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EXTRICATION AND SIGNIFICANCES OF CURCUMINOIDS FROM TURMERIC {CARCUMA LONGA L.}

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ABSTRACT

Traditionally "Indian saffron" comes from the root of turmeric (Curcuma longa) with a tough brown skin and deep orange flesh. It also called golden spice mainly grown in Asia and central America, which is greatly inspired by many cultures due its great power of anti-inflammatory, medicinal, dye, and as an ingredient attributed throughout the history. Turmeric is rich in curcumenoids, which is a linear diarylheptenoid , with molecule such as "curcumin" in different chemical groups that have been formed to enhance the solubility to make them suitable for drug formulation.

The extraction of curcuminoids is performed by soxhelet extractor. Isolation and purification of curcumminoid (Curumin, dimithoxycurcumin, bisdemethoxycurcumin) is carried by column chromatography and their quantification of curcuminoid in maximum resultant extract by (methanol) is performed used by HPLC methodology. Percentage yield curcumin by HPLC was 18%.Separation of curcuminoids were tested in TLC (Choloroform: Methanol at 95:5) showed Rf value 0.65,0.68,0.55 as curcumin, demethoxycarcumin, bisdemethoxy curcumin respectively.

I. INTRODUCTION

Turmeric is commonly known for its medicinal values in the Indian traditional system of medicine. turmeric has been used traditionally in "ayurvedic medicine" as an anticeptic,wound healing and anti-inflammetry compounds. Curcumin, dimethoxycurcumin, and bis demethoxy curcumin is a dietary photochemical obtained from dried rhizomes of the turmeric plant(Curcuma longa).curcummin is a main colouring substance in curcuma longa and two related compounds, demethoxycurcumin (DMC) and bis demethoxycarcumin(BDMC),are all together known as curcuminoid. The value of turmeric product is based on the quantity of curcuminoid content. Quantitative estimaton of curcuminoid can be carried out out photometrically based on its absorbance at 420 nm.The principal colouring component of curcumin exhibit a keto-enol tautomerism and antioxidative properties.

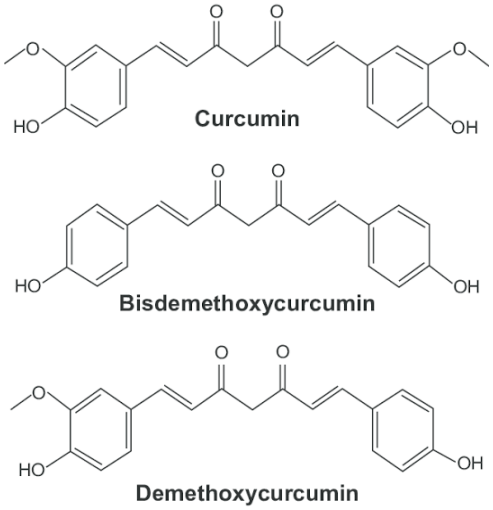

Fig.no-1 (A) curcumin,(B)demethoxy curcumin(C)bis demethoxy curcumin

II. METHODOLOGY AND ANALYSIS

Ordinary strategy for removal of carcumin has been Soxhlet extraction mind warming time extended as long as up to 12h. the Soxhlet extraction could be a time devouring, difficult and makes utilize of bulk sum proceeds long hours, the approach conceivably include tall chances of warm decay of target molecules .Soxhelet extraction strategy work careful cell penetration taken after by solublizing the dynamic constituents by

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extracting dissolvable. Curcumin display interior the oleorosin cell which intern is secured by firmly pressed club cells likely makes the passage course for the dissolvable and time devouring .A number of things about are embrassed to isolated curcuminoids coloured by thin layer chromatograpy (TLC),column chromatography. HPLC strategy is very sensitive, exact and precise for location and evaluation of curcuminoids within the extricate of rhyozmes curcuma longa ..Curcumin acts as an effective antiinflammetoryagent, ehich is known to prevent the inflammetory pathway by blocking several pro inflammatory cytokinin, TNF-α and TGF-ß pathway. some reports have been sujjested that intraperitonial(IP)administration of curcumin should regulate the BLM-induced lung epithelial iinjury and its progression to pulmonary fibriosis. Recent scientific report said that the normal intake of curcumin in the form of turmeric powder is not a preferable way of curcumin comsuption. Theletrature suggested that the direct intake of isolated curcumin could increase the bioavaibility of curcumin and its action related to various heathhazards. Preferaby, curcumin consumption is most appropiate to take with milk, pepper, honey or with any edible oil. It has been noticed that curcumin is fat souluble compound and also the intake with pepper increase the curcumin absorption by interacting with the peprin, which increase the curcumin absorption by 2000%.Curcumin is also known for its beneficial effects on gene regulation,it also invloved in the modulation of cell proleferation and apoptosis regulating the cancer related heath issues.

It also been observed that the curcumin is actively involved in the prevention of platelet clumping to improvise circulatory system,it may happen via-inhibition of thromboxane synthesis and potentiation of prostacyclin synthesis. Curcumin is also known for its anti-mutagenic properties ,can effectively inhibit metastasis of skin cancer cells and deactivate the carcinogenic produced for smoke or tabaccochewing. The specific acton against the microbes also can be seen by using curcumin ,which can acts as a harmfull agent against the microbes and inhibit the growth of bacteria, parasites and pathogenic fungi.

1. The biological source of curcumin

Curcumin genus in the plant family of Zingiberacea, is the biological source for curcuminoids, including curcumin. Curcuma longa, the yellow tuberous root that is referred to as turmeric, was taken from India to Southeast Asia, China, North Australia, West Indies, and South America. Subsequently, its cultivation spread to many African countries. India, however, remains the largest producer of tiormeric in the world, with a figure of 4,87,000 metric tones in production, of which 27 ,750 metric tones are exported. The yellow pigmented fraction of Curcuma longa contains curcuminoids, which are chemically related to its principal ingredient curcumin. The three main curcuminoids isolated from turmeric are curcumin, demethoxy curcumin, and bisdemethoxycurcumin curcumin (Figure I). Curcuminoids are present in 3-5% of turmeric. Curcumin is the important active ingredient responsible for the biological activity of turmeric. Curcumin, C2H20O6 (m.p. 1 84°C), or diferuloyl methane was first isolated in 1815. The crystalline form of curcumin was obtained in 1910, and Lampe solved its structure in 1913. It is insoluble in water, but soluble in ethanol and acetone].

2. Biological activities of curcumin

Anti-inflammatory property Inflammation is a necessary process for fighting infections. It results from a series of complex reactions, triggered by the host immunological response. Uncontrolled inflammatory responses may lead to undesirable effects, such as tissue damage. Many of the diseases, such as Peptide in turmeric Besides the phenolics, turmeric is also a source of a water-soluble peptide with antioxidant properties. This compound has been identified as turmeric, a heat-stable noncyclic peptide which has 40 amino acid residues, and is resistant to the proteolytic action of the enzymes trypsin and pepsin. Turmeric, which is found in turmeric at a concentration of 0.1 percent, has been shown in some experiments to be a more potent antioxidant than curcuminoids or butylated hydroxy anisole (BHA). Turmeric is rich in methionine, the sulfur containing amino acid, and a known antioxidant, which may in part explain the strong antioxidant properties of this compound. [Turmeric and the healing curcuminoids. Muhammed Majeed, Ph.D., Vladimir Badami, M.D., Ph.D. And Frank Murray] 119 Chapter VI Isolation turmeric rheumatoid arthritis, are the result of sustained production of inflammatory mediators causing physical damage to joints. Many inflammatory mediators have been implicated in these complex reactions, some of which are modulated by curcumin Antioxidant properties.

The discovery of the antioxidant properties of curcumin explains many of its wide ranging pharmacological activities. Curcumin is an effective antioxidant and scavenges superoxide radicals, hydrogen peroxide, and nitric oxide from activated macrophages. It inhibits the inducible nitric oxide synthase activity in macrophages.

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Human keratinocytes are protected from Xanthine xanthine oxidase injury by virtue of the antioxidant property of curcumin. Oral administration of 30mg/kg body weight of curcumin in rats for 10 days reduces the iron induced hepatic damage by lowering lipid peroxidation. Protection from radiation by dietary curcumin administered to mice is also attributed to the antioxidant property of curcumin. Curcumin protects renal cells and neural glial cells from oxidative stress. Interestingly, curcumin not only exhibits antioxidative and free radical scavenging properties, but also enhances the activities of other antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase. Lipid peroxidation is lower in liver, kidney, spleen, and brain microsomes from retinol deficient rats that are fed with 0.1% dietary curcumin for three weeks. Another mechanism by which curcumin protects against oxidative stress in endothelial cells is by the induction of heme oxygenase-1.

❖ **Materials and Methods:**

Fresh roots of rhizomes were collected from market and grinded into small pieces using motor piston. For the extraction of curcumin we require weighting machine, measuring cylinder, beaker, filter paper, glass road, conical flask.

The ground turmeric is soaked in 99% ethanol for a day or two. the solid is filtered away using filter paper results a nice amber colored ethanol solution which is shown as:

Fig no-2(extraction of curcuminoids by filtration)

Crystallization of carcumin

Crystallization of curcumin is performed by dissolveing a 500 mg sample in 50 ml of methanol at 60-c.After dissolution,10 to 12 ml of distilled water is added, and the mixture is kekpt at 5-c for 2 hour.The Curcumin crystal were separated from the mother liquor by filteration.

5.2. Chemical Degradation and Metabolism Curcumin undergoes chemical degradation in aqueous-organic solutions and the degradation increases as the pH is increased, which is of a serious concern in its applications. Most phenols in solution form polymers over time, but the degradation of curcumin is not through the phenolic group but is rather found to be through the α,β-unsaturated β-diketo moiety. In dilute solutions (i.e., in micromolar solutions) 90% curcumin degrades in 30 minutes. However the percentage degradation will decrease at high concentrations. Several important products have been identified as a result of curcumin degradation. They are trans-6(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic aldehyde, ferulic acid, feruloylmethane and vanillin (Scheme 2). Although not fully understood, it is believed that the degradation is by hydrolysis through the diketo moiety. However the degradation is significantly decreased when curcumin is attached to lipids, liposomes, albumins, cyclodextrin, cucurbituryl, surfactants, polymers and many other macromolecular and microheterogenous systems .Thus has been found to be of great use that stable curcumin solutions could be prepared in culture medium containing 10% Fetal Bovine Serum (FBS) and also in human blood. Curcumin undergoes much faster degradation when exposed to sunlight .It is one common observation that curcumin/turmeric stains can be quickly removed on exposure to sunlight The colorless products identified during photodegradation of curcumin are vanillin, ferullic acid, and other small phenols, indicating a similar product distribution during photochemical degradation as in chemical degradation in solution. This photodegradation involves formation of the excited states of curcumin. Some reports indicate that curcumin

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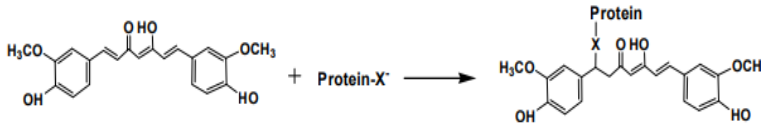
generates singlet oxygen and other ROS on photoexcitation and this is actually responsible for the photobiological and photodynamic activity of curcumin .In such case the degradation of curcumin after photoexcitation must proceed though the triplet excited state of curcumin .Photophysical studies reported the lifetime of triplet excited state of curcumin to be in microseconds, suggesting that the degradation may proceed very fast and compete with singlet oxygen formation. The photodegradation is accelerated in presence of TiO2 nanoparticles, and this method can be employed to remove turmeric stains from cotton fabrics .The metabolism of curcumin in rats and humans produces different products .Two major pathways have been identified in curcumin metabolism, like O-conjugation and reduction. The O-conjugation products are curcumin glucuronide and curcumin sulfate. The reduction products are tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin. Other minor products are dihydrocurcumin glucuronide, tetrahydrocurcumin glucuronide, ferulic acid and dihydroferulic acid. The formation of these products has been confirmed by HPLC and mass spectrometry. Although it has been reported that these processes occur enzymatically, the exact enzymes involved in all these specific reaction products are still a matter of debate. Sulfonation of curcumin through human phenol sulfur transferase enzymes and the formation of reduction products through alcohol dehydrogenase is proposed. Comparing these metabolic products with the degradation products, it appears that simple hydrolytic degradation is prevented in biofluids. Since the degradation may occur through the β-diketo structure, one can presume that in these systems curcumin is not in free form but rather in conjugated form bound to some proteins or other biomolecules, and as the diketo moiety is involved in binding to the proteins [5,6], it is not available for hydrolytic degradation. It may also be implied that the specific enzymatic reactions are probably much faster and do not allow the slow hydrolytic degradation, therefore the latter process cannot compete with the former reaction. This leaves a bigger challenge for chemists to understand the differences between degradation and metabolic reactions in terms of kinetic parameters and also identify the crucial mechanism in these reactions. reaction products are still a matter of debate. Sulfonation of curcumin through human phenol sulfur transferase enzymes and the formation of reduction products through alcohol dehydrogenase is proposed. Comparing these metabolic products with the degradation products, it appears that simple hydrolytic degradation is prevented in biofluids. Since the degradation may occur through the β-diketo structure, one can presume that in these systems curcumin is not in free form but rather in conjugated form bound to some proteins or other biomolecules, and as the diketo moiety is involved in binding to the proteins [5,6], it is not available for hydrolytic degradation. It may also be implied that the specific enzymatic reactions are probably much faster and do not allow the slow hydrolytic degradation, therefore the latter process cannot compete with the former reaction. This leaves a bigger challenge for chemists to understand the differences between degradation and metabolic reactions in terms of kinetic parameters and also identify the crucial mechanism in these reactions.



Scheme 4: Michaels products of curcumin with protein thiols and selenols were x=S or se The methylenic hydrogen of the diketo/enol moiety of curcumin can also act as a nucleophile and participate in Michael addition reactions with stronger electrophiles , but such reactions may not have significance in biological systems. Chemically modified curcumin derivatives have been prepared by condensation/addition reactions like e.g., semicarbazone derivatives and oxime derivatives of curcumin. These stable products prepared independently have been examined for anti-cancer activity. In most of these studies, it has been reported that these derivatives are more cytotoxic to cancer cells than free curcumin. This prompts us to speculate that probably the glutathione conjugate of curcumin may also act as a cytotoxic agent and contribute to the overall anti-tumor activity of curcumin.

Chemistry of Curcumin-Metal Ion Interactions

Curcumin forms strong complexes with most of the known metal ions. The α,β-unsaturated β-diketo moiety of curcumin is an excellent chelating agent. In the last one and half decades, many papers have been published on metal-curcumin complexes .Curcumin is a monobasic bidentate ligand and forms stable complexes with almost

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all the metals and non-metals. In general, stable structures with 2:1 (ligand:metal) stoichiometry are observed. There are very few reports on 3:1 ligand:metal complexes, e.g., an octahedral complex reported with Fe3+ .Metal coordination of curcumin occurs through the enolic group, where the enolic proton is replaced by the metal ion and the o-methoxy phenolic moiety remains intact in the complexes. The metal-oxygen bond is characterized by IR spectroscopy signals at 455 cm−1 and the carbonyl peaks in the complexes show a small shift of ~10 cm−1 on coordination to metals. Changes in NMR chemical shifts of curcumin have also been reported on metal coordination. The shifts however depend on the affinity and thermodynamic stability of the resulting complexes. In the case of strong complexes, the resonances of protons attached to the double bonds of the alkyl chain show significant downfield shifts, while the enolic protons show negligible shifts in the 1 H-NMR spectra ,and the 13C-NMR spectrum shows down- and up-field shfts of carbons near the coordination site. There are several papers published in the literature on complexes of curcumin with transition metals like Fe3+, Mn2+, Ni2+, Cu2+, Zn2+, Pb2+, Cd2+, Ru3+, Re3+ and many others. Complexes with non-transition metal ions and rare earth ions like Al3+, Ga3+, Sm3+, Eu3+, Dy3+, Y3+, Se2+ and metal oxides like VO2+ have also been synthesized. The structure and physical properties of these complexes depend on the nature of the metal ion, as well as the stoichiometry of the reaction conditions, which in turn decides their stability and reactivity. Stable 2:1 complexes of some transition metals can be prepared by mixing stoichiometric amounts of curcumin and metal salts in suitable organic solvents and refluxing for few hours, the complex can be separated as a precipitate, and purified either by column chromatography and repeated crystallization. The general chemical structure of 2:1 complexes of curcumin with metals is given in Figure 4a.

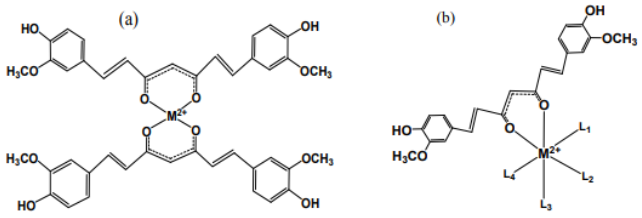

Figure 4(a) structure of 2:1 curcumin:metal complex,(b)mixed ligand curcumin:metal complex.

Curcumin-metal complexes not only modify the physico-chemical properties of curcumin but they also affect the biological reactivity of the metals. In general it has been observed that complexation with curcumin reduces the toxicity of themetals and some curcumin complexes with metals like Cu2+, Mn2+, act as new metal-based antioxidants .Due to the reversible electron transfer reactions with superoxide ions, Cu2+ and Mn2+ complexes of curcumin act as superoxide dismutase enzyme mimics. Metal complexes of curcumin have greater significance in view of the pathology of Alzheimer's disease, where it has been found that due to its lipophilic nature, curcumin can cross the blood brain barrier and chelate metal ions that are toxic to the neurons. It has also been observed that the incidence of Alzheimer's disease is significantly reduced among people that are known to regularly consume turmeric in their diet. Curcumin forms stable complexes with all the metals involved in Alzheimer's disease .The interaction of curcumin with Al3+, earlier considered to be responsible for development of Alzheimer's disease, has been studied extensively. Curcumin forms three different types of complexes with Al3+, depending on the stoichiometry of the reaction. The 1:1 Al3+-curcumin complex showed less affinity to DNA binding than free Al3+, which has been attributed to its ability to reduce development of Al3+ induced Alzheimer's disease .Many other applications of curcumin metal complexes have been reported. Ga3+ curcumin complexes are being developed as innovative bioceramics .Zn2+-curcumin complexes showed anti-cancer, gastroprotective and antidepressant effects in rats .In vivo antiarthritic activity was reported for five co-ordinated curcumin-gold (Au3+) complexes [80]. Vanadyl-curcumin (VO(Cur)2) 2+ complexes show antioxidant and anti-rheumatic activity .Through metal co-ordination, curcumin reduces the toxicity of heavy metals like Hg2+, Cd2+, Pb2+ where significant reduction in heavy metal-induced oxidative stress is reported through complex formation. Owing to their positive charges, most of the metal complexes of curcumin bind to DNA. Some reports indicate that due to binding, the curcumin-metal complexes induce DNA damage and thereby exhibit pro-oxidant behavior. Accordingly, curcumin-metal complexes are also being explored as better anti-tumor agents than curcumin itself .Therefore there is still some confusion about the role of metal-curcumin complexes, in biology whether they act as antioxidants or pro-oxidants. It appears that some metal complexes

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act as antioxidants, while some others may be pro-oxidants. This antioxidant/pro-oxidant activity of a metal complex depends on several factors such as nature of metal ion, co-ordination number, structure, stability and electrochemical potential of the complex. More systematic classification and chemistry of metal-curcumin complexes is necessary in the future. Biologic of the curcumin-metal complexes is very low and the experiments are difficult to perform due to the lower solubility either in organic solvents like DMSO or in biological fluids. However, it is beyond any doubt that this in vivo complexation of curcumin with metals plays a significant role in reducing metal-induced toxicity. Recently a few mixed ligand complexes of curcumin with unique chemical and biological activities have been reported. In these complexes (Figure 1b), the curcumin to metal ratio is 1:1 .A few examples of these mixed ligand complexes are summarized here. Porphyrin-bridged curcumin complexes of Cu2+, Ni2+ and Zn2+ showed improved photodynamic activity in plasmid DNA models . Complexes of curcumin-and 4,4'-bipyridine with Zn2+ were more effective than curcumin to kill neuroblastoma cells. Curcumin-terpyridyl-lanthanum (La3+) complexes showed enhanced photocytotoxicity in HeLa cells. Bipyridyl-curcumin complexes of Pd2+ inhibit the growth of human prostate cancer cells . Mixed ligand-curucumin complexes with rare earth metals like Sm3+, Eu3+ and Dy3+ showed antibacterial activity. The absorption spectrum of curcumin is altered on complexation with metals. The 1:2 transition metal complexes of curcumin showed a blue shift of the absorption maximum. The fluorescence quantum yield of curcumin is drastically reduced on metal chelation. However the Al3+ complexes and rare earth metal complexes of curcumin are more fluorescent and also in mixed ligand complexes, reports suggest that the fluorescence of curcumin remains unaffected. Fluorescent curcumin-metal complexes are being explored for imaging of cancer cells. Rare earth complexes of curcumin and pyridine exhibit two-photon absorption in the wavelength range 700 to 800 nm and such complexes have been used for the imaging of MCF-7 cells. Re(CO)3(Curcumin)(H2O) complexes are fluorescent and show affinity to beta-amyloid plaques, which has potential to be explored in microscopic imaging of the tissue of Alzheimer's disease patients [94]. Similarly 99T(CO)3(curcumin)(H2O) complexes have been produced in high (>90%) radiochemical yield, and showed significant affinity to β-amyloid plaques and such systems are being developed as novel radiodiagnostic agents for Alzheimer's disease. Recently 2:1 complexes of curcumin with 68Ga3+ have been prepared with high radiochemical purity. These compounds have also been reported to be binding to β-amyloid fibrils very strongly ,with possible applications in Alzheimer's disease. Detailed studies on the chemistry and spectroscopy of different curcumin-metal complexes would be helpful for future development of curcumin-metal complexes as imaging agents.

New Curcumin Delivery Systems

The water insolubility and low bioavailability of curcumin in cells have prompted researchers to develop new formulations based on biocompatible organic substances like liposomes, polyethylene glycols, biopolymers, cellulose, corn oil, hydrogels etc.Supramolecular assemblies of curcumin with cyclodextrins, and cucurbyturyl have also been reported. All these systems have not only shown improved water solubility but also increased curcumin bioavailability. In these systems, curcumin is solubilised by getting entrapped in hydrophobic pockets, mainly through hydrophobic interactions. Interestingly the fluorescence of curcumin gets is enhanced once solubilised in any of these systems, making it easy to estimate its binding efficiency. Due to their biocompatibility all these systems could be successfully investigated for anti-cancer activity in cancer cells, and in vivo systems, where significant increase in the anticancer activity due to improved bioavailability of curcumin was reported. Liposomal curcumin was found to be the best for improving the bioavailability of curcumin in cells and products based on liposomal formulations are being marketed for different dietary applications of curcumin. Till recently the word nanocurucmin referred to curcumin-loaded organic formulations only. Since there are already several reviews published on organic nanoformulations, in this review, application of inorganic nano formulations in curcumin delivery is discussed. With the same aim of improving anti-cancer activity of curcumin, in the last few years, researchers have been preparing formulations in which curcumin is bound to novel metal and oxide nanoparticles. Such systems can be easily manipulated for improved delivery, activity and specificity. Mesoporous silica nanoparticles (MSN) are one of the most employed nanosystems for improving the bioavailability of poorly water soluble .Due to their ordered nanoporous structures, high surface areas, large pore volumes and high surface densities of hydroxyl groups, MSNs can be functionalized easily. They are biocompatible and they are commonly used in many biomedical applications. Curcumin binds covalently through a silicon-oxygen bond at the diketo moiety (Figure).

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Curcumin-loaded MSNs have been prepared and employed in several studies. In these systems, curcumin release could be controlled for even up to several hours along with improvement in the stability and bioavailability of curcumin.

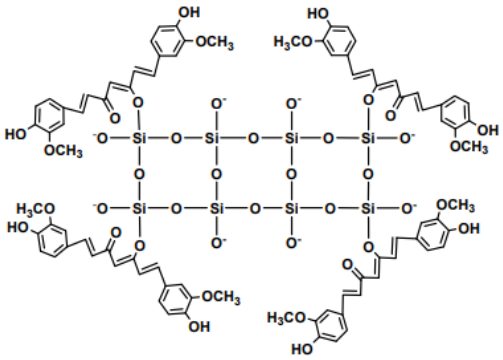

Fig no:5

Curcumin loaded MSNs

The fluorescence of curcumin is enhanced on MSN conjugation and therefore has the potential to be employed for imaging biomolecules/organelles. Novel cyclodextrin functionalized MSN have been found to be photothermally controlled on exposure to light to release curcumin on demand in zebrafish larve. Large amount of curcumin could be loaded in to spherical microcapsules containing L-lysine, trisodiumcitrate and silica sol (colloidal suspension). These microcapsules could be triggered to release curcumin by adjusting the pH to acidic conditions .MSN-curcumin conjugates, increased the cytotoxicity of curcumin in Hela cell lines and also in normal fibroblast cell lines .They also increased photocytotoxicity of curcumin in human oral cancer cells, on exposure to light .Other formulations like guanidine functionalized, and PEGylated MSN-curcumin conjugates showed improved bioavailability and controlled release of curcumin in vitro systems .

Gold nanoparticle-based curcumin formulations have been prepared and reported recently. Gold nanoparticles find application in biology and medicine, for drug delivery, diagnosis and cancer treatment .In a simple method, curcumin-gold composites were prepared by mixing alkaline curcumin solutions with gold salts, where the ionized curcumin acts both as a reducing agent and also as the capping agent .In this case, both the phenolic-OH and enolic-OH donate hydrogen for reduction of Au3+ ions. Such gold-curcumin conjugates were reported to be hemocompatible and non-toxic. Singh et al. also prepared gold-curcumin conjugates by mixng the gold salt with curcumin at high temperature. Such conjugates have been reported to exhibit antixodiant activity by DPPH assay. In another study, curcumin was first conjugated to hyaluronic acid (HA) and this conjugate is treated with gold salt, where HA acted as the reductant .These gold-HA-curucmin composites were biocompatible and exhibited more cytotoxicity in cancer cell lines than pure curcumin. Other gold-curcumin composites were also prepared by conventional methods employing sodium citrate as reducing agent and polymeric systems as capping agents. Such systems can also be employed for the delivery of curcumin .

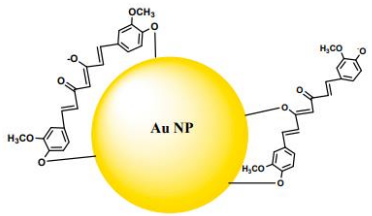

Fig no:6

Gold nano particles capping by curcumin molecules

Curucmin-nanoconjugates of cobalt and silver nanoparticles have been shown to exhibit antimicrobial activity. New antimicrobial films are being fabricated with silver nanocomposites along with curcumin for potentially treating microbial infections. Recently magnetic nanoparticles (MNP) are attracting the attention of researchers. MNP, mostly of iron oxide, are used as drug delivery systems and MRI contrasting agents. They can be targeted magnetically and applied for local hyperthermia. Recently a few reports on preparation and

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charactirisation of MNP-curucmin conjugates are reported. They have also been evaluated for diagnosis and anticancer activity. MNP is coated with pluronic polymers or other biopolymers to load hydrophobic drugs like curcumin . MNP-curucmin can be magnetically and selectively accumulated in cancer cells. In one such study, Yellapu et al.prepared curcumin-loaded MNPs modified with cyclodextrin and reported inhibition effects in ovarian, breast, and prostate cancer cells and also in human pancreatic xenografts. MNP-curucmin has been found to cause apoptosis in MDA-MB-231 breast cancer cell lines along with loss of mitochondrial membrane potential and increased ROS production. MNP-curucmin improved the serum bioavailability by 2.5-fold. The formulation showed good MRI imaging characteristics. MNP formulations along with porous silica nanoparticles have also been employed to encapsulate curcumin, for efficient delivery of curcumin. Due to their selective accumulations and ability to cause hyperthermia, MNP-curcumin with suitable modifications are attracting the attention of many researchers for specific application in cancer therapy and diagnosis.

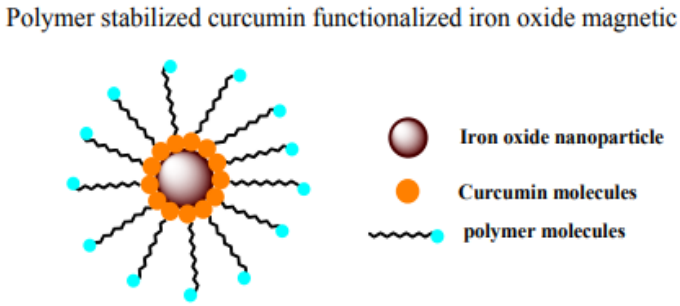

Figure no:7

COLUMN CHROMATOGRAPHY :- Sample preparation Six gram of fine powdered rhizomes were subjected to Soxhlet extraction and solvent used were methanol for seven hours. The extract was concentrated in rotary evaporator .this crude curcuminoids mixture contained curcumin, demethoxycurcumin and bis demethoxycurcumin

(a) (b)

Fig no:8 (a)column chromatography and (b)three different curcuminoid

B) Silica gel Column chromatography: Methanol extract was subjected to Column chromatography in silica gel glass column. About 1gm of crude curcuminoids were mixed with 1 ml methanol and loaded on to the column (34×1.5cm) and eluted with chloroform: Methanol followed by methanol with increasing polarity. All the collected fractions were subjected to TLC and detected as yellow Spot as shown in fig(9).

iii) Separation of curcuminoids by TLC: Methanol extracts were tested on TLC for presence different curcuminoids. The TLC pre-coated silica gel. Plate were developed using glass beaker , which was pre-saturated with mobile phase for 20 min and each plate was developed up to a height of about 6.8 cm. chloroform : methanol mobile phase was used with composition 95: 5. After development, plates were removed and dried. Spots were analyzed.

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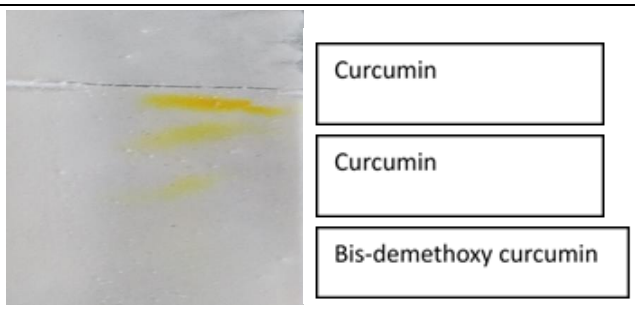

Fig no-9 (separation of curcuminoids by TLC)

1. Estimation of curcuminoid by sphectrophotometric analysis:

i) Preparation of standard: 1mg of pure curcumin was dissolved into methanol and water such that concentration was 60µg/ml, got reading at 420 nm. They are shown in graph no (3). Refer this graph as standard.

ii) Sphectrophotometric analysis : To find out concentration of extracted sample by using spectrophotometer, 1 mg of sample were mixed with methanol and water same as standard solution, OD was taken at 420 nm. Because all three components of curcuminoids has λmax at 420 nm

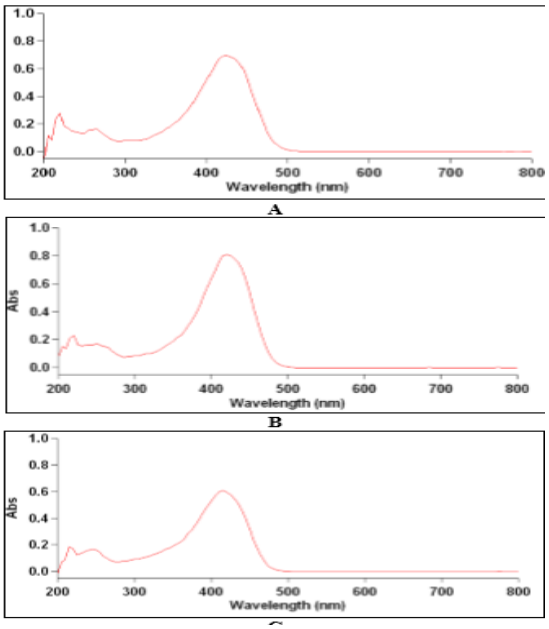

Figure no:9, UV spectrum of A,curcumin crystallized B,demethoxy curcumin crystallized C,bis demethoxy curcumin crystallized.

Soxhelet extraction of curcumin:

Selection of solvents for extraction The selection of the solvents for successive extraction was done by trial and error method. Small quantity of Curcuma caesia roxb rhizome powder is transferred to the series of test tubes. Various organic solvents are added to the each test tube containing the Curcuma caesia roxb rhizome powder. The solutions are mixed thoroughly and warmed for 5min. The solvents selected were n-hexane, methanol and water of increasing polarity. Process of extraction The curcuma caesia roxb rhizome powder was previously macerated overnight with Methanol in soxhlet apparatus. Then, the macerated drug was kept for extraction in soxhlet apparatus at 500C. The continuous hot percolation with Methanol was completed in 5 hours. Then, the extract was collected and concentrated by evaporating the solvent. The concentrated semi-solid extract yield was determined by weighing. % yield of extract from crude drug = (weight of extract/weight of crude drug) X 100.

Parameters of Extraction:

Solvent: Methanol

Temperature : 500C

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Period of extraction : 5 hours

Weight of crude drug : 50 grams

Colour of extract : Yellowish brown

% yield : 12.54% (w/w)

III. RESULT AND DISCUSSION

Maximum concentration of curcuminoids was obtained in methanol extract in the form of dark black orange colour. Concentrations of extracted dried sample were analyzed on spectrophotometer at 420 nm with respect to standard graph (fig 3). We got regration values such as 0.65, 0.68,and 0.55 for methanol (with conc. 60µg/ml), acetone (100µg/ml), ethyl acetate (200µg/ml) and chloroform (100µg/ml) respectively. Sample was run on TLC (fig.4), we got three different spots of C, DMC, BDMC. RF values for these spots were calculated and found to be similar that of reported values [(Table no.1) . According to the RF values curcumin was analyzed by running standard:

Rf value= The migration of sample

The migration of solvent

The Rf value by the separation of curcuminoid by TLC is given by

Table no:1

S.no	TLC mobile phase	ratio	Rf value		
			C	DMC	BDMC
1.	Choloform: methanol	19:1	0.65	0.68	0.55

The HPLC profile of spent turmeric oleoresin (STO) taken for isolation had 18% total curcuminoids, of which curcumin (C), and its analogues DMC and BDMC are 4.4%, 4.9% and 8.7% respectively. Curcuminoid in the spent turmeric oleoresin enriched from 18% to 34.1% by purification using Diaion HP 20 as adsorbent and 80% methanol as eluent. The curcuminoid content of eluted fractions are shown in table 2.

Based on the HPLC profile the eluted fractions from 3 to 8 was combined and concentrated to obtain curcuminoid enriched oleoresin. The HPLC profile of this curcuminoid enriched oleoresin contains the total curcuminoid of 34.1% with curcumin 9.6%, DMC 9.2% and 15.3% BDMC. This was further purified by dissolving the curcuminoid enriched oleoresin in methanol and precipitated in excess hexane.

Hexane precipitated product filtered and dried, to obtain curcuminoid enriched fraction with a yield of 31% with respect to spent turmeric oleoresin.

Table 2: Diaion Hp 20 Column Elution Profile

Fraction Numbers	Total Volume Collected	Curcuminoids			
		Curcumin (%)	Demethoxy curcumin	Bis demethoxy curcumin	Total curcuminoids (%)
1	100ml	3.8	5.2	7.6	16.6
2	100ml	3.9	5.4	7.7	17
3	100ml	8.1	9.4	15.8	33.3
4	100ml	8.44	9.82	16.10	34.37
5	100ml	8.60	9.22	16.26	34.08
6	100ml	8.8	9.7	16.5	35
7	100ml	9.0	9.9	16.8	35.7
8	100ml	7.8	8.2	14.3	30.3
9	100ml	2.7	2.9	3.1	8.7
10	100ml	2.52	2.25	2.45	7.23

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Table 3: 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

Table 4: Silica Gel Column Chromatography Elution Profile

Fraction Numbers	Total Volume Collected	Curcuminoids			
		Curcumin (%)	Demethoxy curcumin	Bis demethoxy curcumin	Total curcuminoids (%)
1	100ml	3.8	5.2	7.6	16.6
2	100ml	3.9	5.4	7.7	17
3	100ml	8.1	9.4	15.8	33.3
4	100ml	8.44	9.82	16.10	34.37
5	100ml	8.60	9.22	16.26	34.08
6	100ml	8.8	9.7	16.5	35
7	100ml	9.0	9.9	16.8	35.7
8	100ml	7.8	8.2	14.3	30.3
9	100ml	2.7	2.9	3.1	8.7
10	100ml	2.52	2.25	2.45	7.23

These fractions on recrystallization gives Curcumin, DMC, BDMC crystals having purities 98.4%, 97.1%, 97.3% by HPLC with an yield of 2.8%, 3.6%, 7.2% with respect to the spent turmeric oleoresin. Melting point obtained for recrystallized material of curcumin 183oC, DMC 172oC, BDMC 222oC respectively.

Table 5: Physical And Chemical Properties Of Crystallised Curcuminoids

Tests done	Curcumin	Demethoxy curcumin	Bisdemethoxy curcumin
Colour and appearance	Yellowish crystals	Yellowish crystals	Yellowish orange crystals
Melting point	183 ^{0}C	172 ^{0}C	222 ^{0}C
Solubility	Soluble in acetone, methanol	Soluble in acetone, methanol	Soluble in acetone, methanol
Purity by HPLC	98.4	97.1	97.3
Rf value	0.70	0.40	0.27
UV ABS MAXIMUM	425nm	420nm	416nm

IV. CONCLUSION

Demethoxy curcumin and Bis demethoxy curcumin in pure form commercially not available. A method developed for separation of 3 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.

Curcumin is a specially gifted molecule provided by Mother-Nature to protect humans from chronic health problems. Looking at the simple chemical structure of curcumin, it is natural to presume that chemistry of curcumin is also very simple, however with increasing scientific understanding it appears to be more complex, unique and difficult to comprehend. It is a symmetric molecule abundant in turmeric with relatively high stability in natural form. It has an intense yellow color, that changes to deep red in basic pH solution. In simple aqueous and aqueous-organic solutions, it is susceptible to fast degradation, which increases as the basicity of

the solutions increases, and also on exposure to sunlight. The metabolic products of curcumin are different from the degradation products, where O-conjugation and reduction are the important processes initiated through the enzymatic reactions. Interestingly unlike the degradation products, the metabolic products are much more difficult to synthesize in the laboratory. The presence of α,β-unsaturated structure makes curcumin participate in nucleophilic addition reactions with protein thiols and selenols, that play important role in modulating cellular oxidative stress. It is still not clear if these processes are reversible under physiological conditions. Future chemical research on these aspects is necessary to elucidate the kinetics and mechanism of all these reactions, so that a meaningful conclusion can be made on the role of these different processes in curcumin biology. Recently there is a surge of activity on preparation and characterization of curcumin-metal complexes due to the strong affinity of β-diketo moiety as an efficient metal chelator. Although it is confirmed that curcumin reduces metal toxicity in living systems through complexation, the actual role of these metal complexes in curcumin biology appears to be complex and unclear. Detailed research is warranted on structure-activity evaluation of the curcumin-metal-complexes in solution. Problems associated with curcumin bioavailability could be overcome to a great extent through formulations with natural biopolymers, which find practical application as nutritional products. Recent research is now focused on developing conjugates of curcumin with metal and metal oxide nanoparticles and some of these formulations have promising potential in nanomedicine with additional effects of inducing targeted hyperthermia in cancer cells. They are also attracting interest as diagnostic tools for Alzheimer's disease and also as MRI contrast agents. Overall it appears that even though there has been significant progress in the chemistry of curcumin, a great deal can still be expected from chemists to exploit this divine natural product as a therapeutic remedy for many chronic diseases.

Abbreviation and Symbols

DMC	Demethoxy curcumin
BDMC	bis demethoxy curcumin
HPLC	High profile liquid chromatography
TLC	Thin layer chromatography
TNF	Tumor necrosis factor
TGF	Transforming growth factor
IP	Interperitonial
BLM	Bleomycin
MCF	Michigan cancer foundation
AuNP	Global nano particals
MRI	Magnetic resonance imaging
Rf	Retention factor
MSNR	Mesophorous silica nano particles

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