reducing inflammation, and stimulating the growth of new red blood cells, turmeric essential oil can protect against atherosclerosis, fatigue, weakness, anaemia, weakened blood vessels, heart attack, stroke and other cardiovascular diseases.

- **Culinary Uses**

  Turmeric oil is not solely used for medicinal purposes; it also has a rich, aromatic flavour that can complement many different dishes. Turmeric is often used as a spice, but concentrated doses of turmeric essential oil might also be desired in your kitchen.

PHARMACOLOGICAL ACTIVITY

Turmerone Induces Neural Stem Cell Proliferation

Curcumin an active compound found in the rhizome (underground stem) of turmeric (Curcuma longa)ar-turmerone and a great many other active turmeric compounds have not been widely explored. Although there has been a lot of research about curcumin, comparatively little is known about turmeric itself. Turmeric, a golden spice, has been used since ancient times to give color and taste to food. Over the millennia, it has also been used in Ayurveda medicine for the treatment of many ailments, such as those that affect the stomach and the liver. Also, for reproductive problems, infectious diseases, and blood disorders.

In recent years, science has provided the basis for the use of turmeric against such disorders and more. Turmeric is chemically diverse in composition. Various chemical constituents have been isolated from this spice that fall into a number of distinct categories. To date, around 235 compounds, primarily phenolic compounds and terpenoids, have been identified from this spice. Of these compounds, 22 are diarylheptanoids and diarylheptanoids, 8 phenylpropene and other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, 5 diterpenes, 3 triterpenoids, 4 sterols, 2 alkaloids, and 14 other

compounds. Curcumin, which constitutes 2 – 5% of turmeric rhizome, is perhaps the most-studiedcomponent. However, while some of the activities of turmeric can be mimicked by curcumin, other activities are curcumin-independent. Among the curcuminoids, which belong to the group of diarylheptanoids, are found a few of most studied bioactive ingredients of turmeric. The most common curcuminoid present in turmeric is curcumin, and commercial curcumin is typically a mixture of three curcuminoids.

Ar-Turmerone Enhances Bioavailability of Curcumin

Dried turmeric rhizomes usually yield 1.5 – 5% essential oils, which are dominated by sesquiterpenes and are responsible for its aromatic taste and smell. Among the most common sesquiterpenes is ar-turmerone. The essential oil from Curcuma longa L. was analyzed by gas chromatography – mass spectrometry. The major components of the oil were ar-turmerone (33.2%), α-turmerone (23.5%) and β-turmerone (22.7%).2 In another study, the ar-turmerone amount in turmeric essential oils was found to be as high as 62%.3 Thus, in some samples of whole turmeric, the amount of turmerone approaches that of curcumin.

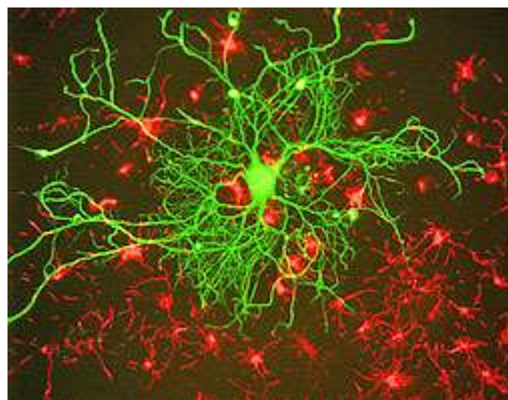
In a 2005 study, the curcuminoids and ar-turmerone were found to have hypoglycemic effects (the ability to reduce blood sugar) by activating PPAR-gamma* as one of the mechanisms.4 While turmeric's most well-studied component, curcumin, has been shown to be a valuable antioxidant, separated from turmeric it exhibits poor bioavailability in animal studies and clinical trials. In a 2012 study, the poor bioavailability of curcumin was enhanced through the

use of ar-turmerone, so much so that the researchers concluded that the combination, rather than curcumin alone, was necessary for treating diseases.

Self-Repair and Recovery of Brain Function

Judging from a new study published in the journal Stem Cell Research & Therapy, data indicate that ar-turmerone induces neural stem cell (NSC) proliferation.6 By promoting endogenous NSC, which are stem cells found within adult brains and which differentiate into neurons, ar-turmerone may play an important role in self-repair and recovery of brain function in neurodegenerative diseases.

Neural Stem Cells Produce Neurons



Neural stem cells in red

The study's scientists discovered that when they put neural stem cells in petri dishes, and bathed them in ar-turmerone, up to 80 percent more of the stem cells grew into neurons or others cells, compared to control experiments where ar-turmerone wasn't used.Previous studies of ar-turmerone have shown that it can block activation of neuroinflammatory microglia cells, thus helping to prevent assorted neurological disorders.7,8 Until this recent study, however, ar-turmerone's effect on the brain's capacity to self-repair was unknown.

Thus, ar-turmerone shows great promise as a contestant to support regeneration in neurologic disease. Ar-turmerone may therefore be poised as a nutrient candidate for treating neurological disorders, such as stroke and Alzheimer's disease.

Up to 80% Increase in Neural Stem Cells

In the study, researchers from the Institute of Neuroscience and Medicine in Jülich, Germany, studied the effects of ar-turmerone on NSC proliferation and differentiation both in vitro and in vivo. In vitro, rat fetal NSC were cultured and grown in six different concentrations of ar-turmerone over a 72-hour period. At certain concentrations, ar-turmerone was shown to increase NSC proliferation by up to 80%, without having any impact on cell death. The cell differentiation process also accelerated in ar-turmerone-treated cells compared to untreated control cells. To summarize, rodent neural stem cells grew when they were bathed in a solution of ar-turmerone and appeared to specialize into certain types of brain cells more rapidly.

Sub-ventricular Zone Widened and Hippocampus Expanded

To test the effects of ar-turmerone on NSC in vivo, the researchers injected adult rats with ar-turmerone. Using positron emission tomography (PET) imaging† and a tracer to detect proliferating cells, they found that the sub-ventricular zone (SVZ) was wider, and the hippocampus more expanded, in the brains of rats injected with ar-turmerone than in control animals. In adult mammalian brains, the SVZ and hippocampus are the two sites where the growth of new neurons is known to occur.

ISOLATION OF TURMERONES

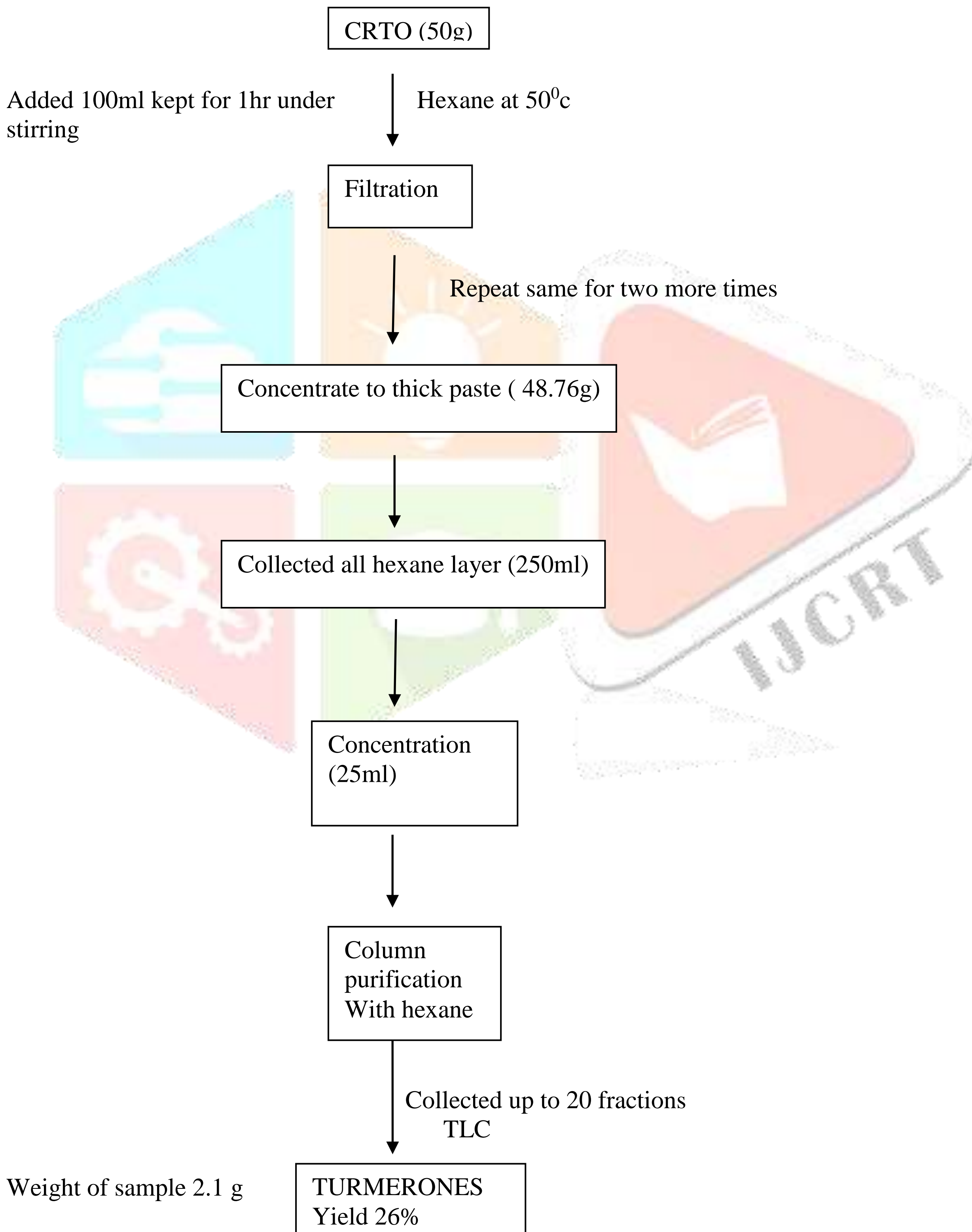
After the extraction of curcumin, it is repeatedly washed with hexane to remove the turmeric oil. This hexane layer is separated by decantation.

The residue obtained contains Curcuminoids which crystallised by using IPA (isopropyl alcohol). This solution is filtered to get the pure crystals of curcuminods. The filtrate is concentrated which forms a thick gummy paste that is Curcumin removed turmeric oleoresin. This paste is brownish in colour. Obtained CRTO was kept in Water bath at 60^0C for 1 hour to remove excess IPA and then cooled to room temperature .

It is washed with hexane to remove excess of oil content that is nothing but turmerones. Now both hexane layers which contain turmerones are concentrated by using rotary evaporator.

This concentrated hexane extract is passed through Glass column which is packed with silica gel. The fractions were collected based on TLC. Pure Turmerone rich fractions were pooled and concentrated to remove hexane layer by using rotaryevaporator at 50°c under reduced pressure. The yellow coloured oil was obtained having total turmerones.

Process Flowchart:

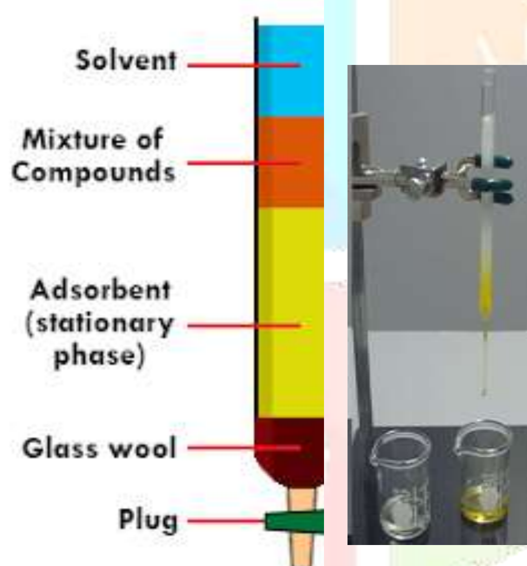


CHROMATOGRAPHY

Chromatography has been developed into new method of separation of mixture of substance mainly when they are available in small amount. This method is very useful when the components of the mixture have almost same physical and chemical properties and hence cannot be separated by other usual methods of separations. The term chromatography means writing in color

The method of separation in chromatography is based on distribution of the components in a mixture between a fixed (stationary) and a moving (mobile) phase. The stationary phase maybe column of adsorbent, a paper, a thin layer of adsorbent on a glass plate etc, through which mobile phase moves on the mobile phase that may be liquid,gas. When a solid stationary phase is taken as a column it is known as column chromatography.

COLUMN CHROMATOGRAPHY



Column chromatography is one of the most useful methods for the separation and purification of both solid and liquid. This is a solid –liquid technique in which stationary phase is solid and mobile phase is liquid. The principle of column chromatography is based on differential adsorption of substance by the adsorbent.

The usual adsorbent employed in column chromatography are silica,alumina, calciumcarbonate, calcium phosphate, magnesia, starch etc, selection of solvent is based on the nature of both the solvent and the adsorbent. The rate at which the components of a mixture are separated depends on the activity of the adsorbent and polarity of the solvent.

If the activity of the adsorbent is very high and polarity of the solvent is very low, then the

separation is very slow but gives a good separation. If the activity of adsorbent is low and polarity of the solvent is high the separation is rapid but only gives poor separation.

PRINCIPLE

When a mixture of mobile phase and sample to be separated are introduced from top of column, the individual components of mixture move with different rates.

EXPLANATION: - Those with lower affinity and adsorption to stationary phase move faster and eluted out first while those with greater adsorption affinity move or travel slow and get eluted out last

The solute molecules absorb to the column in a reversible manner. The rate of movement of the component is given asR= rate of movement of components/rate of movement of mobile phase, that is., it is the distance moved by solute to the distance moved by solvent.

PROCEDURE:-

The stationary phase material is suitably moistened with mobile phase and packed sufficiently in the column with a cotton or asbestos pad at the bottom. The extract material or sample to be separated is placed on the top of packed stationary phase with a second cotton or asbestos pad in between. The mobile phase is poured into the column over the sample. A collecting beaker is placed at the bottom of column near the end to collect the elute. The mobile phase percolates through entire stationary phase researches the bottom of the column. From there it elutes out and gets collected in the beaker placed below. When the mobile phase flows through, different components of the sample travel with different rates through the silica gel. This rate of travel is decided by the adsorption and affinity of molecules towards the stationary phase and mobile phase. The fractional components of the mixture with greater affinity to mobile phase travels fast and reach the bottom early. Those with higher affinity to stationary phase travel slow and reach bottom late. Thus, coloured bands of sample are formed. Each colour is an indicator of one set of compound in the sample mixture. Then by differential mobile phase different components are taken out of column by further flow of solvents. This elution is drop by drop and the process may take few hours to days based on the sample size, length of the column, mobile phase used and the packing material used.

COLUMN CHROMATOGRAPHY APPLICATIONS:-

- Column chromatography is best suited to separate active principle from plant material. Since plants contains many ingredients like alkaloids, resins, glycosides, tannins, flavonoids, and other bio molecules,the individual constituents arte to be separated. Since, the plant extract is bulk this method is best to separate them
- In separation of compounds after organic synthesis to obtain desired molecules.
- To separate or purify natural compound mixture likealkaloids, glycosides.

PARAMETERS

1. THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is chromatography technique used to separate non-volatile mixtures. TLC performed on a sheet of glass, plastic or aluminium foil, which is coated with thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known is known as stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC place at different rates, separation is achieved.

The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non polar mobile phases such as heptanes are used. The mobile phase may be a mixture, allowing chemist to fine – tune the bulk properties of the mobile phase.

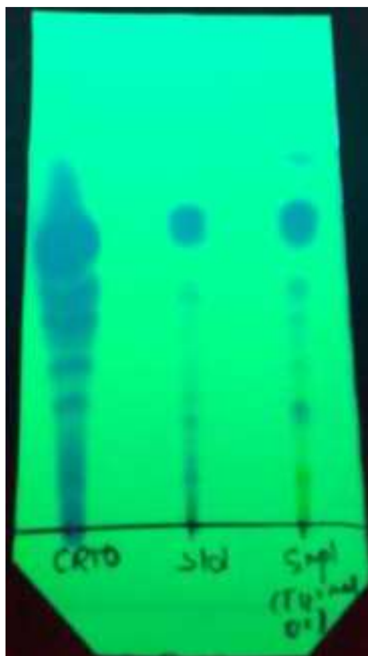
After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light on the sheet; the sheets are treated with a phosphor, and dark spots appear on sheet where compounds absorb the light impinging on a certain area. Chemical process can also be used to visualize spots anisaldehyde, for example, forms coloured adducts with many compounds, and sulphuric acid will char most organic compounds, leaving a dark spot on the sheet.To quantity the results, the distance travelled by the substance being considered is devided by the total distance travelled by the mobile phase. (the mobile phase must not be allowed to reach the end of the stationary phase). The ratio is called the Retention factor or $R_{f.}$

Preparation of mobile phase :

7ml of Hexane + 3ml of Ethyl acetate

Detection:

UV light Chamber254 nm.



2. **SPECIFIC GRAVITY:**

Specific gravity is the ratio of the density of a substance to the density of a reference substance; equivalently, it is the ratio of the mass of a substance to the mass of a reference substance for the same given volume. Apparent specific gravity is the ratio of the weight of a volume of the substance to the weight of an equal volume of the reference substance. The reference substance for liquids is nearly always water at its densest (at 4 °C / 39.2 °F); for gases it is air at room temperature (20°C / 68° F).The temperature and pressure must be specified for both the sample and the reference. Pressure is nearly always 1 atm (101.325 kPa).

Specific gravity varies with temperature and pressure; reference and sample must be compared at the same temperature and pressure or be corrected to a standard reference temperature and pressure. Substances with a specific gravity of 1 are neutrally buoyant in water. Those with SG greater than 1 are denser than water and will, disregarding surface tension effects, sink in it. Those with an SG less than 1 are less dense than water and will float on it.

In scientific work, the relationship of mass to volume is usually expressed directly in terms of the density (mass per unit volume) of the substance under study. It is in industry where specific gravity finds wide application, often for historical reasons.

Analytical Methods

Pycnometer (Typical ASTM Test method D1840)

Specific gravity can be measured in a number of value ways. A pycnometer is simply a bottle which can be precisely filled to a specific, but not necessarily accurately known volume. Placed upon a balance of some sort it will exert a force where using the mass of the bottle filled with the product being tested and the mass of the bottle full of water the specific gravity can be calculated.

Digital density meters (Typical ASTM Test method D4052)

Vibrating Element Transducers: This type of instrument requires a vibrating element to be placed in contact with the fluid of interest. The resonant frequency of the element is measured and is related to the density of the fluid by a characterization that is dependent upon the design of the element. In modern laboratories precise measurements of specific gravity are made using oscillating U-tube meters. These are capable of measurement to 5 to 6 places beyond the decimal point and are used in the brewing, distilling, pharmaceutical, petroleum and other industries. The instruments measure the actual mass of fluid contained in a fixed volume at temperatures between 0 and 80 °C but as they are microprocessor based can calculate apparent or true specific gravity and contain tables relating these to the strengths of common acids, sugar solutions, hydrocarbons etc.

$$S = \frac{W}{W_1 - W_2}$$

3. REFRACTIVE INDEX

Theoretical introduction:

Refractive index is a dimensionless physical quantity, which is specific for a certain medium, and its value characterizes the speed of light in this medium. We distinguish between the relative and absolute refractive index. The absolute refractive index is defined as a ratio of the speed of light in vacuum and in selected medium.

Generally, the refractive index depends on the wavelength of incident light. Relative refractive index is defined as a ratio of speeds of light in two different media. Usually it characterizes properties of an interface between these media. If light impacts the interface between two media, it is partially reflected and partially refracted. Snell's law describes the relation between the angles of incidence and refraction.

DETERMINATION OF REFRACTIVE INDEX

Refractometer:

The temperatureof the refractometer should be controlled to within ±O' I°0 and for thispurpose it should be provided with a thermostatically controlled water-bathand a motor-driven pump to circulate water through the instrument. Theinstrument should be standardized, following the manufacturer's instructions. with a liquid of known purity and refractive index or with a glassprism of known refractive index. Distilled water, which has a refractiveIndex of 1'333 0 at 20'O°C, is a satisfactory liquid for standardization.

Light Source - If the refractometer is equipped with a compensator, a tungsten lamp or a daylight bulb may be used. Otherwise, a monochromatic light, such as an electric sodium vapour lamp, should beused.

Procedure - Melt the sample, if it is not already liquid, and filter through a filter paper to remove any impurities and the last traces of Moisture. Make sure that the sample is completely dry. Adjust the temperature of the refractometer to 40'0 ± O'l°C or any other desired temperature. Ensure that the prisms are clean and completely dry, and then place a few drops of the sample on the lower prism. Close the prisms, tighten firmlywith the screw-head, and allow to stand for one or two minutes. Adjustthe instrument and light to obtain the most distinct reading possible and determine the refractive index.

4. DETERMINATION OF ACID VALUE:

The acid value (AV) is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil.

Determination of acid value

(a) **Reagents**

Phenolphthalein indicator

Weigh 1 g of phenolphthalein and dissolve in 100 mL of ethanol.

Sodium hydroxide titrant

Weigh accurately 4.0 g of sodium hydroxide and place it in a 1000-mL volumetric flask. Make up to the mark with water.

Ethanol-ether solution

Prepare a mixture of ethanol and diethyl ether (1:1, v/v). Neutralize with sodium hydroxide titrant and add 1.0 mL of phenolphthalein indicator until pink colouration is observed. Freshly prepare the solution.

(b) **Standardization of sodium hydroxide titrant**

Weigh accurately 0.6 g of potassium hydrogen phthalate, previously dried to constant weight at 105^0 C and place it in a 250-mL conical flask, then add 50 mL of water. Shake it well. Add 2 drops of phenolphthalein indicator. Titrate the solution with the sodium hydroxide titrant until pink colouration can be observed. Towards the end of titration, potassium hydrogen phthalate should be completely dissolved. Calculate the concentration of the sodium hydroxide titrant according to the following equation:

$$C_{NaOH} = \frac{W_{C_8H_5KO_4} \times P_{C_8H_5KO_4} \times 1000}{V_{NaOH} \times Mw_{C_8H_5KO_4}}$$

Where,

C_{NaOH} = Molarity of sodium hydroxide titrant (mol/l)

V_{NaOH} = Volume of sodium hydroxide titrant used (ml)

$Mw_{C8H5KO4}$= Molecular weight of potassium hydrogen phthalate (204.22 g)

$W_{C8H5KO4}$ = Weight of potassium hydrogen phthalate used (g)

$P_{C8H5KO4}$ = Purity of potassium hydrogen phthalate (%)

Titration of test solution

Unless otherwise specified, weigh accurately a quantity of the fatty oil being examined as indicated in Table 1 and place it in a 250-mL conical flask, then add 50 mL of ethanol-ether solution. Shake it well. If necessary, reflux the mixture gently until the substance is completely dissolved. Titrate the solution with sodium hydroxide titrant until pink colouration can be observed which persists for 30 s. Measure the volume of sodium hydroxide titrant used and calculate the acid value according to the following equation:

$$\text{Acid value} = \frac{V_{NaOH} \times 5.61}{W}$$

Where,

V_{NaOH}= Volume of sodium hydroxide titrant used (ml)

W = Weight of the fatty oil being examined (g)

When the acid value is less than 10, it is suggested that a 10-mL semi-micro burette may be used for the titration.

ESTER VALUE

The ester value is the number of mg of potassium hydroxide required to saponify the esters in 1.0 g of the substance. If the saponification value and Acid value have been determined, the difference between these two represents the Ester value.

PROCEDURE: -

Place 1.5 g to 2 g of the substance in a tared, 250 ml flask, weigh accurately, add 20 ml to 30ml of neutralized alcohol and shake. Add 1ml of phenolphthalein TS and titrate with 0.5N alcoholic potassium hydroxide VS until the free acid is neutralized. Add 25.0 ml of 0.5N alcoholic potassium hydroxide VS and as proceed as directed under Saponification Value, beginning with " Heat the flask " and omitting the further addition of phenolphthalein TS. The difference between the volumes in ml of 0.5N hydrochloric acid consumed in the actual test in

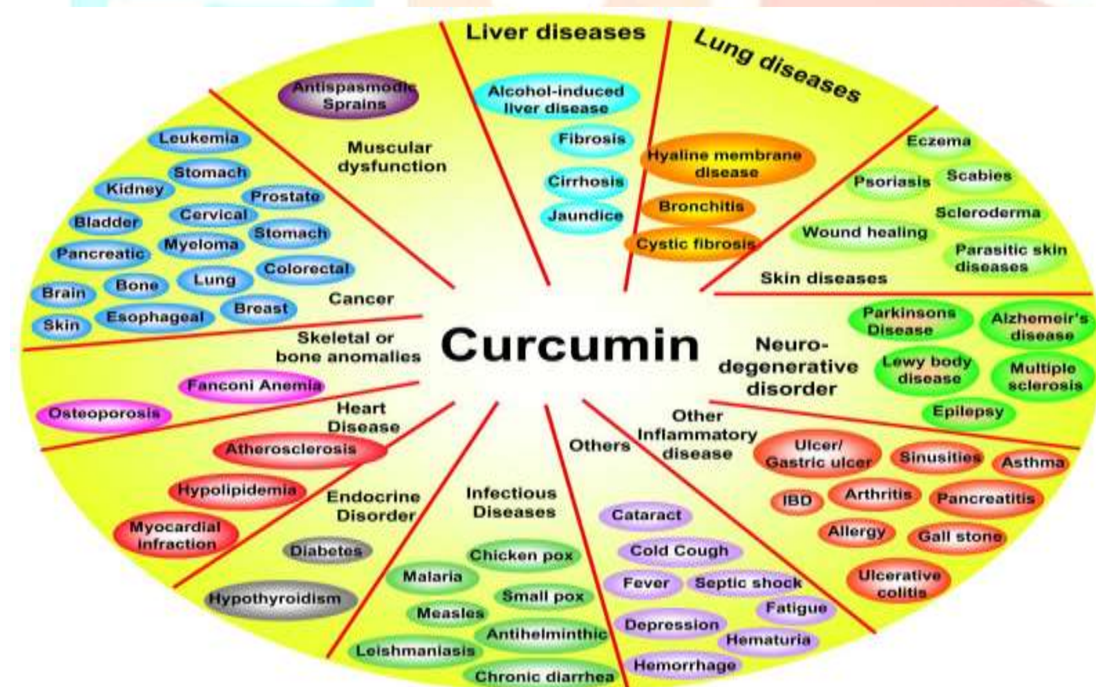
the blank test, multiplied by 28.05 and divided by the weight in g of the specimen taken, is the Ester Value

RESULT

Physical and Chemical properties	Values
Boiling point	330^0 C
Specific gravity	0.9234
Refractive Index	1.5154
Acid Value	3.4
Ester Value	32.98

PHARMACOLOGY ANDINSTRUMENTATION

MEDICINAL AND PHARMACOLOGICALPROPERTIES OF TURMERIC



Anti-inflammatory properties

Oral administration of curcumin in instances of acute inflammation was found to be as effective as cortisone or phenylbutazone. Oral administration of Curcuma longa significantly reduced inflammatory swelling [8]. C. longa's anti-inflammatory properties may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states. Curcuminoids also inhibit LOX, COX, phospholipases, leukotrienes, prostaglandins, thromboxane, nitric oxide elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1,

interferon inducible protein, TNF and interleukin-12. They also decrease prostaglandin formation and inhibit leukotriene biosynthesis via the lipoxygenase pathway. An RCT investigated the effect of a combination

of 480mg curcumin and 20mg quercetin (per capsule) on delayed graft rejection (DGR) in 43 kidney transplant patients. Of 39 participants who completed the study, two of 14 in the control group experienced DGR compared to zero in either treatment group.

Early function (significantly decreased serum creatinine 48 hours post-transplant) was achieved in 43% of subjects in the control group, 71% of those in the low dose treatment group. Since the amount of quercetin in the compound was minimal, the majority of benefit is thought to be due to curcumin's anti-inflammatory and antioxidant activity. Likely mechanisms for improved early function of transplanted kidneys include induction of the hemeoxygenase enzyme, and proinflammatory cytokines, and scavenging of free radicals associated with tissue damage.

Antioxidant Effects

Water and fat-soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity, comparable to vitamins C and E. A study of ischemia in the felineheart demonstrated that curcumin pre-treatment decreased ischemia-induced changes in the heart. An *in vitro* study measuring the effect of curcumin on endothelial home oxygenase-1 an inducible stress protein, was conducted utilizing bovine aortic endothelial cells. Incubation (18 h) with curcumin resulted in enhanced cellular resistance to oxidative damage.

Rheumatoid Arthritis

In an animal model of streptococcal cell wall-induced rheumatoid arthritis, a turmeric extract devoid of essential oils was given to Wistar female rats. Intraperitoneal injection of an extract containing 4 mg total curcuminoids/ kg/day for four days prior to arthritis induction significantly inhibited joint inflammation in both the acute (75%) and chronic (68%) phases. To test the efficacy of an oral preparation, a 30-fold higher dose (to allow for possible low gastrointestinal absorption) of the curcuminoid preparation, given to rats four days prior to arthritis induction, significantly reduced joint inflammation by 48% on the 3rd day of administration.

Pancreatitis

In two rat models of experimentally - induced pancreatitis, curcumin decreased inflammation by markedly decreasing activation of Nuclear factor-kappa B and Activating Protein-1 as well as inhibiting mRNA induction of interleukin-6, tumor necrosis factor-α, and inducible Nitric Oxide Synthetase in the pancreas. Both cerulean induced and ethanol inducedpancreatitis, curcumin showed inhibitory effect on the inflammatory mediators resulted in improvement in disease severity as measured by histology, serum amylase, pancreaticrypsin, and neutrophil infiltration.

Cancer

Numerous animal studies have explored curcumin anti-inflammatory mechanisms and their influence on the carcinogenesis. Table 3 lists animal studies in which oral or dietary curcumin inhibited carcinogenesis through anti-inflammatory mechanisms.

Hepatoprotective Effects

Turmeric has been found to have a hepatoprotective characteristic similar to silymarin. Animal studies have demonstrated turmeric's hepatoprotective effects from a variety of hepatotoxic insults, including, carbon tetrachloride (CCl4), galactosamine,acetaminophen (paracetamol) and Aspergillusaflatoxin.Turmerichepatoprotective effect is mainly a result of its antioxidant properties, as well as its ability to decrease the formation of pro-inflammatory cytokines. In rats with CCl4-induced acute and subacute liver injury,curcumin administration significantly decreased liver injury in test animals compared to controls. Turmeric extract inhibited fungal aflatoxin production by the 90% when given to ducklings infected with Aspergillusparasiticus. Turmeric and curcumin also reversed biliaryhyperplasia, fatty changes, and necrosis induced by aflatoxin production. Sodium curcuminate, a salt of curcumin, also exerts choleretic effects by increasing biliaryexcretion of bile salts, cholesterol, and bilirubin, as well as increasing bile solubility, therefore, possibly preventing and treating cholelithiasis.

Antimicrobial Effects :

Turmeric extract and the essential oil of C. longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caecal parasite Eimera maxima demonstrated that diets supplemented with 1% turmeric resulted in a reduction in small intestinal lesion scores and improved weight gain. Another animal study, in which guinea pigs were infected with either dermatophytes, pathogenic moulds or yeasts found thattopically applied turmeric oil inhibited dermatophytes and pathogenic fungi, but neither curcumin nor turmeric oil affected the yeast isolates. Improvements in the lesions were observed in the dermatophytes and fungi-infected guinea pigs and at 7 days post-turmeric application the lesions disappeared. Curcumin has also been found to have moderate activity against Plasmodium falciparum and Leishmania major organisms.

Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. The extensive survey of the literature revealed that C. longa is highly regardedas a universal panacea in the herbal medicine with diverse pharmacological activity spectrum. This versatile medicinal plant is the unique source of various types of chemical compounds, which areresponsible of the various activities of the plant. Hence, extensive investigation is needed toexploit their therapeutic utility to combat diseases. A drug development program should beundertaken to develop modern drugs. Although crude extracts from leaves of the plant have medicinal applications from time immemorial, moderndrugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics and toxicity after proper standardization and clinical trials.As the global scenario is now changing towards the use of non-toxic plant products having traditional medicinal use, development of modern drugs from C. longa shouldbe emphasized for the control of various diseases.

INSTRUMENTATION :

ROTARY EVAPORATOR

A **rotary evaporator** (or **rotavap/rotovap**) is a device used in chemical laboratories for the efficient and gentle removal of solventsfrom samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (e.g., "the sample was evaporated under reduced pressure"

THEORY:

Vacuum evaporators as a class function because lowering the pressure above a bulk liquid lowers the boiling points of the component liquids in it. Generally, the component liquids of interest in applications of rotary evaporation are research solvents that one desires to remove from a sample after an extraction, such as following natural product isolation or a step in an organic synthesis. Liquid solvents can be removed without excessive heating of what are often complex and sensitive solvent-solute combinations

Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents such a n-hexane or ethyl acetate from compounds which are solid at room temperature and pressure. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there is minimal co-evaporation (azeotropic behavior), and a sufficient difference in boiling points at the chosen temperature and reduced pressure.

Solvents with higher boiling points such as water (100 °C at standard atmospheric pressure, 760 torr or 1 bar), dimethylformamide (DMF, 153 °C at the same), or dimethyl sulfoxide (DMSO, 189 °C at the same), can also be evaporated if the unit's vacuum system is capable of sufficiently low pressure. (For instance, both DMF and DMSO will boil below 50 °C if the vacuum is reduced from 760 torr to 5 torr [from 1 bar to 6.6 mbar]) However, more recent developments are often applied in these cases (e.g., evaporation while centrifuging or vortexing at high speeds). Rotary evaporation for high boiling hydrogen bond-forming solvents such as water is often a last recourse, as other evaporation methods or

freeze-drying (lyophilization) are available. This is partly due to the fact that in such solvents, the tendency to (("bump" is accentuated. The modern centrifugal evaporation technologies are particularly useful when one has many samples to do in parallel, as in medium- to high-throughput synthesis now expanding in industry and academia.

Evaporation under vacuum can also, in principle, be performed using standard organic distillation glassware — i.e., without rotation of the sample. The key advantages in use of a rotary evaporator are that the centrifugal force and the frictional force between the wall of the rotating flask and the liquid sample result in the formation of a thin film of warm solvent being spread over a large surface.

The forces created by the rotation suppress ((bumping. The combination of these characteristics and the conveniences built into modern rotary evaporators allow for quick, gentle evaporation of solvents from most samples, even in the hands of relatively inexperienced users. Solvent remaining after rotary evaporation can be removed by exposing the sample to even deeper vacuum, on a more tightly sealed vacuum system, at ambient or higher temperature (e.g., on a Schlenk line or in a vacuum oven).

HPLC [HIGH PRESSUER LIQUID CHROMATOGRAPHY]

The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios .

changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature at which the separation is performed.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography), is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts

slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

HPLC has been used for manufacturing (e.g. during the production process of pharmaceutical and biological products), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g. detecting vitamin D levels in blood serum) purposes.

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid particles (e.g. silica, polymers, etc.), 2–50 micrometers in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the absorbent particles. The pressurized liquid is typically a mixture of solvents (e.g. water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50–350 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length. Also HPLC columns are made with smaller sorbent particles (2–50 micrometre in average particle size). This gives HPLC superior resolving power (the ability to distinguish between compounds) when separating mixtures, which makes it a popular chromatographic technique.

Principle:

HPLC is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase & the stationary phase used in the separation.

Types: there are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. Normal phase HPLC: This separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase & non-polar mobile phase. Therefore, the stationary phase is usually silica & typical

mobile phases are hexane, methylene chloride, chloroform, diethyl ether and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2. Reverse phase HPLC: The stationary phase is non-polar (hydrophobic) in nature , while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more non-polar the material is, the longer it will be retained.

3. Size-exclusion HPLC: The column is filled with material having precisely controlled pore sizes, & the particles are separated according to its molecular size. Larger molecules are rapidly washed through the column ; samaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-exchange HPLC: The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionisable samples. The stronger the charge on the sample , the stronger it will be attached to the ionic surface & thus , the longer it will take to elute. The mobile phase is an aqueous buffer, where both p^H& ionic strength are used to control elution time. HPLC instrumentation includes a pump ,column, injector, detector, and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

Solvent reservior:

Mobile phase contents are present in a glass reservoir . The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending upon the composition of the sample.

Pump:

A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressure of upto 42000 Kpa (about 6000 psi) can be generated.

Sample injector:

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100ml of volume with high pressure (upto 4000 psi).

Column:

Columns are usually made of polished stainless steel, are between 50 & 300mm long and have an internal diameter of between 2 & 5mm. They are commonly filled with a stationary phase with particle size of 3-10 micrometer. Columns with internal diameters of less than 2mm are often reffered to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

Detector:

Detector is located at the end of the column, detects the analyte as they elute from the chromatographic column. Commonly used detectors are UV-Spectroscopy, Fluorescence, Mass spectrometric & electrochemical detectors.

Data Collection Devices:

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.Applications:

*Pharmaceuticalapplications:

Tocontroldrugstability

Tablet dissolution study of pharmaceuticaldosage form

Pharmaceutical qualitycontrol

*Environmentalapplications:

Detection of phenolic compounds in drinking water

Bio-monitoring of pollutants

*Application in Forensics:

Quantification of drugs in biological samples

Identification of steroids in blood, urine etc

Forensic analysis of textile dyes

Determination of cocaine and other drugs of abuse in blood, urine etc

*Food and flavours:

Measurement of quality of soft drinks and water

Sugar analysis in fruit juices

Analysis of polycyclic compounds in vegetables

Preservative analysis

*Application in clinical tests:

Urine analysis, antibiotics analysis in blood

Analysis of bilirubin, biliverdin in hepatic disorders

Detection of endogenous neuropeptides in the extracellular fluid of the brain, etc.

HPLC METHOD

Assay:

Total Curcuminoids

Mobile phase A: 0.1 ^{0}/0 v/v Trifluro acetic acid Mobile phase B: Acetonitrile (HPLC grade)

(Gradient program)

Time (Min)	Solvent -B
0.01	50
10.00	50
15.00	95
25.00	95
28.00	50
35.00	50
35.01	stop

Column - Phenomenex Luna C 18 (250 X 4.6mm)I particle size-511

Flow rate - 1.0 ml/min

Wavelength - 420 nm for Curcuminoids

Injection volume10 Ill

Column Temperature350C

Run time - 35 minutes

Details of working standards:

Name of working standard	% Purity
Curcuminoids	99.47%w/w

Preparation of Mobile phase-A (On 1 ^{0}/0 v/v TFA): Take 500 ml purified water into a 1000 ml volumetric flask, add 1 ml of Trifluro acetic acid and make the volume up to the mark with purified water, shake vigorously and sonicate for 5 minutes.

Preparation of Mobile phase-B (Acetonitrile): Take 100 % Acetonitrile.

Diluent preparation (1 00^{0}/0 Methanol): Take Methanol (HPLC grade) and use as a diluent-

Standard stock solution of Curcuminoids (1.0 mg/ml): Weigh about 100 mg equivalent of standard Curcuminoids and transfer in a 100 ml volumetric flask. Add 70 ml methanol and sonicate for 10 minutes, make the volume up to the mark with methanol and mix well.

Working mixed standard:. Take 10m' of curcuminoides standard stock solution to this 100ml volumetric flask and make the volume up to the mark with methanol and mix well. Discard the first 2mL of the filtrate and use subsequent filtrate for the analysis.

Sample Preparation (04 mg/ml):

Weigh accurately about 400mg of powdered test substance (passed through 60#) in to 100 ml volumetric flask. Add 70 ml of Methanol and dissolve by sonication for 10 min.. Make the volume up to the mark with methanol. Pipeete out Iml of this solution into 10ml volumetric flask and Make the volume up to the mark with methanol Filter the solution through 0.451-1. syringe nylon filter.Discard the first 2mL of the filtrate and use subsequent filtrate for the analysis.
Chromatographic procedure: Stabilize the instrument with the mobile phase till the baseline is satisfactory. Inject the standard solution two times and record the chromatogram. The % RSD between the results should be less than 2 ^{0}/0. Inject the sample solution and record the Chromatogram.

The analysis should comply with the following system suitability parameters. Theoretical plate: More than 2000; and tailing factor: Less than 2 Calculation:
Percentage of Total Curcuminoids in w/w content can be calculated using the formula

$$\%\text{w/w of Total Curcuminoids} = \frac{A1}{A2} \times \frac{W2}{1OO} \times \frac{10}{1OOW1} \times 1OO \times \%\ \text{Purity of standard curcuminoids}$$

Where,

Al =AUC of peak in the sample solution corresponding to standard Curcuminoids (sum of three major peaks)

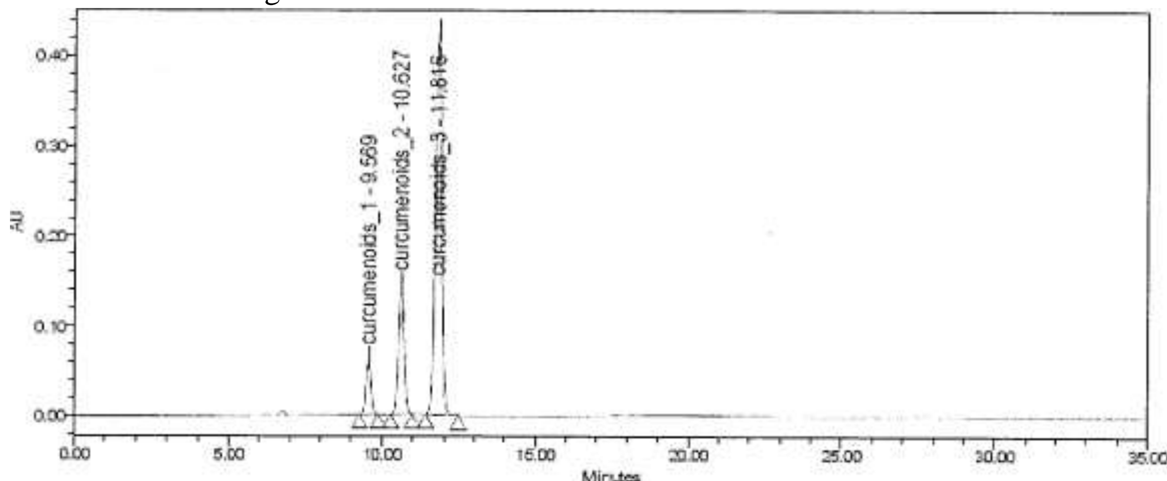
A2 = AUC standard Curcuminoids (Sum of three major peaks)

WI =Weight of sample (mg)

W2 =Weight of standard Total Curcuminoids (mg)

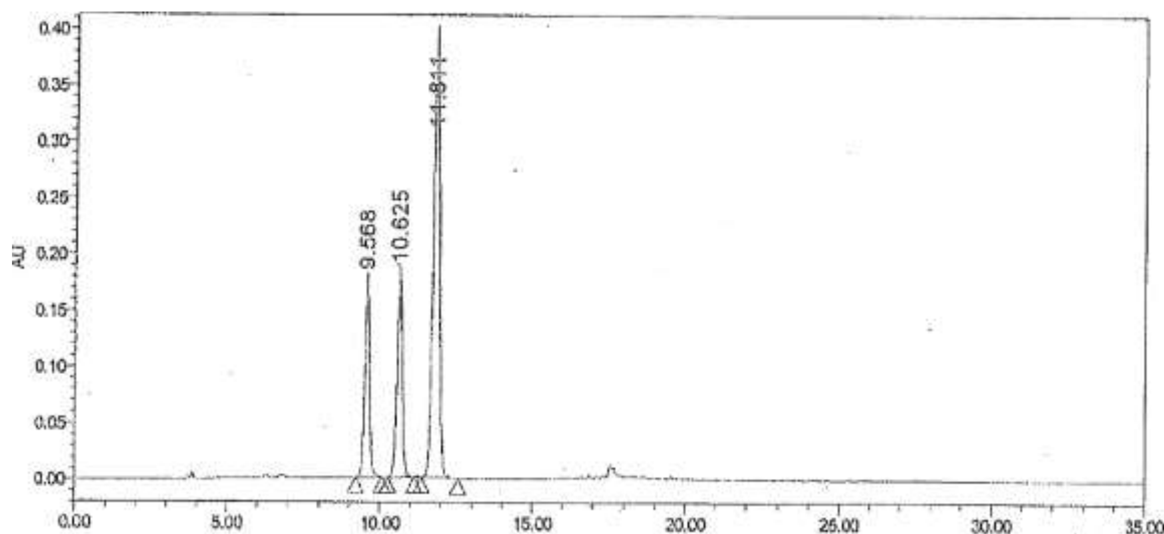
Standard chromatogram of Curcuminoides

Auto-scale chromatogram



Sample chromatogram of Curcuminoides

Auto Scaled Chromatogram



TLC fingerprint:

Chromatographic system:

TLC plate type: Pre coated thin layer silica plate 60 F254' 10 x 10 cm, E-Merck

Mobile phase Chloroform: Methanol (90: 10) Spotting volume 10 PI

Sample preparation: Weigh accurately about 2 g of finely powdered sample in a 100ml beaker. Add 10 ml of methanol and sonicate for 10 minutes, Filter the extract through Whatman No. 1 filter paper.

Standard preparation: Weigh accurately about 1 g of standard in a 100ml beaker. Add 10 ml of methanol and sonicate for 10 minutes Filter the extract through Whatman No. 1 filter paper.

Curcuminoids standard: 1 mg/ml Curcuminoids of standard prepared for HPLC assay.

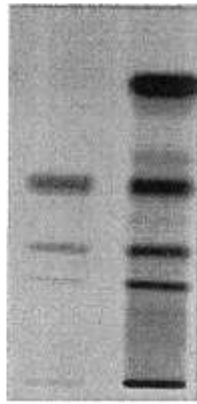
Application: Apply the sample and standard solutions as 10 - 12 mm band, in a distance of 12 mm from the bottom of TLC plate. Make a mark up to a distance of 8u5 cm from the application point as a development mark using pencil

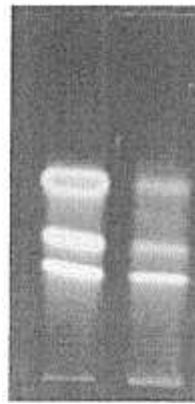
Preparation of development tank: Use Camag twin trough development tank (10 x 10 cm). Cover one side of the inside chamber with the required size of WhatmanNo,,l filter paper. Measure about 20 ml of mobile phase and transfer into the chamber along the side of the filter paper.

Tank saturation: Cover the development t:ank with a lid to saturate the chamber for 30 minutes. At the end of 30 minutes, open the lid to keep the spotted plate and develop the plate up to the pencil marking in the solvent systern. Soon after the development, remove the plate and dry in air at room temperature (27 ± 2 ^{0}C) for about 10 minutes.

Visualization and documentation: Visualize the dried plate under UV 254 nm and 366 nm using UV cabinet. The image of the plate to be captured under UV 254 nm and 366 nm. Observation: The fingerprint obtained with the sample solution should match with the fingerprint obtained with standard solution,,

TLC Images:





At 254nm At 366nm

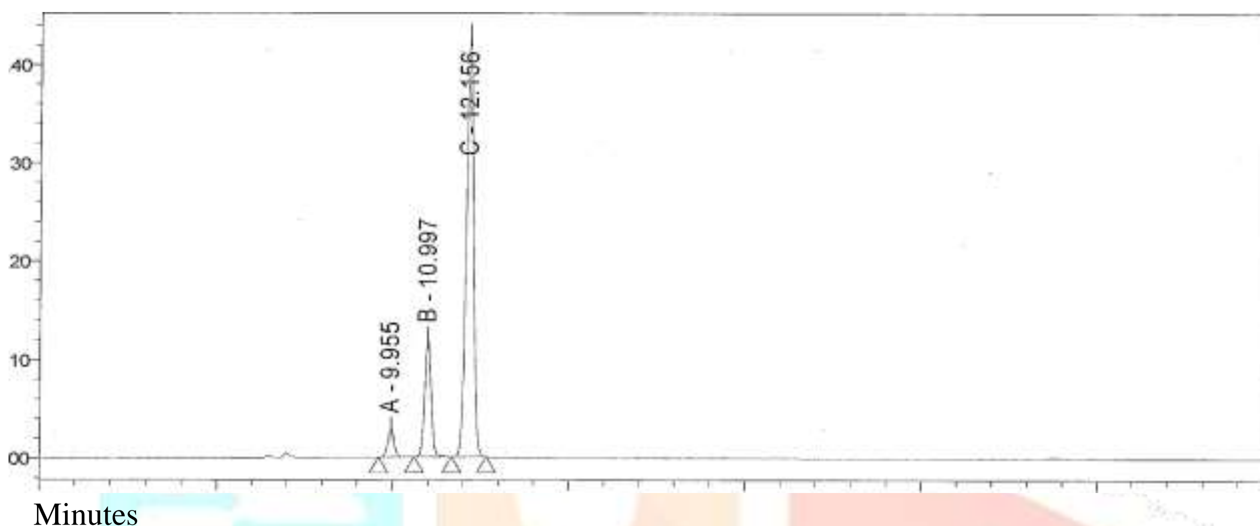
SAMPLE INFORMATION

Sample Name: MIX ST D-1 (curcuminiods+turmeron)

Sample Type: Unknown
Vial: 26
Injection #: 1
Injection Volume: 1 0.00 ul
Run Time: 35.0 Minutes

Acq. Method Set: Curcumenoids_lM
Processing MethocCURCUMINOIDS 420PM
Channel Name: 2998 Ch2 420nm@1 .2nm
Proc. Chnl. Descr..2998 Ch2 420nm@1 .2nm

AutoScaled Chromatogram



Minutes

Peak Result

	Name	RT	AREA	USP Plate Count	USP Tailing	Resolution
1	A	9.95	345545	16835	1.12	
2	B	10.9	1544875	17695	1.04	3.3
3	C	12.1	5944945	17972	1.01	3.4

SAMPLEINFORMATION

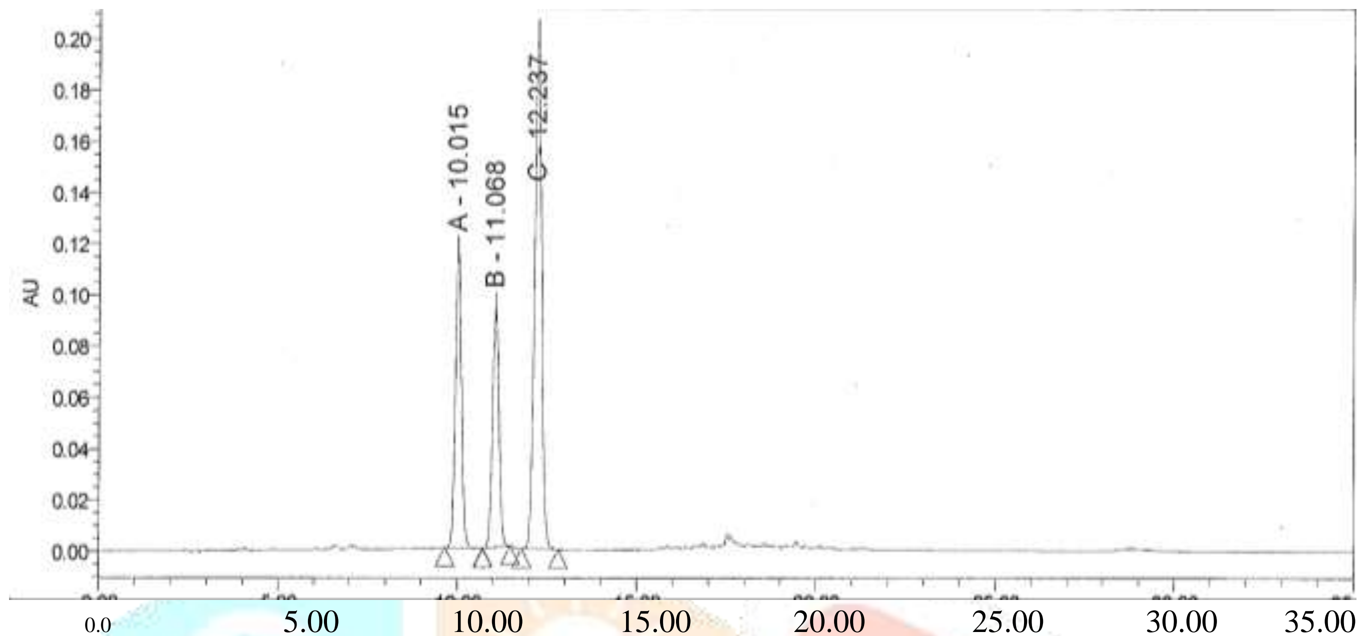
Sample Type: Unlmown
Vial: 39
Injection #: 1
Injection Volume: 10.00 ul

Acq. Method Set: Curcumenoids_lM
ProcesingMethoc CURCUMINOIDS 420PM
Channel Name: 2998 Ch2 420nm@1 .2nm

Run Time: 35.0 Minutes

Proc. Chnl. Descr.. 2998 Ch2 420nm@1 .2nm

AutoScaled Chromatogram



0.0 5.00 10.00 15.00 20.00 25.00 30.00 35.00

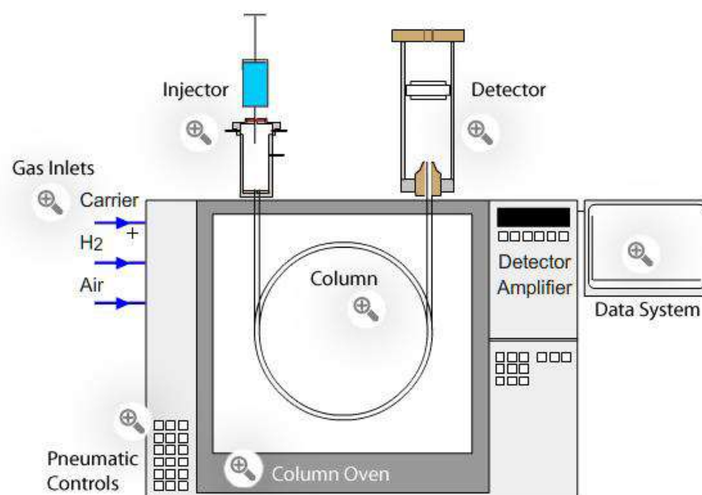
Minutes

Peak result

GAS CHROMATOGRAPHY

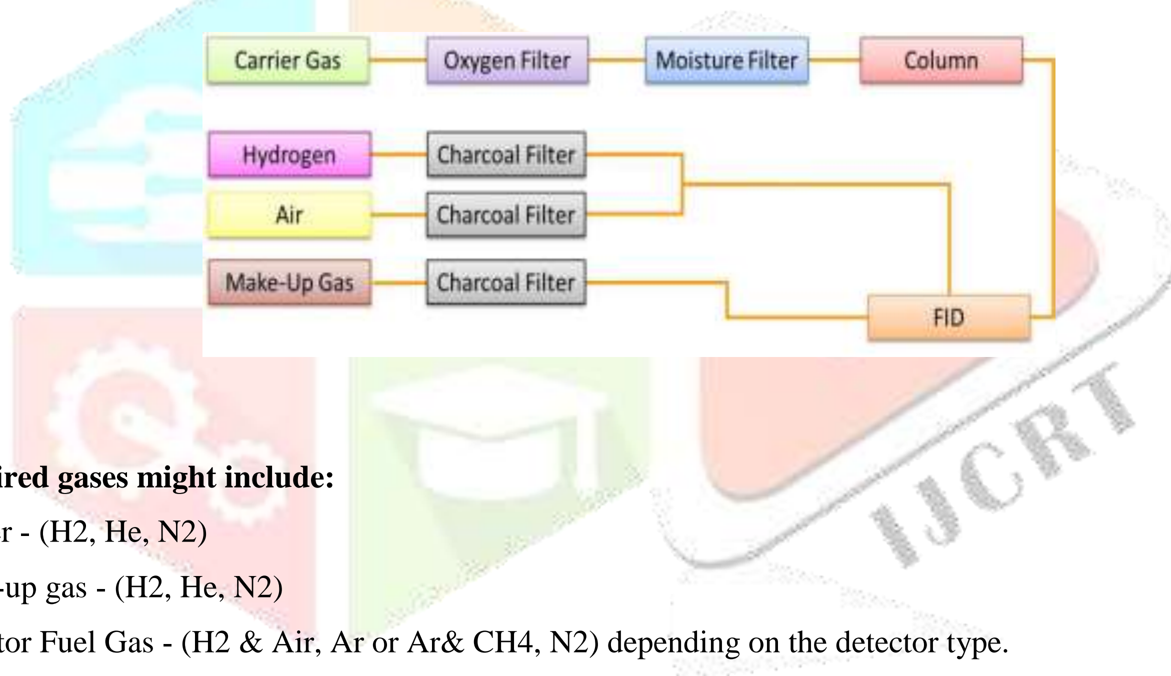
Gas chromatography - specifically gas-liquid chromatography - involves a sample being vaporised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

SL.NO	NAME	RT	AREA	USP Plate Count	USP Tailing	Resolution
1	A	10.0	1337200	17904	1.08	
2	B	11.0	1163393	18339	1.02	3.4
3	C	12.2	2774174	18418	1.03	3.4



Gas Inlets:

Gas is fed from cylinders through supply piping to the instrument. It is usual to filter gases to ensure high gas purity and the gas supply may be regulated at the bench to ensure an appropriate supply pressure



Required gases might include:

Carrier - (H2, He, N2)

Make-up gas - (H2, He, N2)

Detector Fuel Gas - (H2 & Air, Ar or Ar& CH4, N2) depending on the detector type.

<u>Carrier gas</u>

The role of the carrier gas -GC mobile phase- is to carry the sample molecules along the column while they are not dissolved in or adsorbed on the stationary phase. The carrier gas is inert and does not interact with the sample, and thus GC separation's selectivity can be attributed to the stationary phase alone. However, the choice of carrier gas is important to maintain high efficiency. The effect of different carrier gases on column efficiency is represented by the van Deemter (packed columns) and the Golay equation (capillary columns).The van Deemter equation, (2), describes the three main effects that contribute to band broadening in packed columns and, as a consequence, to a reduced efficiency in the separation process.

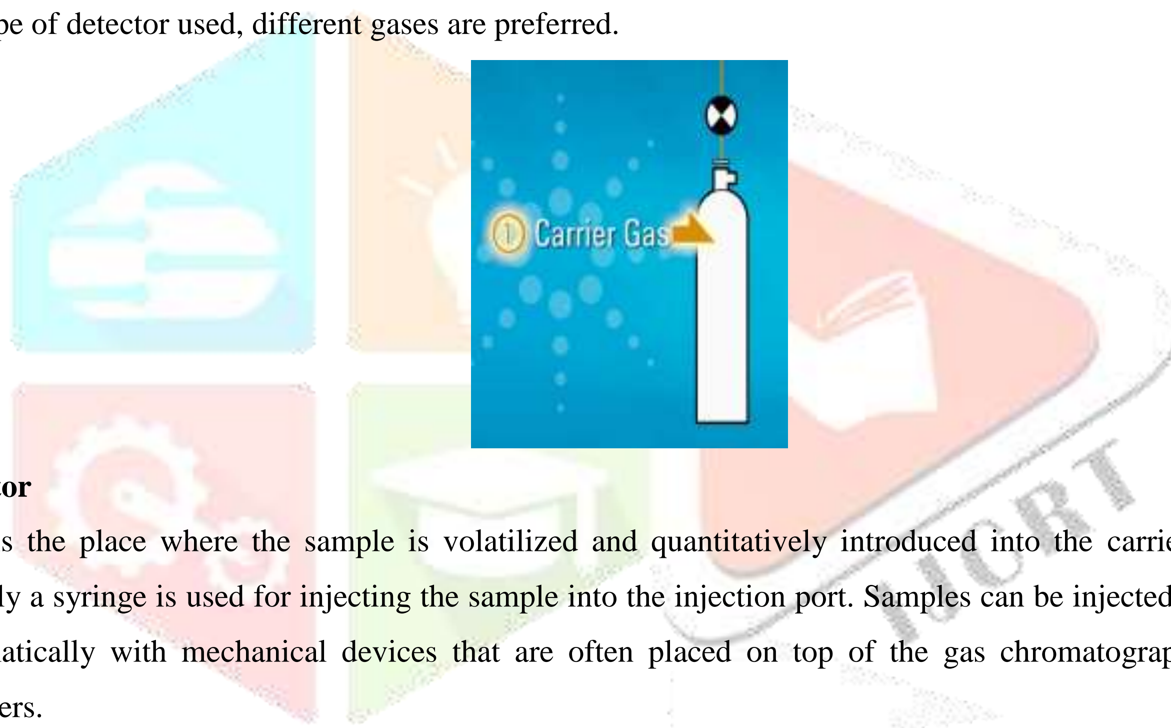
These three factors are:

1. the eddy diffusion (the A-term), which results from the fact that in packed columns spaces betweenparticles along the column are not uniform. Therefore, some molecules take longer pathways thanothers, and there are also variations in the velocity of the mobile phase.

2. the longitudinal molecular diffusion (the B-term) which is a consequence of having regions with differentanalyte concentrations.

3. The mass transfer in the stationary liquid phase (the C-term) The broadening is described in terms of the height equivalent to a theoretical plate, HEPT, as a function ofthe average linear gas velocity, u. A small HEPT value indicates a narrow peak and a higher efficiency.Since capillary columns do not have any packing, the Golay equation, (3), does not have an A-term.TheGolay equation has 2 C-terms, one for mass transfer in then stationary phase (Cs) and one for mass transferin the mobile phase (CM).

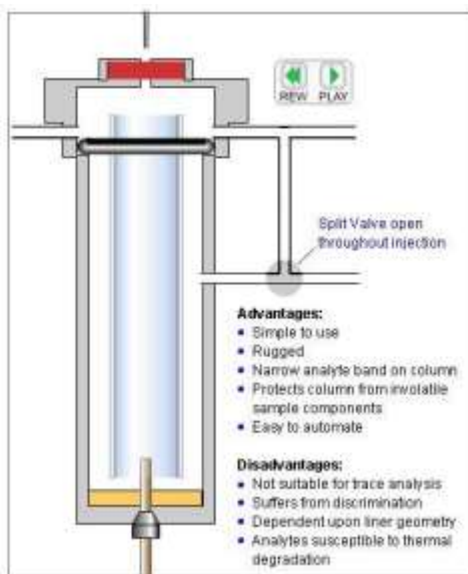
High purity hydrogen, helium and nitrogen are commonly used for gas chromatography. Also, depending on the type of detector used, different gases are preferred.



Injector

This is the place where the sample is volatilized and quantitatively introduced into the carrier as stream. Usually a syringe is used for injecting the sample into the injection port. Samples can be injected manually or automatically with mechanical devices that are often placed on top of the gas chromatograph: the auto-samplers.

The COC injector introduces the sample into the column as a liquid to avoid thermal decomposition or improve quantitative accuracy



Column

The gas chromatographic column may be considered the heart of the GC system, where the separation of sample components takes place. Columns are classified as either packed or capillary columns. A general comparison of packed and capillary columns.

A typical GC ColumnA Glass Packed GC Column.

Since most common applications employed nowadays use capillary columns, we will focus on this type of columns. To define a capillary column, four parameters must be specified:

1. The stationary phase is the parameter that will determine the final resolution obtained, and will influence other selection parameters. Changing the stationary phase is the most powerful way to alter selectivity in GC analysis.

2. The length is related to the overall efficiency of the column and to overall analysis time. A longer column will increase the peak efficiency and the quality of the separation, but it will also increase analysis time. One of the classical trade-offs in gas chromatography (GC) separations lies between speed of analysis and peak resolution.

3. The column internal diameter (ID) can influence column efficiency (and therefore resolution) and also column capacity. By decreasing the column internal diameter, better separations can be achieved, but column overload and peak broadening may become an issue.

4. The sample capacity of the column will also depend on film thickness. Moreover, the retention of sample components will be affected by the thickness of the film, and therefore its retention time. A shorter run time and higher resolution can be achieved using thin films, however these films offer lower capacity.

Detector

The detector senses a physicochemical property of the analyte and provides a response which is amplified and converted into an electronic signal to produce a chromatogram. Most of the detectors used in GC were invented specifically for this technique, except for the thermal conductivity detector (TCD) and the mass spectrometer. In total, approximately 60 detectors have been used in GC. Detectors that exhibit an enhanced response to certain analyte types are known as "selective detectors"

During the last 10 years there had been an increasing use of GC in combination with mass spectrometry(MS). The mass spectrometer has become a standard detector that allows for lower detection limits and does not require the separation of all components present in the sample. Mass spectroscopy is one of the types of detection that provides the most information with only micrograms of sample. Qualitative identification of unknown compounds as well as quantitative analysis of samples is possible using GC-MS. When GC is coupled to a mass spectrometer, the compounds that elute from the GC column are ionized by using electrons (EI, electron ionization) or a chemical reagent (CI, chemical ionization). Charged fragments are focused and accelerated into a mass analyser: typically, a quadrupole mass analyser. Fragments with different mass to charge ratios will generate different signals, so any compound that produces ions within the mass range of the mass analyzer will be detected. Detection limits of 1-10 ng or even lower values can be achieved selecting the appropriate scanning mode.

Many different detector types exist and the choice is based mainly on application, analyte chemistry and required sensitivity – also on whether quantitative or qualitative data is required.

Detector choices include:

Flame Ionization (FID)

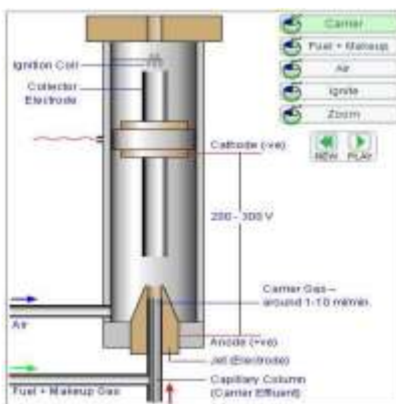
Electron Capture (ECD)

Flame Photometric (FPD)

Nitrogen Phosphorous (NPD)

Thermal Conductivity (TCD)

and Mass Spectrometer (MS)



Flame ionization (FID) detector.

Sample preparation techniques

Derivatization

Gas chromatography is primarily used for the analysis of thermally stable volatile compounds. However, when dealing with non-volatile samples, chemical reactions can be performed on the sample to increase the volatility of the compounds. Compounds that contain functional groups such as OH, NH, CO2H, and SH are difficult to analyse by GC because they are not sufficiently volatile, can be too strongly attracted to the stationary phase or are thermally unstable. Most common derivatization reactions used for GC can be divided into three types:

1. Silylation.
2. Acylation.
3. Alkylation & Esterification.

Samples are derivatized before being analyzed to:

- Increase volatility and decrease polarity of the compound
- Reduce thermal degradation
- Increase sensitivity by incorporating functional groups that lead to higher detector signals
- Improve separation and reduce tailing

Advantages and disadvantages

GC is the premier analytical technique for the separation of volatile compounds. Several features such as speed of analysis, ease of operation, excellent quantitative results, and moderate costs had helped GC to become one of the most popular techniques worldwide.

Advantages of GC

- Due to its high efficiency, GC allows the separation of the components of complex mixtures in a reasonable time.

- Accurate quantitation (usually sharp reproducible peaks are obtained)
- Mature technique with many applications notes available for users.
- Multiple detectors with high sensitivity (ppb) are available, which can also be used in series with a mass spectrometer since MS is a non-destructive technique.

Disadvantages of GC

- Limited to thermally stable and volatile compounds.
- Most GC detectors are destructive, except for MS.

GAS CHROMATOGRAM OF ISOLATED TURMERONE

Gas chromatogram of isolated turmerone

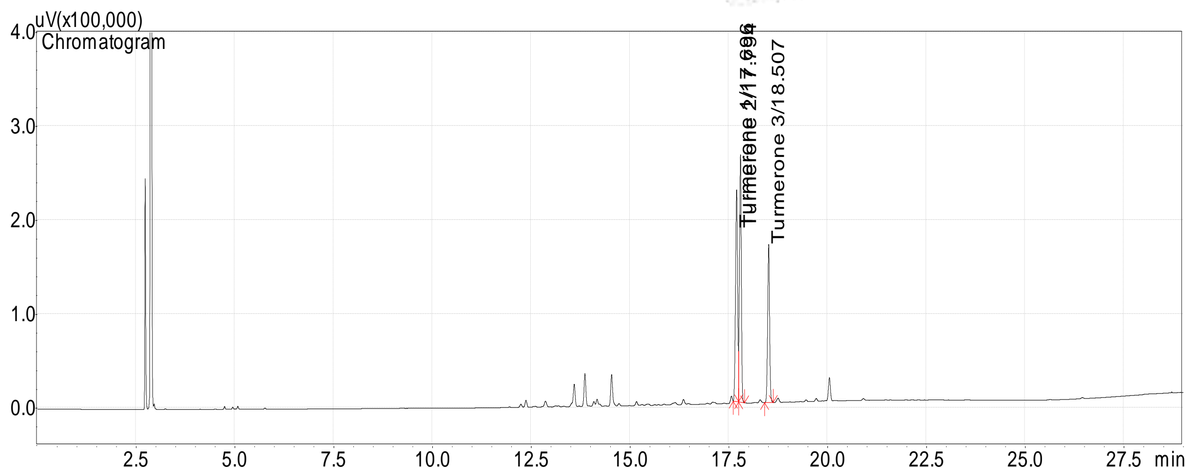
Column: HP 5 30 m X 320 µm X 0.25 µm

Run time 44 min

Injection volume: 2 µL

Temperature: 2500 C, hydrogen: 30ml/min, Air: 300ml/min Carrier gas: Nitrogen.

Gas Chromatographic analysis of Turmeric oil and its column fractions we prepared withchloroform solvent and were analyzed using Chemito Ceres 800 Plus chromatography equipped with FID detector, using HP 5 30 m X 320 µm X 0.25 µm packed column using nitrogen as carrier gas with the carrier gas flow rate of 3.6 bars. Injector port temperature was 280oC; detector temperature was 290^{0}C with the holding time of 44 min at 250°C



RESULT:

The main volatile constituents in the rhizome of Curcuma longa were basically the same. Among these Major constituents, ar-Turmerone and α- Turmerone. The relative content of Turmerone was 26.35% in the CRTO (Hexane extract).

CONCLUSION

Based on the results of the tests carried out and from Chromatographic studies, the observed data was found to match well with that of reported data for turmerone and the isolated compound was identified as turmerone**.** GC-MS spectra of the isolated compound in positive ionization mode showed molecular ion peaks at m/z: 217.2 and 319.2 which correspond to molecular weight of Ar-turmerone and α-turmerone.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR):

Definition of NMR:

Nuclear magnetic resonance is defined as a condition when the frequency of the rotating magnetic field becomes equal to the frequency of the processing nucleus.

If ratio frequency energy and a, magnetic field are simultaneously applied to the nucleus, a condition as given by the equation $v = \gamma H0/2\pi$ is met. The system at this condition is said to be in resonance [v — frequency of radiation associated with transition from one state to the other; γ = proportionality constant and H0 = magnetic field]'.

Principle of NMR:

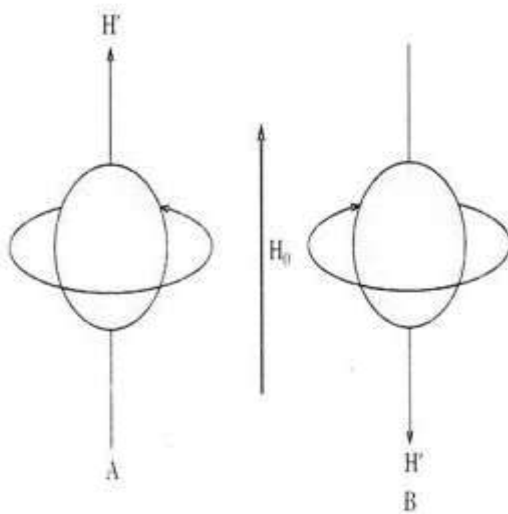
The principle of nuclear magnetic resonance is based on the spins of atomic nuclei. The magnetic measurements depend upon the spin of unpaired electron whereas nuclear magnetic resonance measures magnetic effect caused by the spin of protons and neutrons. Both these nucleons have intrinsic angular momenta or spins and hence act as elementary magnet.

The existence of nuclear magnetism was revealed in the hyper fine structure of spectral lines. If the nucleus with a certain magnetic moment is placed in the magnetic field, we can observe the phenomenon of space quantization and for each allowed direction there will be a slightly different energy level.

Theory of NMR:

The hydrogen nucleus or protons can be regarded as a spinning positively charged unit and so it will generate a tiny magnetic field HO along its spinning axis (as shown in figure 1). Now if this nucleus is placed in an external magnetic field H0, it will naturally line up either parallel A or antiparallel B to the direction of external field. The A will be more stable, being of lower energy.

The energy difference AE between two states will be absorbed or emitted as the nucleus flips from one orientation to the other.



Then,

AE = hv

where v = a radiation frequency and h = Planck's constant

If correct frequency is applied to the sample containing hydrogen nuclei and sample is placed in the external field HQ, then low energy nuclei A will absorb AE = hv, and flips to B. Thus, on flipping back down, they remit hv as a radiation signal which is picked up by the instrument.

In other words, if both radio frequency and magnetic field are simultaneously applied to the nucleus, transition from lower to higher level will occur when equation (1) will be equal to (2).

$\Delta E = \partial hH/2\pi$ … (1)

$\Delta E = hv$ … (2)

or $v = \delta H/2\pi$ … (3)

δ = Gyromagnctic ratio, a constant characteristic of a particular nucleus.

Where ΔE = energy difference between two spin states, h = Planck's constt, v = frequency of resonance absorption, H = strength of applied magnetic field at nucleus. The system at this condition is said to be in resonance and hence the name nuclear magnetic resonance. The observed value of H is therefore a function of molecular environment of proton affording the signal.

(1) Relaxation Process:

Relaxation processes are defined as different types of radiation-less transitions by which a nucleus in an upper spin slate returns to a lower spin state.

Generally there are two types of relaxation processes:

(a) Spin-spin Relaxation:

It is affected by mutual exchange of spins by two processing nuclei in close proximity to each other.

(b) Spin Lattice Relaxation (lattice term refers to frame work of molecules containing the precessing nuclei):

This process maintains an excess of nuclei in a lower state, which is the essential basic condition for the observation of nuclear resonance phenomenon.

In a NMR spectroscopy the sharp resonance lines are observed for stales of extended excitation, and broad lines are observed for short-lived excited stales. Both the processes, spin-spin relaxation and spin lattice relaxation contribute to he width of a spectral line.

(2) Condition of Resonance Signals:

The atoms like O16 and C12 which have even number of protons and neutrons have no magnetic moment and hence refuse to give resonance signals. While atoms such as P21, F19, which have odd number of protons and even numbers of neutrons, if any, generate nuclear magnetic moments and "hence give resonance signals.

(3) Units of NMR:

The nuclear magnetic resonance values are expressed in any of three ways:

(a) δ-the reference compound be quoted (δ denotes that chemical shift is independent of oscillator frequency).

(b) Cps — the reference compound must be quoted and the oscillator frequency given.

(c) τ-TMS (tetra methylsilane) or DSS (2, 2 dimethyl-2 silapentane-5 sulphonate) is assumed independent of both oscillator frequency and reference compound.

Nuclear Magnetic Resonance Spectrometers:

The basic elements of a typical n.m.r. spectrometer consist of the main parts;

(1) A magnet with strong, stable homogeneous field. The field must be constant over the area of the sample.

(2) A radio frequency oscillator (transmitter) connected to coil which transmits energy to the sample in a direction perpendicular to the magnetic field.

(3) A sample container, usually a glass tube spun by an air driven turbine to average the magnetic field over the sample dimensions.

(4) A radio frequency receiver connected to a coil encircling the sample. The two coils are perpendicular to each other and to the magnetic field.

(5) A read out system. The other supporting parts are— consisting of an amplifier, recorder and additional components for increasing sensitivity, accuracy or convenience.

(6) A sweep generator which supplies a variable d-c current to a secondary magnet so that the total applied magnetic field can be varied (swept) over a limited range.

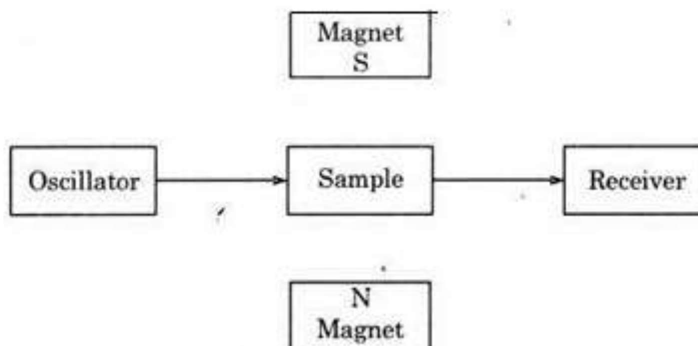
Experimental Technique:

Always a dilute solution is analysed. The compound to be studied is generally mixed with a solvent like CC14 or etramclhylsilane and the dilute solution is filled in a tube.

Now when a sample under investigation is placed in the magnetic field and subjected to rf field of oscillator then at particular combinations of the oscillator frequency and field strength, the rf. energy is absorbed by certain nuclei and an rf. signal is picked up by the detector.

Two ways have been employed in NMR experiments for getting the desired particular combinations:

In one way, the magnetic field remains constant and radio frequency is varied.



Simple Line of NMR Spectrometer

In second, the radio frequency remains unchanged and magnetic field is varied till resonance conditions are obtained and there is detectable absorption by the nucleus.

Instrument:

In block diagram, the blocks labelled N and S represent the poles of the large HO magnet, which is generally an electromagnet operated through a stabilized power supply. A field of up-to 1400 gauss and a pole of about 1.75 — 1.8 inch is necessary for high resolution spectra. The frequency and field strength are related to each other by Larmor condition.

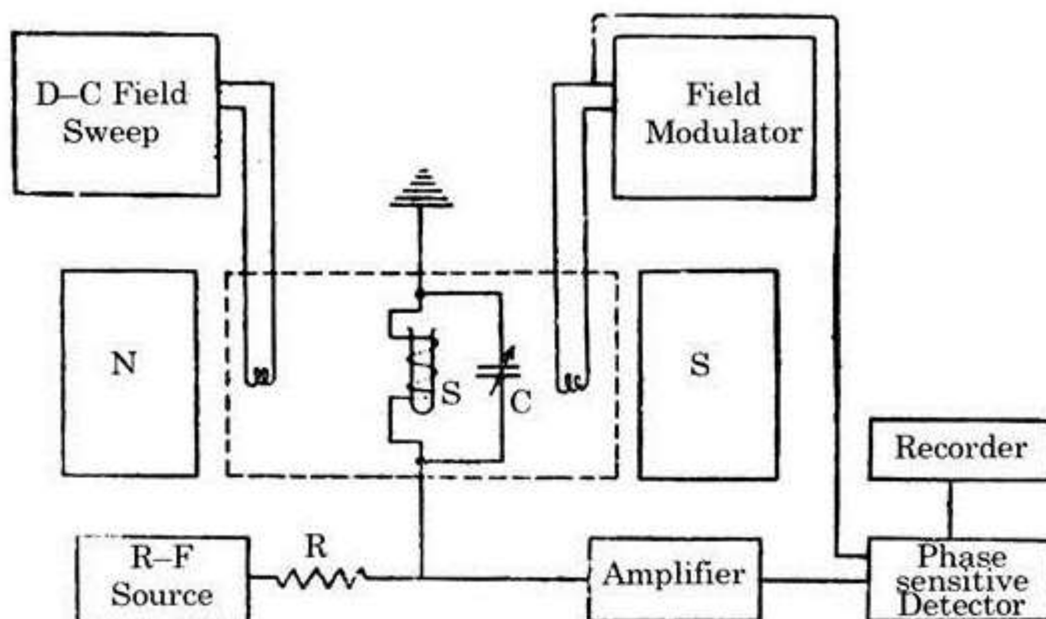
$v = \gamma H0/2\pi$

[This equation represents the condition of resonance.]

where HO = magnetic field,

v = is the frequency of radiation associate with transition from one state to another. It is generally known as Larmor frequency,

γ = proportionality constant or gyromagnetic ratio.



Experimental Parameter (Chemical shift):

The most important molecular parameter determined by NMR is the chemical shift. The chemical shift is defined as a measure of the resonance frequency of the nuclei in a given chemical environment.

The magnitude of the chemical shift is proportional to the strength of applied field and is caused by the circulations of surrounding electrons about the protons.The chemical shift parameter 6 is defined

$\delta = (Hr - Hs)/Hr \times 106$ ppm

where Hr and Hs are field strengths corresponding to resonance for a particular nucleus in the sample (Hs) and reference (Hr).But as spectra are usually calibrated in cycles per second (cps), the equation can be written as:

$\delta = \Delta v \times 106/$Oscillator frequency (cps)

where Δv = Difference in absorption frequencies of the sample and the reference in cps;

oscillator frequency is the characteristic of the instrument: For a 60 MHz instrument, the oscillator frequency is 60×106 cps. The factor 106 has been included for convenience.

Units:

The units of 5, is expressed as parts per million (ppm). The tetra methyl silane (TMS) is generally taken as acceptable standard (because of low boiling point 27°C).

If the compound has a symmetrical structure, each proton is identical to all others and is found in an identical electronic environment which gives a very high shielding. As a result, TMS gives a single sharp resonance line.

Chemical shift is also designated by τ where τ = 10 – δ.

The standard (CH3)4Si protons appear between 0 on 5 scale and 10 on τ scale.

Measurement of Chemical Shift:

In fact the measurement of chemical shift gives information about the various types of magnetic environments. The chemical shift in simple molecules is fairly characteristics and may be used for analysis and characterization.

Factors which influence δ:

Actually, the chemical shift parameter 8 is a function of electron density around the nucleus as the electrons are directly involved in the diamagnetic shielding which acts to attenuate the applied magnetic field.

Hence following factors are responsible for influencing its value:

(a) Specific solvent,

(b) Bulk diamagnetic susceptibility effect,

(c) Temperature (only when change in temperature causes changes in some type of association equilibrium or changes in amplitude of torsional vibrations),

(d) Electron density,

(e) Inductive effect,

(f) Vander Waal deshielding, and

(g) Hydrogen bonding.

Interpretation of NMR Spectrum:

The number spectrum gives several kinds of information:

(1) The number of signals (peaks) tells us how many kinds of protons (protons with different chemical environments) are present in a molecule.

(2) The position (chemical shift) of the signal informs about the bonding environment of each proton.

(3) The area under each signal tells us how many protons of each kind are in the molecule.

(4) All hydrogens with identical environments in a molecule have same chemical shift, e.g., (a) all the three protons of a methyl CH3; (b) the protons of a methylene — CH2; (c) one identical.

(5) Protons on heteroatoms (H—S, H —N, H—O etc.) show highly variable chemical shifts and sometimes broad peaks.

(6) Hydrogen on different carbons yields the same absorptional signal if they are structurally indistinguishable.

(7) Sometimes a proton exhibits an absorption signal which is split into several peaks because of coupling with its neighboring protons. In such cases a coupling constant J is calculated.

(8) The number of peaks (N) into which a proton signal is split equals one more than the number of vicinal protons (n) (number of equivalent neighbors causing splitting):

$N = n + 1$

N = 2 (one vicinal H) = doublet (d)

N= 3 (two vicinal H's) = triplet (t)

N= 4 (three vicinal H's) = quartet (q).

Applications of N.M.R. Spectroscope:

Quantitative Analysis:

The area of peak is directly proportional to the number of nuclei responsible for that peak. Thus the concentration of species can be determined directly by making use of signal area per proton. The signal area per proton can easily be calculated by use of a known concentration of an internal standard.

Similarly, (he concentration of new species formed during the reaction can also be calculated from the spectrum of parent compound.

Qualitative Analysis:

The qualitative analysis of the compound can easily be made by knowing:

(i) Chemical shift 8 values of hydrogen containing groups,

(ii) The presence of particular functional group,

(iii) The relative position of these groups and

(iv) The relative number of nuclei in these groups.

Nuclear Magnetic Double Resonance (NIVIDR):

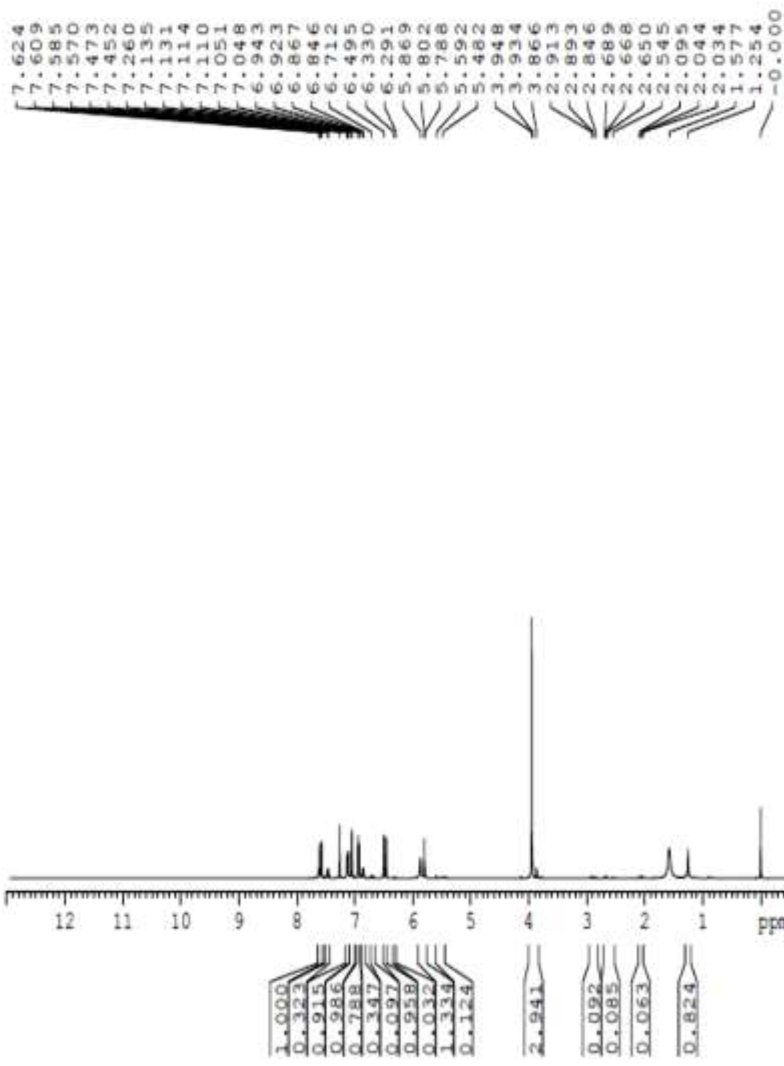
When two oscillating magnetic fields are simultaneously applied to the sample, the experiment is called double resonance, double irradiation, or spin decoupling. In the usual nuclear magnetic double resonance experiment, a strong rf. field H2 is used to irradicate the sample while a weak rf. field H1 induces the transitions to be observed. We can sweep the magnetic field holding HI and H2 constant.

Electron Paramagnetic resonance (EPR):

The electron paramagnetic resonance (EPR) differs from NMR principally because in that the frequencies of electron resonance occur in microwave region for magnetic fields of the order of several thousand gauss. Therefore EPR spectrometer uses such components as Klystrons, wave guides and resonance cavities for the sample.EPR method is applicable whenever the compound displays at least one unpaired electron, i.e., in free radicals, crystalline and amorphous solids subjected to irradiation or containing transition element ions and rare earths had some chelates. The other different examples are metals, odd molecules, graphite's and impurities in semiconductors.

NMR Spectra

sample-2



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DE 6.50 usec
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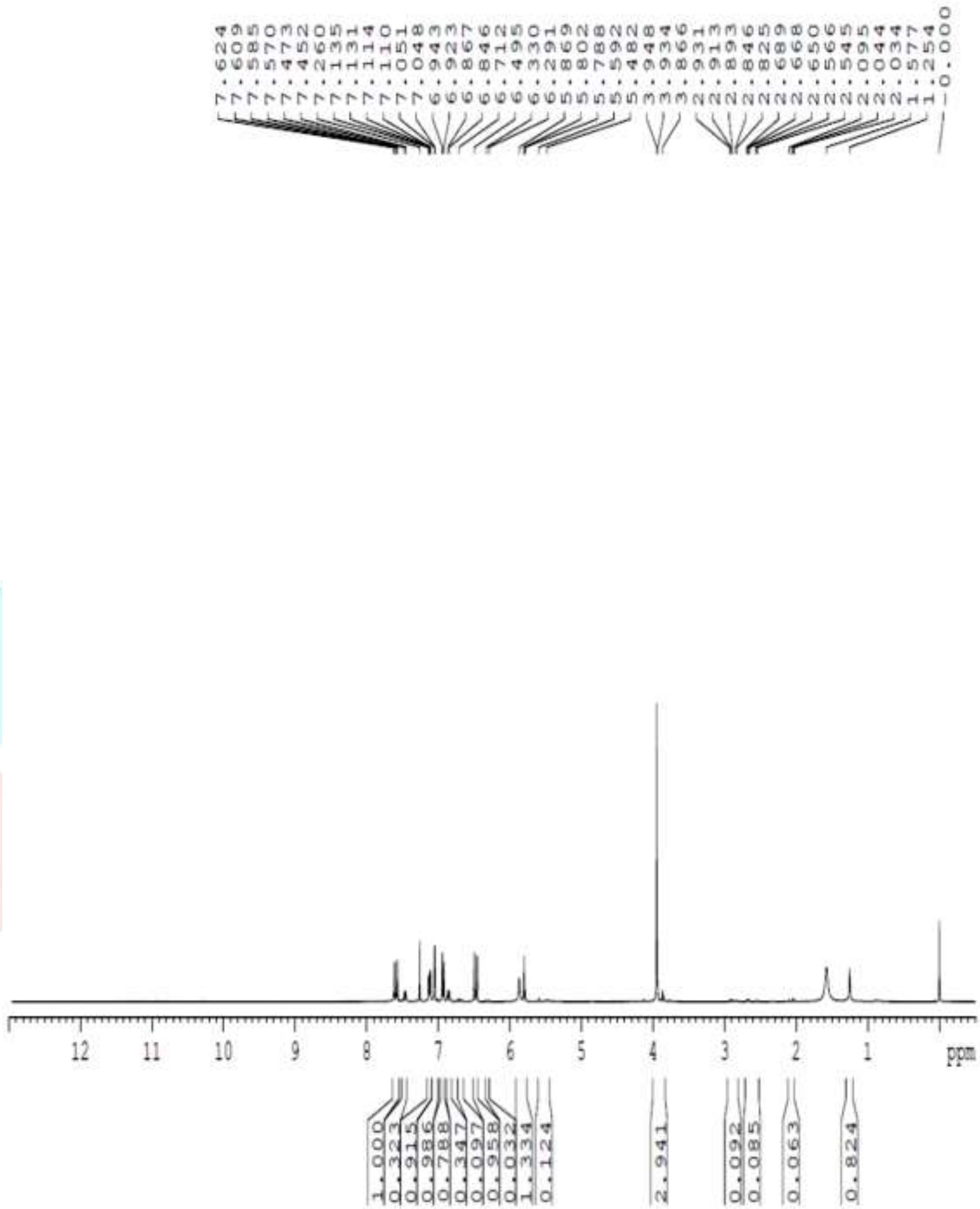
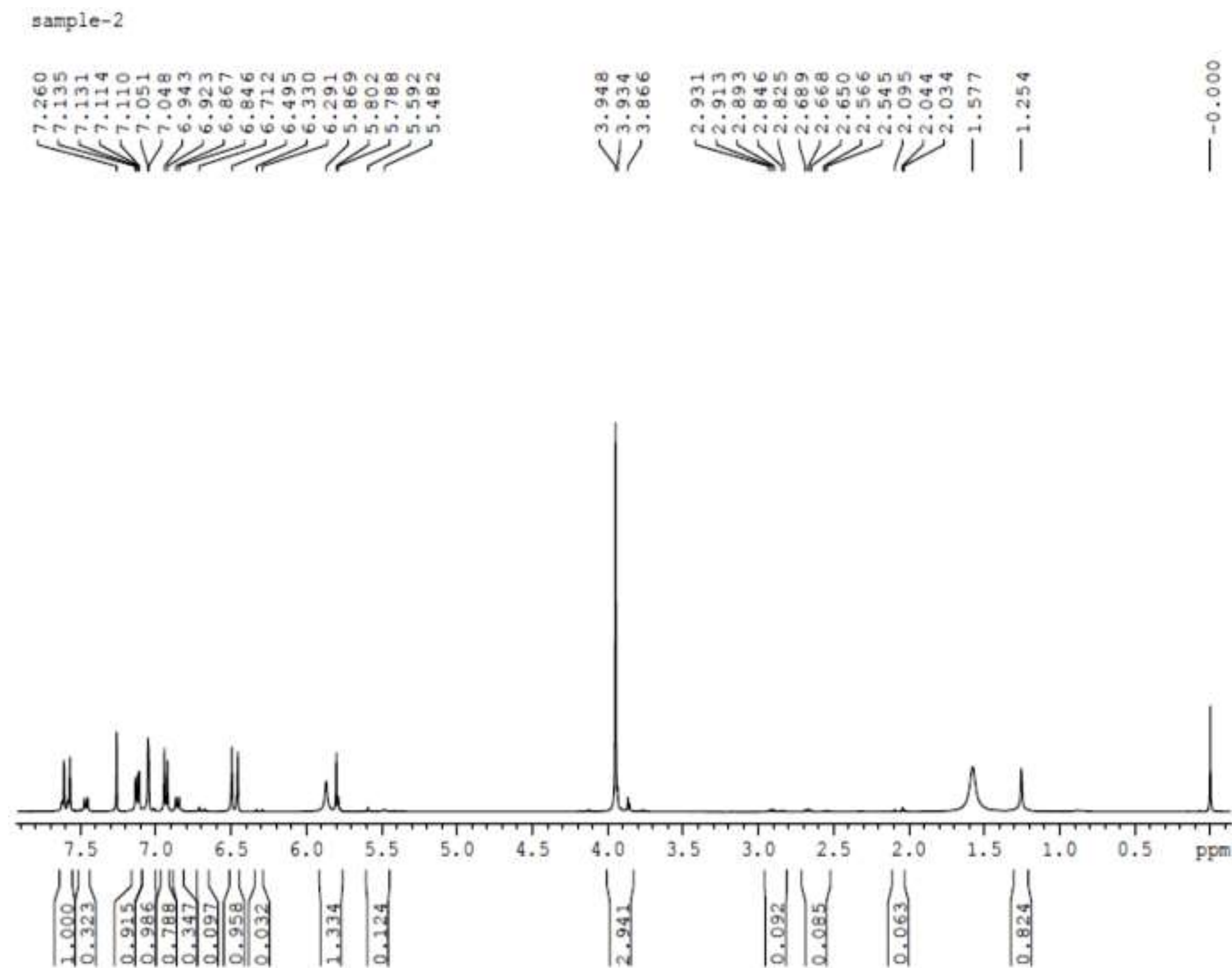

sample-2
ppm

sample-2



CONCLUSION:

The comprehensive study on the physicochemical analysis, isolation, purification, and identification of curcuminoids and turmerones from turmeric root and spent turmeric oleoresin (CRTO) has provided valuable insights into the extraction and characterization of these bioactive compounds. Turmeric, known for its rich

composition of curcuminoids and turmerones, holds significant promise in various biomedical and pharmaceutical applications due to its potent antioxidant, anti-inflammatory, and antimicrobial properties.

Physicochemical Analysis and Extraction of Curcuminoids and Turmerones: The initial stages of this study involved the meticulous selection and preparation of turmeric roots. Solvent extraction, a critical step in the process, was optimized to efficiently recover curcuminoids and turmerones from the raw material. Ethyl acetate was chosen for its ability to selectively dissolve these compounds while minimizing the extraction of unwanted constituents. This process was followed by concentration and solvent removal to obtain a crude extract rich in curcuminoids and turmerones.

Isolation and Purification of Curcuminoids: The purification of curcuminoids from the crude extract, particularly curcumin, demethoxycurcumin, and bisdemethoxycurcumin, was achieved through column chromatography using silica gel as the stationary phase and a gradient of chloroform as the mobile phase. This method allowed for the separation of individual curcuminoids based on their differential affinity for the stationary and mobile phases. Each fraction obtained was characterized using analytical techniques such as HPLC, confirming their purity and identity through comparison with authentic standards and spectral data.

Separation of Turmerones from CRTO:Spent turmeric oleoresin (CRTO), a by-product of curcumin extraction, contains residual turmerones that were targeted for isolation in this study. Extraction of turmerones from CRTO was achieved using hexane as the solvent, employing Soxhlet extraction to ensure efficient recovery. The resulting crude turmerone extract underwent further purification steps to remove impurities and concentrate the target compounds, thereby enhancing the yield and purity of isolated turmerones.

Identification and Characterization Using Analytical Techniques:The identification and characterization of curcuminoids and turmerones relied on a combination of advanced analytical techniques. Nuclear Magnetic Resonance (NMR) spectroscopy played a pivotal role in elucidating the chemical structure of isolated compounds, confirming their molecular composition and structural integrity. High-Performance Liquid Chromatography (HPLC) provided quantitative analysis, determining the concentration and purity of curcuminoids and turmerones in the extracts.

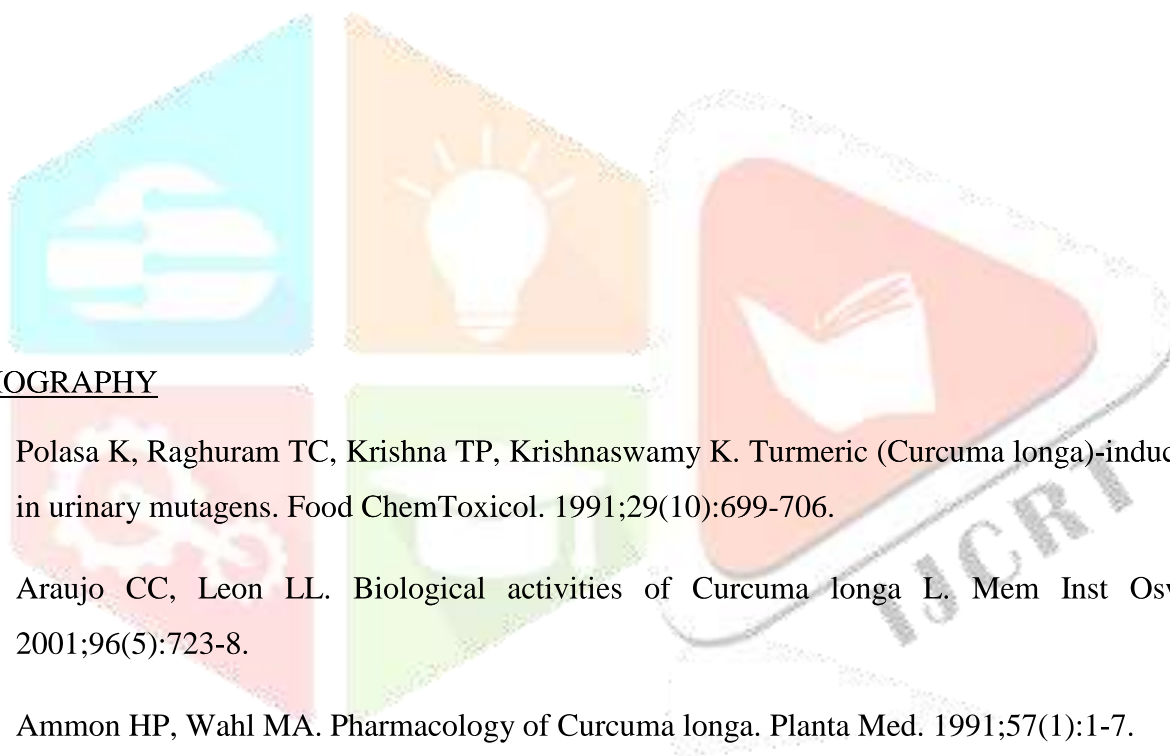
Thin Layer Chromatography (TLC) served as a rapid qualitative tool for assessing the separation and purity of isolated compounds, complementing the results obtained from NMR and HPLC analyses. Gas Chromatography (GC) offered insights into the volatile components of turmerones, aiding in the identification and characterization based on their retention times and mass spectra.

Implications and Future Directions:

The findings from this study underscore the potential applications of curcuminoids and turmerones in pharmaceuticals, nutraceuticals, and biomedical research. Their demonstrated antioxidant, anti-inflammatory,

antimicrobial, and hepatoprotective properties highlight turmeric as a valuable source of natural compounds with therapeutic benefits.

Future research directions should focus on optimizing extraction protocols to enhance yield and purity, exploring additional biological activities of curcuminoids and turmerones, and conducting clinical studies to evaluate their efficacy and safety in human health. Furthermore, the development of standardized extraction methods and quality control measures is essential for ensuring consistent and reliable production of turmeric extracts for commercial applications. In conclusion, this study contributes significant insights into the extraction, purification, and characterization of curcuminoids and turmerones from turmeric, paving the way for their potential use in various health-promoting applications and pharmaceutical formulations.



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