بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Extraction and Characterization of Curcumin from Turmeric and Using it as Color Coating Matrial for Metronidazole Tablets

استخلاص وتشخيص الكركميين من الكركم واستخدامه مادة ملونه لتغليف حبوب الفلاجيل

A Thesis Submitted in Partial Fulfillment for the Requirement of the Degree of M.Sc. in Chemistry

By

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الآية

بسم الله الرحمن الرحيم

قال تعالي :

(وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ)

سورة التوبة الآية [105]

Dedication

I dedicate this effort to my father, mother, brother and sisters.

Acknowledgment

In the beginning I would like to thank Allah ,Almighty, who assisted me to complete this research.

I would like to extend my sincere thanks to Dr. Mohammed Sulieman, who provided me with the valuable information that enabled me to accomplish this work.

Thanks to Unimed pharmaceutical Factory, in particular laboratory staff, my thank extends to Azal pharmaceutical Factory and Mr. Musa Omer who help me geratly.

Abstract

As it is known that curcumin has several medicinal characteristics, such as an anti-oxidant, anti-cancer, anti- inflammatory, and also has many benefits in cosmetics field.

Several studies have been proposed to study the curcumin extraction from turmeric in a simple and easy way and to use it in several areas. However, applying curcumin in coating Metronidazole tablets curcumin pills has not been shown in the previous studies.

At the present work, curcumin was extracted in higher yield and the obtained extract was characterized using infrared red (IR), thin layer chromatography (TLC). The obtained curcumin has a melting point(M.P) of 183°C,182°C and 184°C. The ash content found to be 1.5%, 3.09%, and 3.17% . Curcumin material was used in the pharmaceutical field as a colorful agent used in coating Metronidazole tablet curcmin pills.

The experimental results showed that the coated Metronidazole tablets curcumin pills have no side effects. In addition it also showed stability of color and even at high temperature .and the coated tablets showed that the taste of bitterness ratio in Metronidazole pills wasa reduced or disappearanced completely.

المستخلص

كما هو معروف أن الكركمين له عدة خواص طبية مثل انه مضاد للأكسدة والسرطانات ،مضاد للالتهابات وهذا النبات واسع الانتشار خصوصا في الهند .

لذا اهتمت العديد من الدراسات باستخلاص الكركمين من الكركم وكانت أهم الأهداف التي هدفت لها تلك الدراسات استخدام طرق سهلة وبسيطة والاستفادة من الكركمين في عدة مجالات . من أهداف هذا البحث ، استعمال الكركمين في مجال الصناعات الصيدلانية كمادة ملونه تستخدم في تغليف حبوب الفلاجيل .

أظهرت النتائج التجريبية أن الاستخلاص تم بكفاءة من خلال النسبة المئوية العالية للكركمين وتم وصف الراسب باستخدام طرق الاشعة تحت الحمراء وكروموتغرافيا الطبقة الرقيقة واظهرت النتائج ان للكركمين درجة انصهار عند183 وله محتوي رماد يساوى كما اظهرت ان تغليف حبوب الفلاجيل بمادة الكركمين انها فعاله وذات درجة لون ثابتة ولا تتأثر بالحرارة وليس للكركمين اثار جانبية.

ومن النتائج التجريبية ايضآ لهذا البحث اختفاء نسبة طعم المرارة عند تناول حبوب الفلاجين وذلك نسبة للاثر الذى لعبته مادة الكركمين.

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CHAPTER One

Introduction

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1. Introduction

As a dried rhizome of a herbaceous plant, turmeric is closely related to ginger. The spice is also sometimes called "Indian saffron" thanks to its yellow color [1]. The underground rhizome imparts a distinctive flavor to food but it is also used to provide food with a deep , indelible orange color. In the form of this fine, dried, yellow powder, turmeric is mostly sold to customers in developed countries.

Turmeric is used in a wide variety of foods of the cuisines of Southern Asia but locally it also applies as an antiseptic for skin abrasions and cuts [2].

1.1 Global production and trade

While there is speculation that turmeric may have originated from South or South-East Asia, its center of domestication is certainly the Indian subcontinent. Currently, India is the major producer of turmeric, and it is also the major user of its own production (Table 1). Turmeric is part of Indian's culture: it is an important ingredient in curry dishes; it is also used in many religious observances, as a cosmetic, a dye, and it enters in the composition of many traditional remedies [3][4][5].

Figure 1& 2: Shows Turmeric (Curcuma Longa L)

Other producers in Asia include Bangladesh, Pakistan, Sri Lanka, Taiwan, China, Burma (Myanmar), and Indonesia. Turmeric is also produced in the Caribbean and

Latin America Jamaica, Haiti, Costa Rica, Peru, and Brazil [5][6].Turmeric and curry powder exports from India are listed in Table 2.

Table 1: Turmeric production in India[5]

	1995-96a	1998-99a	2000b
Area (hectares)	65,320	73,830	145,000
Production (metric tons)	252,437	329,436	600,000

1 a: Indian Spice Board Statistics

2 b: Estimate, from Weiss, 2002

1.2 Main consumption areas and trends:

Asian countries consume much of their own turmeric production, except for Japan and Sri Lanka. Major importers are the Middle East and North African countries, Iran, Japan and Sri Lanka . These importing countries represent 75% of the turmeric world trade , and are mostly supplied by the Asian producing countries. Europe and North America represent the remaining 15%, and are supplied by India and Central and Latin American countries.

Taiwan exports mostly to Japan. The United States imports of turmeric come from India at 97%, and the rest is supplied by the islands of the Pacific, and Thailand. Tables 2 and 3 show turmeric imports by the United States, United Kingdom and Japan. Quantities and prices for these countries were stable over the period 1997-2002. However, the increasing demand for natural products as food additives makes turmeric an ideal candidate as a food colorant, thus increasing demand for it. Additionally, recent medical research demonstrating the anti-cancer and anti-viral activities of turmeric may also increase its in demand Western countries.

As an indication of its value, the delivered price of turmeric on the New York market was 1,300 $/ton (Indian Madras fingers) and 1,455 $/ton (Indian Alleppey fingers) in mid-2001.

Table 1.2: Turmeric imports in the US in the period 1998-2002 (metric tons; US$1,000)a[5]

	1998	1999	2000	2001	2002
Turmeric (MT)	2,284	2,641	2,427	2,404	2,383
Value (US $1,000)	3,849	3,614	2,904	2,488	2,955

1.3 Primary Products:

There are two dominant types of turmeric found on the world market: 'Madras', and 'Alleppey', both named after the regions of production in India. The orange-yellow fleshAlleppey turmeric is predominantly imported by the United States, where users prefer it as a spice and a food colorant.[5] Alleppey turmeric contains about 3.5% to 5.5% volatile oils, and 4.0% to 7.0% curcumin. In contrast, the Madras type contains only 2% of volatile oils and 2% of curcumin. The Madras turmeric is preferred by the British and Middle Eastern markets for its more intense, brighter and lighter yellow color, better suited for the mustard paste and curry powder or paste used in oriental dishes.[7-8] Turmeric produced in the Caribbean, Central and South America has low curcumin and volatile oil contents, and is darke; it is not sired by dethe U.S. importers . The Bengal type is preferred for use in dyes in India.19It is interesting to note that in the United States, turmeric is considered as a spice by the food industry, whereas it is classified as a food colorant by the Food and Drug Administration (FDA)[1][2][3][4][5].

1.3.1 Dried Rhizome

Turmeric is mostly imported as a whole rhizome, which is then processed into powder or oleoresin by flavor houses and the industrial sector. Rhizomes come as fingers, ulbs and splits. Fingers are the secondary branches from the mother rhizome, the bulb, and splits are the bulbs cut into halves or quarters before curing. The fingers are 2 to 8 cm long and 1 to 2 cm wide, and are easier to grind than the more fibrous bulbs and splits, and therefore command a higher price. Rhizome quality is judged by a clean and smooth skin, uniform skin and flesh colors, and a clean snap (or "metallic twang" as described by the Indian Ministry of Agriculture standards, Agmark) when broken. Turmeric cleanliness specifications for import pertain to whole rhizomes[5].

Figure 3. Dried rhizomes[39]

1.3.2Turmeric Powder

Ground turmeric is mostly used on the retail market, and by the food processors. Rhizomes are ground to approximately 60-80 mesh particle size. Since curcuminoids, the color constituents of turmeric, deteriorate with light and to a lesser extent, under heat and oxidative conditions, it is important that ground turmeric be packed in a UV protective packaging and appropriately stored. Turmeric powder is a major ingredient in curry powders and pastes. In the food industry, it is mostly used to color and flavor mustard. It is also used in chicken bouillon and soups, sauces, gravies, and dry seasonings. Curcumin powder has also been used as a colorant in cereals.

1.4 Secondary and Derived Product

Curry Powder

Turmeric is such an important ingredient in curry powder that it merits special mention. In its export statistics of spices, the Indian Spice Board specifically lists curry powder exports.

The turmeric content in curry powder blends ranges from 10-15% to 30%.Typical Indian curry powder for meat and fish dishes contains 20-30% turmeric, 22-26% coriander, 12% and 10% cardamom and cumin, respectively, 4% or 10% fenugreek, ginger, cayenne, cloves and fennel in proportions from 1% to 7%.Curry mixes for vegetarian dishes contain less turmeric, in the range of 5 to 10%, because of the bitter flavor it would impart to the dish[5].

Figure 4: Turmeric powder

1.5. Chemical and Physical Properties of Curcumin

The chemical structure of curcumin is shown in Figure 1. Curcumin is a major secondary metabolite of the perennial Asian plant turmeric (Curcuma longa L).

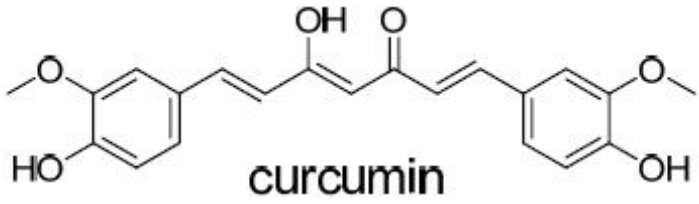


Figure 5: Structure of curcumin

Curcumin was identified as the active principle of turmeric in 1815, and its Structure determined after crystallization in 1870 [9].Turmeric is only one representative of more than 80 curcuma species of the ginger family, Zingiberaceae [10]. Turmeric is widely cultivated in many Asian countries particularly in India where it is grown mostly for dietary use, and is a major component of the spice curry. Additionally, turmeric is recognized for its medicinal properties, and has been used for centuries in the treatment of a variety of ailments including eczema, arthritis, ulcers, asthma, anemia and many others [11] Resulting from extensive studies over the last few decades, curcumin has emerged as a promising anti-cancer agent and has been shown to target multiple and diverse signaling pathways involved in disease causation and

progression[9]. The attractiveness of curcumin as a therapeutic agent is enhanced by its safety, affordability, and history of long-term use [12]. The IUPAC name of curcumin, also referred to as diferuloylmethane, is (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.

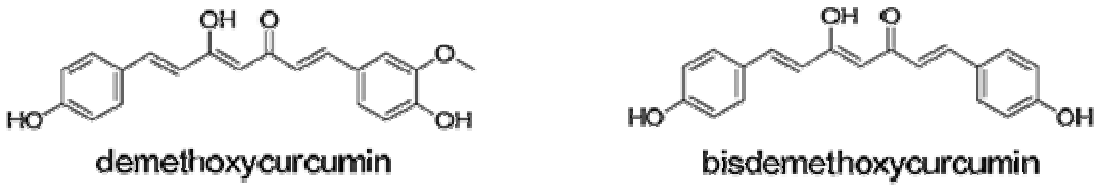


Figure 6: Structure of the minor curcuminoids

molecular formula of $C_{21}H_{20}O_6$, corresponding to a molecular weight of 368.37. Curcumin is a yellow-orange crystalline powder with maximum absorbance at 430 nm and melting point of 183 °C [11]. Curcumin exhibits hydrophobic and (slight) hydrophilic properties owing to its aliphatic heptadienone linker and polar β-dicarbonyl and phenolic groups, respectively[13]. Curcumin is sparingly soluble in water, but shows greater solubility in some organic solvents such as acetone, ethyl acetate, acetonitrile and ethanol. Its reported partition coefficient (Log P) ranges from 2.5 to 3.3 [14]. Curcumin is a bis-α,β-unsaturated β- diketone, and exists in equilibrium with its enol tautomer [15]. Studies involving 1H, C NMR, and infrared spectroscopy have shown that the enolate form predominates in alkaline solution [16]. Further, the enolate form is energetically favored since curcumin then exists as a completely conjugated and planar structure[16][17]. In the enolate conformation in alkaline conditions, the phenolic hydroxyl is the major site of curcumin reactivity; curcumin donates a proton and an electron from either of its phenolic hydroxyls via the 'Sequential Proton Loss Electron Transfer' (SPLET) mechanism, resulting in a phenoxyl radical. [19].The donation of a phenolic H underlies curcumin's antioxidant activity and its instability at alkaline pH [20][21][22]. In contrast, under acidic conditions, the bis-keto form of curcumin predominates and it becomes a potent H donor from the weak central C–H bond [17][19][23][24]. Up to eight different conformational isomers of the enolic curcumin have been proposed from crystallization studies, with three crystalline forms exhibiting varying degrees of solubility identified depending on the type of solvents used for extraction and

purification. All three crystalline forms exist as the β-keto–enol tautomer but differ in their hydrogen bonding, molecular packing, and the relative orientation of theketo–enol groups in neighboring molecules. Form 1, the type typically seen in commercial preparations of curcumin, has a slightly twisted conformation. Forms 2 and 3 show a linear, planar conformation and have a slight increase in solubility compared to form 1 ([14][25].

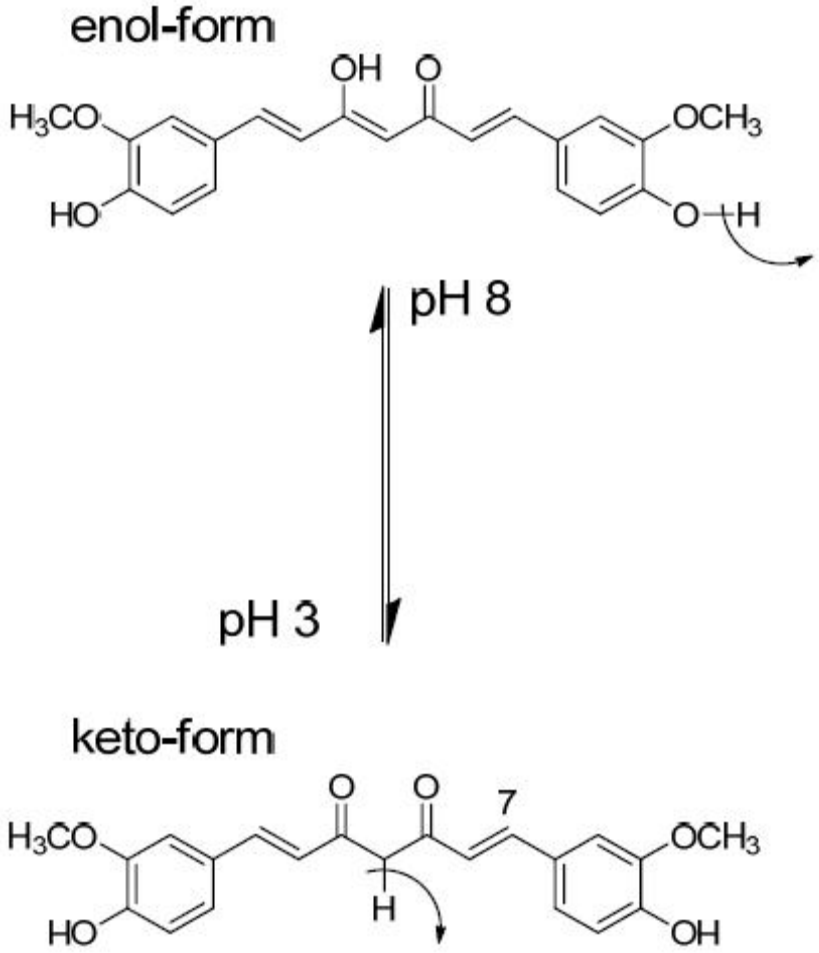


Figure 7: Enol (pH 8) versus bis-keto (pH 3) tautomers of curcumin, and their different site of H-donation.

1.6 Therapeutic potential of curcumin

Like many other natural products with 'roots' in ethno-medicine, curcumin is now the subject of extensive research and is among the most studied plant derived medicinal chemicals [14]. Curcumin is widely recognized for its potent anti-inflammatory effects, and is associated with reduction in neutrophil and macrophage infiltration, inhibition of pro-inflammatory chemokines, and suppression of NF-κB, COX-2,

iNOS, IL-8 and other pro-inflammatory targets in vitro and in animal models [26][27][28]. Inflammation is central to the progression of many chronic diseases such as cancer, cardiovascular disease, diabetes, neurodegenerative disease, and arthritis, and curcumin is being investigated in the treatment and prevention of these diseases [12][27][29][30][31]. Suppression of prostaglandin (PGs) synthesis through the inhibition of COX-2, for example, is associated with decreasing inflammation and cancer cell proliferation [32][33][34][35][36]. Epidemiological studies have linked the high consumption of curcumin/turmeric in India (up to 1.5 g per person daily) to the lower over all incidence of colorectal, pancreatic, lung, breast, and prostate cancers when compared to Western countries where little curcumin is consumed [9]. Furthermore, second generation migrants from India show higher cancer prevalence than their first generation counterpart, further solidifying an environmental (or dietary) basis for the discrepancy in cancer Prevalence [26][37]. Pre-clinical studies of curcumin in rodent models show efficacy against colonic, stomach, liver, breast, pancreatic, brain and lung cancers [16][38][39][40][41][42]. Curcumin exhibits chemo preventive effects against cancer caused from germline mutation in the APC gene in C57BL/6J-Min/+ mice that show spontaneous intestinal adenomas, or cancer caused from the carcinogens DMBA, benzo[a]pyrene, and azoxymethane that induce lymphomas, stomach and colon carcinogenesis, respectively [43][45]. The chemo preventive effects of curcumin in mouse models support its role in the low incidence of cancers in regions where turmeric forms a major part of the diet. Curcumin is also able to reduce tumors once they are formed in animals.

Studies conducted in tumor xenograft mice shows that curcumin can reduce or cure brain tumors, pancreatic tumors, lymphomas, and others [40][41][46][47]. In mouse models of diabetes, curcumin treatment results in improvements in obesity, blood glucose levels, as well as glucose and insulin tolerance. Curcumin was shown to significantly decrease body weight and fat content even with increasing caloric intake, and protects pancreatic islets against disease related damage. The improvements in glycemic status were coupled with decreased NF-κB signaling, reduced macrophage infiltration into adipose tissue, and higher circulating adiponectin levels [27][30]. Curcumin also shows efficacy in models of depression, Alzheimer's disease, and cardiovascular disease [31][48][49].

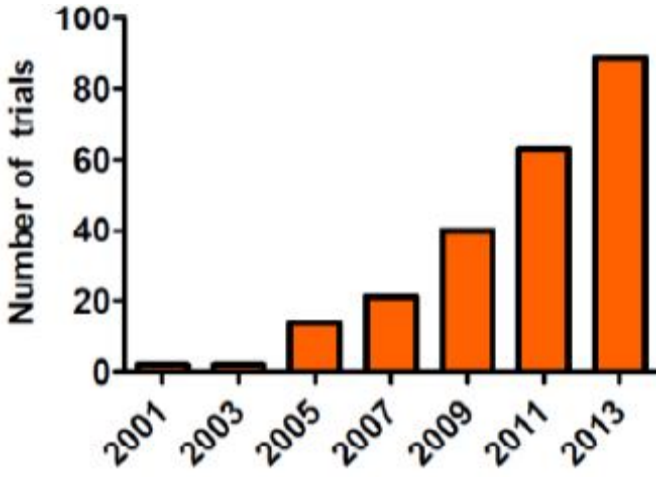


Figure 8: Number of curcumin clinical trials registered at www.clinicaltrials.gov. every other year since 2001.

Extensive preclinical data showing efficacy for curcumin led to studies in human diseases. There is a growing number of clinical studies involving curcumin, reaching 92 as of March 2014, up from 2 studies in 2001 (Figure 8). A study by Hishikawa et al. investigated turmeric in three Alzheimer's disease (AD) patients with severe cognitive, behavioral and psychological decline. The patients were given 100 mg/day curcumin for 12 weeks. The authors reported significant improvement in behavioral symptoms with no adverse effects seen even after 1 y of taking the drug[31].The mechanism of action for curcumin in AD has not been elucidated. Current strategies for treating the disease use inhibitors of glutamate and acetyl cholinesterase (AChE). Curcumin has been shown to protect against glutamate excitotoxicity and inhibit AChE in in vitro assays. Furthermore, curcumin shows memory-enhancing effects in rodent models [51], exhibits anti-amyloid activity and can lessen inflammation in the Brain as possible mechanisms of its effects on AD. Studies in arthritis patients report improvements in disease activity and improved over all quality of life in patients taking 200 mg (open label) or 500 mg meriva curcumin per day[12][52].Additionally, curcumin shows efficacy in patients with ulcerative colitis, diabetes, colorectal cancer, and other diseases [50][53][54][55].

1.7 Curcumin as An Antioxidant

The effects of curcumin were first attributed to its antioxidant properties [56]. Curcumin has a uniquely conjugated phenolic structure making it a particularly good

radical trapping antioxidant [57]. Curcumin can reduce oxidative damage in cells by preventing lipid per oxidation, increasing reduced glutathione by inducing its biosynthesis, and scavenging small free radical species like HO• and ROO• [58][59]. There was some contention in the literature over the relative importance of the phenolic hydroxyl versus the central methylenic hydrogen in mediating the antioxidant effect of curcumin. Jovanovic and his collaborators concluded that curcumin donates its H-atom from the central methylenic group in aqueous acidic buffer and in acetonitrile solutions. It was later proposed that under physiological conditions, curcumin is a typical phenolic antioxidant and donates an H-atom from one of its phenolic hydroxyls [58][60][61]. The meta-methoxy groups were suggested to further increase its antioxidant activity. During anti-oxidant reactions, the donation of an H-atom and electron to an oxidized species creates a second radical (albeit a more stable one) in the antioxidant molecule (a phenoxyl radical in the case of curcumin). This secondary radical must then be terminated by another radical species. An important clue to understanding the anti-oxidant mechanism of a compound is the structural elucidation of the terminated antioxidant product. Masuda et al conducted model studies of curcumin oxidation using 2,2‘- azobis(isobutyronitrile) to produce the curcumin radical in acetonitrile. The authors reported isolating chain cleavage products vanillin and ferulic acid, as well as dimers of curcumin. A mechanism was proposed in which curcumin is first converted to the phenolic radical that moves to a carbon centered position along the heptadienone chain resulting in the formation of a quinine methide. The dimers, they further proposed, were formed from coupling of two different carbon-centered radical species, and vanillin and ferulic acid formed from radical chain cleavage. Studies in our lab show that in aqueous solution, the terminal product(s) involves the stable incorporation of oxygen into the molecule to form a bicyclopentadione, with no products from inter-molecular curcumin reactions observed[21][62]. The reaction of curcumin to generate the bicyclopentadione is the main focus of the work presented in this dissertation.

1.8 Intermolecular Interactions of Curcumin:

In addition to its anti-inflammatory and anti-oxidant effects, curcumin exhibits antiviral, antibacterial, antifungal, antineoplastic, and antiangiogenic bioactivities[41][61][63]. Curcumin modulates more than 100 cellular targets in vitro studies using cell-based systems. Curcumin has been shown to affect transcription

factors (AP-1, PPAR-γ), cytokines (IL-1, IL-2, IL-5, IL-12), enzymes (MMP-1, GST, 5-LOX), growth factors (EGF, VEGF, TGF β1), kinases (PKA, PKB, JNK, MAPK) and survival pathways (p53, Bcl-2) in many cell types[38][33][42][56][64][65]. Affecting this many targets is a remarkable feat for any single molecule. The chemical mechanisms whereby curcumin is able to achieve this is one conundrum associated with its biological effects. The chemical properties of the curcumin molecule provide many inferences into the nature of its interactions with protein targets and may explain its interaction with some of its targets. The following features of curcumin are implicated in mediating its intermolecular interactions: lipophilicity; Michael reaction acceptor capacity; H-bond donating capacity of the β-diketo and phenolic hydroxyl moieties; and its rotamerization capacity (Figure 9).

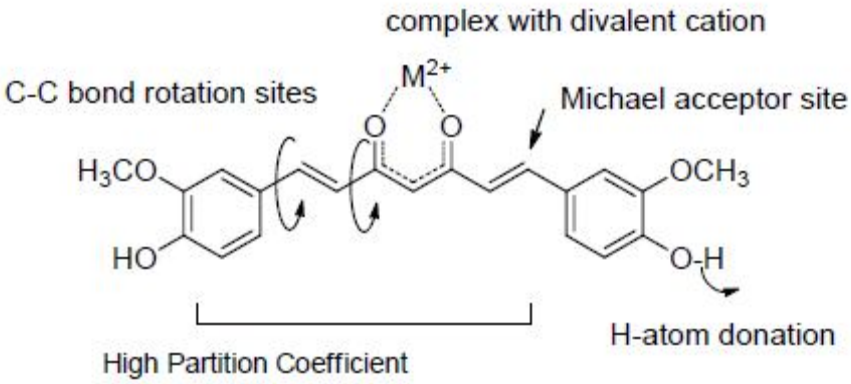


Figure 9: Chemical properties of curcumin

The high lipophilicity of curcumin has been attributed to the aliphatic heptadienone linker connecting the more polar methoxyphenol rings. Lipophilicity allows for non-covalent interactions with hydrophobic residues in proteins. Molecular docking studies show interactions between curcumin and alanine and tyrosine residues in human immunoglobulin G. Docking studies of curcumin in COX-1 active site show that the aliphatic chain region of curcumin is surrounded by hydrophobic amino acids, including leucine, alanine, and valine [66][67]. Curcumin also binds in the minor and major groove of DNA at AT-rich regions [63][68]. The strong hydrophobic binding of curcumin to its target proteins stabilizes further covalent interactions between the two

molecules The Michael acceptor capacity of curcumin allows for covalent interactions with nucleophilic residues in proteins (thiolates and amines) at its α,β-unsaturated carbonyl positions (Figure 1.5). A common Michael reaction involving curcumin is with glutathione (GSH). The GSH tripeptide is composed of γ-glutamate-cysteine-glycine and is known for its nucleophilic reaction with α,β-unsaturated carbonyl compounds [65][69]. Incubation of curcumin with GSH led to a concentration and time-dependent decrease in the curcumin chromophore at 430 nm, due to the disruption in the conjugated system resulting from covalent adduction Curcumin-GSH adducts have been confirmed by HPLC, LC-MS and NMR studies (ON Gordon, unpublished). One study suggesting covalent adduction of curcumin with p300 in cells uses the lack of reactivity of the protein with tetrahydrocurcumin (which lacks the α,β-unsaturated carbonyl) as evidence of Micheal reaction with curcumin Covalent interaction of curcumin with many of its target proteins has not been demonstrated. Lastly, curcumin undergoes rotamerization about multiple a C–C bonds. This conformational flexibility maximizes the number of interactions between curcumin and its targets, as evident from the docking studies in which curcumin tends to adopt different configurations for each molecular target .Curcumin retains its planar structure when interacting with B-form DNA minor grooves, and becomes non planar when bound to amyloid protein and PKC. These interactions highlight the versatility of curcumin in its ability to associate with other molecules, but only its antioxidant and Michael acceptor functions are expected to contribute significantly to its ability to modulate signaling in these proteins. The Michael acceptor capacity and antioxidant effects of curcumin however do not account for the many and varied targets identified. The chemical mechanisms whereby curcumin is able to affect its proteins targets therefore remain to be determined.

1.9 Curcumin Bioavailability:

A second conundrum associated with the biological effects of curcumin is that efficacy is often demonstrated in tissues that show low to undetectable levels of the unconjugated compound. Curcumin is poorly absorbed across the gastrointestinal (GI) tract and is extensively metabolized in the liver and intestines, accounting for the submicromolar levels of the un conjugated compound observed in blood after oral dosage [70][71][72]. Administered 10 mg/kg of curcumin intravenously to rats and reported maximum serum levels 0.36 μg/mL after 45 min. A very high oral dose of

500 mg/kg curcumin gave only 0.06 μg/mL, and 1 g/kg dose produced a maximum serum curcumin level of only 0.5 μg/mL after 45 min. The comparatively low plasma levels of curcumin seen after the oral doses support significant first pass metabolism of curcumin Similar plasma levels of curcumin are reported in other animal studies [70][73]. Curcumin was undetectable in the serum in studies in human subjects given a single oral dose of 0.5 or 10 g curcumin. Only one of three subjects who received a 10 g dose had detectable serum levels of 0.03 to 0.06 μg/mL after 1, 2, and 4 hours. An increase to a 12 g dose resulted in detectable levels in only one of three subjects with comparable serum levels over the same time period (Lao et al. 2006). These results are consistent with data from other studies in which glucuronide conjugates of curcumin and its reduced metabolites are the predominant form of the compound detected in plasma.

Multiple approaches are being employed to improve the uptake of curcumin from the gut, including complexes with nanoparticles, liposomes, micelles and phospholipids. The studies described above used curcumin-C3 complex marketed/developed by Sabinsa. Curcumin-C3 is a mixture of the three curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) in a patented ratio and is reported to enhance the bioavailability of curcumin. Another often-used formulation is meriva curcumin patented by Indena SpA, Milan, Italy. The meriva curcumin formulation contains soy lecithin and microcrystalline cellulose with an overall curcumin content of 20%. A comparison of 340 mg/Kg unformulated curcumin and meriva curcumin corresponding to an equal amount of the compound was conducted in rats. In the first group, 99% of curcumin was present in plasma as glucuronide conjugates, with the remaining 1% being curcumin sulphate and free curcumin. The meriva curcumin group showed 23 fold increase in glucuronides, but only a5 fold increase in curcumin. Curcumin levels in the plasma therefore remains low, and only marginal improvements in efficacy have been reported with these formulations. Even though the low bioavailability is considered the main limitation to the clinical efficacy of curcumin, pre-clinical studies in rodents do report efficacy with plasma exposures that one would consider sub-therapeutic. Examples include systemic effects of dietary curcumin (0.01%–0.25% w/w) in treating inflammatory eye disorders and glioma in the brain. These effects either suggest extraordinary potency for systemic curcumin, or suggest a role for its more abundant metabolites.

1.10 Curcumin Metabolism:

Studies assessing the metabolism of curcumin in rat liver slices, microsomes, and cytosol yields octahydrocurcumin, hexahydrocurcumin tetrahydrocurcumin and dihydrocurcumin . Thus, the phase I metabolism of curcumin involves the successive reduction of the unsaturated heptadieneone chain (Figure 9). Alcohol dehydrogenase is required for the formation of tetrahydrocurcumin and hexahydro curcumin, the abundant reduced Metabolite while unidentified microsomal enzyme(s) catalyze the reduction of hexahydro curcumin to octahydrocurcumin .The reported conjugation metabolites of curcumin are glucuronide and/or sulfate conjugates; the major conjugation metabolites were formed in varied but significant amounts . None of the postulated cytochrome P450 metabolites (demethylation or hydroxylation) of curcumin were detected, suggesting these enzymesare not involved to an appreciable extent in curcumin metabolism of curcuminin the liver .Analyses of curcumin metabolism in vivo are consistent with the in vitro data, indicating reduction of the unsaturated heptadienone chain of curcumin and subsequent conjugation to glucuronic acid or sulfates (usually inferred after hydrolysis with β-glucuronidase and arylsulfatase) forms the major metabolites. The first detailed study on the in vivo metabolism of curcumin was conducted in rats by Holder et al. using [3H]-curcumin [70]. After an oral dose of 0.6 mg [3H] curcumin, about 90% of the radioactivity was detected in the feces, suggesting biliary excretion of curcumin and its metabolites. Similar data were obtained when [3H] curcumin was administered intravenously. Most of the excretion occurred within the first 24 h of administration, with very little compound detected in the tissues beyond 3 days. Glucuronide conjugates represented more than 95% of the metabolites recovered from the bile. Most of the glucuronide was conjugated to tetra- and hexahydrocurcumin. Sulfates accounted for about 2% of the metabolites. After oral administration of 8 g curcumin in human subjects, curcumin glucuronide and curcumin-sulfate were detected in plasma at 1.5 to 1.7 μM and 0.21 to 0.35 μM, respectively. Hexahydro curcumin and hexahydro curcumin glucuronide were also present in minor amounts .Several studies have also explored the tissue distribution of curcumin. Among these are studies conducted by Ravindranath & Chandrasekhara, showing that after oral administration of 400 mg of curcumin to rats, only traces of the parent compound could be found in tissues such as the liver and kidney. After 30 min, about 90% of curcumin was found in the stomach

and small intestine, but only 1% was present at 24 h, with less than 3% of the curcumin found in the tissues. In a similar evaluation of the tissue distribution using [3H] curcumin diluted with cold curcumin, radioactivity was detectable in blood, liver, and kidneys after oral administration of 400, 80, or 10 mg of the compound. With a high dose of 400 mg [3H] curcumin, large amounts of the radioactivity were present in tissues 12 days after dosing. [3H]. Curcumin remained in the body for 72 h to 12 days with doses of 10 mg and 400 mg.

1.11 Chemical Instability of Curcumin in Vitro:

The instability of curcumin at alkaline conditions (pH 7 to pH 10) was reported in 1985 by Tonnesen&Karlsen. The reported products of the degradation were ferulic acid, vanillin, feruloylmethane, and condensation products of feruloylmethane. The products were identified by GC-MS comparison with authentic standards. A more detailed account of this degradation was later provided by Wang et al. The authors showed that curcumin undergoes degradation at alkaline pH in aqueous buffer, cell culture medium, and human blood. In 0.1 M phosphate buffer at pH 7.2, curcumin degrades rapidly with 90% of the chromophore at 423 nm disappearing over 10 minutes. Curcumin was more stable in media containing 10% serum or in human plasma in which it has a half-life of about 8 h[22][74]. Curcumin degradation is also slower in the presence of thiol-based antioxidants such as glutathione, and divalent ions such as Zn2+, Cu2+, Mg2+, and Se2+ [75]. No appreciable degradation of curcumin occurs below pH 7 and feruloyl methane (Figure10) as minor products of this degradation with the major product tentatively identified as trans-6-(4’-hydroxy-3’-methoxyphenyl)-2,4-dioxo-5-hexenal.

The time comparisons with authentic standards. Due to limited sample availability, themajor product was only tentatively identified based on LC–ESI–MS. Its structure

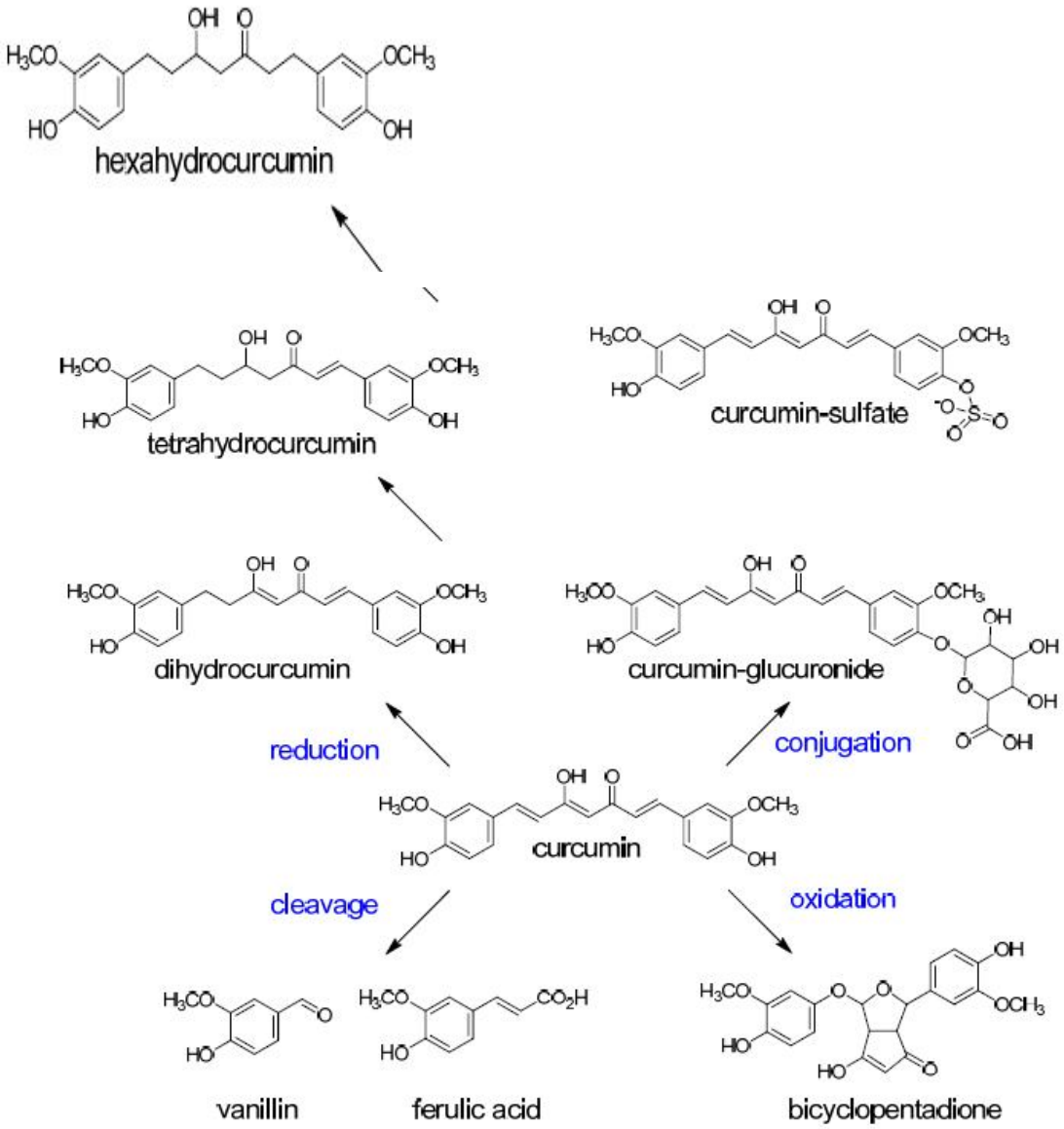


Figure 10: Curcumin Metabolism

1.12 Color Stability of Natural Colorant in Turmeric:

Nowadays, the attractive colors of food products like strawberry jams, blood orange juice, or raspberry jellies is an important quality parameter, which influences consumer behavior. However, there are some problems to remain and to obtain a stable color of fruit products especially while processing and storage them in a long period. In order to improve and maintain the stability of color, scientific research on the chemistry of colors is needed. Natural colorant additives are usually considered as color additives derived from plant or animal sources by extraction or other physical processing. Some examples of natural colorants are include carmine, annatto extract,

grape skin extract, turmeric, saffron, and beta-carotene, which are the major natural color additives used in foods product. Synthetic colorant additives are including chemically synthesized substances and for example are tartrazine, erythrosine and indigo carmine. Natural food colorant additives should be used because there are so many advantages and benefits to consumer. There are no side affect that will give bad condition to consumers. Usage of natural colorant in food industry appears to have multidimensional potential (Dufosse, 2004) and it will help to increase the production of high quality product in food industries. Curcumin (synonyms: turmeric yellow, kurkum, INS No. 100(i)) is an orange-yellow crystalline powder.

Joint Expert Committee for Food Additives (JECFA) specifications define only curcumin extracted from natural source materials. It can also be produced by chemical synthesis. Synthetic curcumin is not used as a food additive.[77]

1.12.1Manufacturing of Curcmin:

Turmeric is subjected to solvent extraction. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) specifications monograph for Curcumin (FNP 52 Add. 9, 2001) lists acetone, methanol, ethanol, and is opropanol as suitable solvents.

The European Commission Directive 95/45/EC lists the following solvents as suitable for the extraction: acetone, carbon dioxide, ethyl acetate, dichloromethane, n-butanol, methanol, ethanol, and hexane. Curcumin is recovered by crystallization from the extract. Minor amounts of oils and resins naturally occurring in turmeric may be present.

1.12.2 Detailed Description:

Curcumin is extracted from the dried root of the rhizome Curcuma Longa. The process of extraction requires the raw material to be ground into powder, and washed with a suitable solvent that selectively extracts coloring matter. This process after distillation of the solvent yields an oleoresin with coloring matter content in the region of 25-35 percent along with volatile oils and other resinous extractives. The oleoresin so obtained is subjected to further washes using selective solvents that can extract the curcumin pigment from the oleoresin.

This process yields a powdered, purified food colour, known as curcumin powder, with over 90 percent coloring matter content and very little volatile oil and other dry

matter of natural origin. The selection of solvents is done with care to meet extractability and regulatory criteria. The following solvents are considered suitable: Isopropanol In the curcumin manufacturing process isopropyl alcohol is used as a processing aid for purifying curcumin.

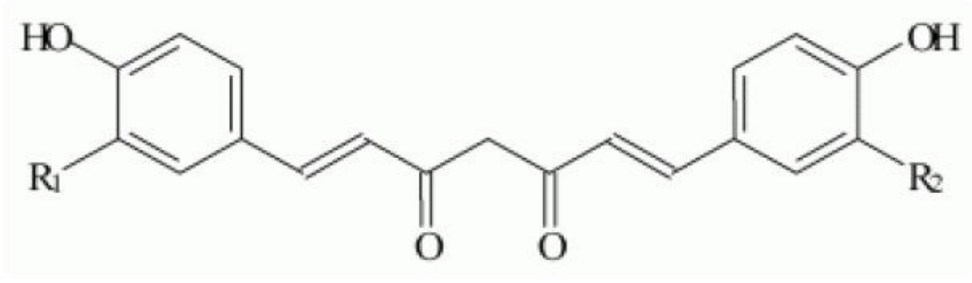
Ethyl acetate with a restriction placed on the use of chlorinated solvents, such as dichloroethane, it is found that ethyl acetate, owing to its polarity, is a reasonable replacement providing acceptable quality of product and commercially viable yields. Acetone this is used as a solvent in the curcumin manufacturing process. Carbon dioxide is not currently used in commercial production. However, it is listed in EC Directive 95/45/EC and has potential as a substitute for chlorinated solvents. Methanol this solvent is used occasionally as a processing aid for purification. Ethanol this solvent is used sparingly because curcumin is completely soluble in ethanol.

1.12.3 Composition of the Food Additive:

The three principal colouring components of curcumin that are present in various proportions are all dicinnamoylmethane derivatives:

1) 1,7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione= iferuloylmethane (Chemical formula: $C_{21}H_{20}O_6$: C.A.S. number: 458-37-7, Formula weight: 368)
2) 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione = p-hydroxycinnamoylferuloylmethane (Chemical formula: $C_{20}H_{18}O_5$: C.A.S. number: 33171-16-3, Formula weight: 338) Chemical and Technical Assessment Curcumin 61st JECFA3 (8)

3)1,7-Bis-(4-hydroxyphenyl)-hepta-1,6-diene-3,5-dione=p,p-dihidroxydicinnamoylmethane (Chemical formula: $C_{19}H_{16}O_4$: C.A.S. number: 33171-05-0, Formula weight: 308)



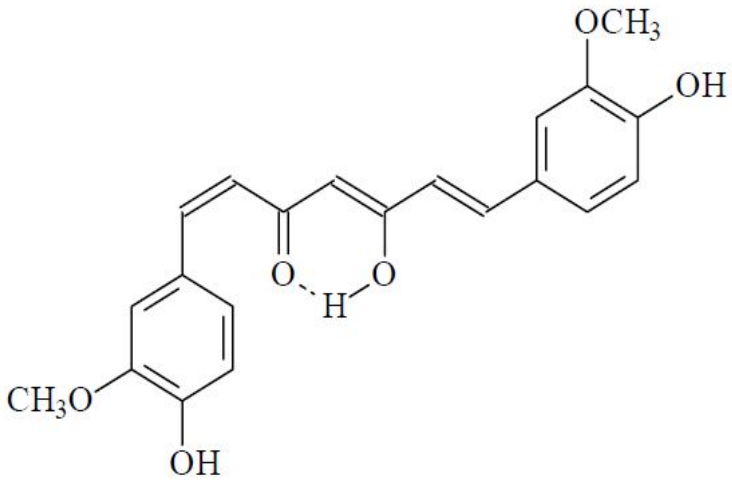
1) $R_1 = R_2 = OCH_3$

2) $R_1 = OCH_3$, $R_2 = H$

3) $R_1 = R_2 = H$

Figure 11: The three Principal Coloring Components of Curcumin

Besides these major constituents, three minor constituents can be isolated which are resumed to be the geometrical isomers of compounds 1-3, above. One of these is assumed to be thecis-trans geometrical isomer of compound 1 (which has the trans-trans configuration) based on its UV spectrum, lower melting point and lower stability in solutions and in the presence of light when compared to compound 1.[78]

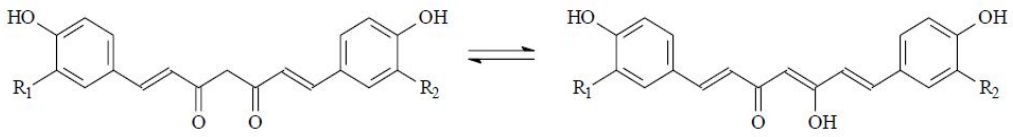


cis-trans geometrical isomer of Compound 1

Figure 12

Minor amounts of oils and resins naturally occurring in turmeric may be present in curcumin. The predominant constituents of these oils and resins appear to be sesquiterpene ketones and

alcohols: α-turmeron, β-turmeron, curlon, zingiberen, ar-turmeron, turmenorol A, turmeronol B etc. (Ohshiro and Kuroyanagi, 1990, Imai and Morikiyo, 1990, Majeed, et all, 2000).



Chemical and Technical Assessment Curcumin 61st JECFA4 (8)

Figure 13: Compunds 1-3 exhibit keto-enoltautomerism

1.12.4 Physico-chemical Properties:

Curcumin is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, and soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (e.g. sodium dodecyl sulfate, cetylpyridinium bromide, gelatin, polysaccharides, polyethylenglycol,cyclodextrins) have been reported[79]. In solutions the principal colouring1985a), products of decomposition at pH 7-10 were determined by HPLC. The initial degradation products are formed after 5 minutes and the chromatographic pattern obtained after 28 h at pH 8.5 is representative for alkaline degradation. Ferulic acid and feruloylmethane are formed initially. Feruloylmethane rapidly forms coloured (mostly yellow to brownish-yellow) condensation products. Degradation products formed by hydrolysis of feruolylmethane are vanillin and acetone and their amount increase with incubation time.

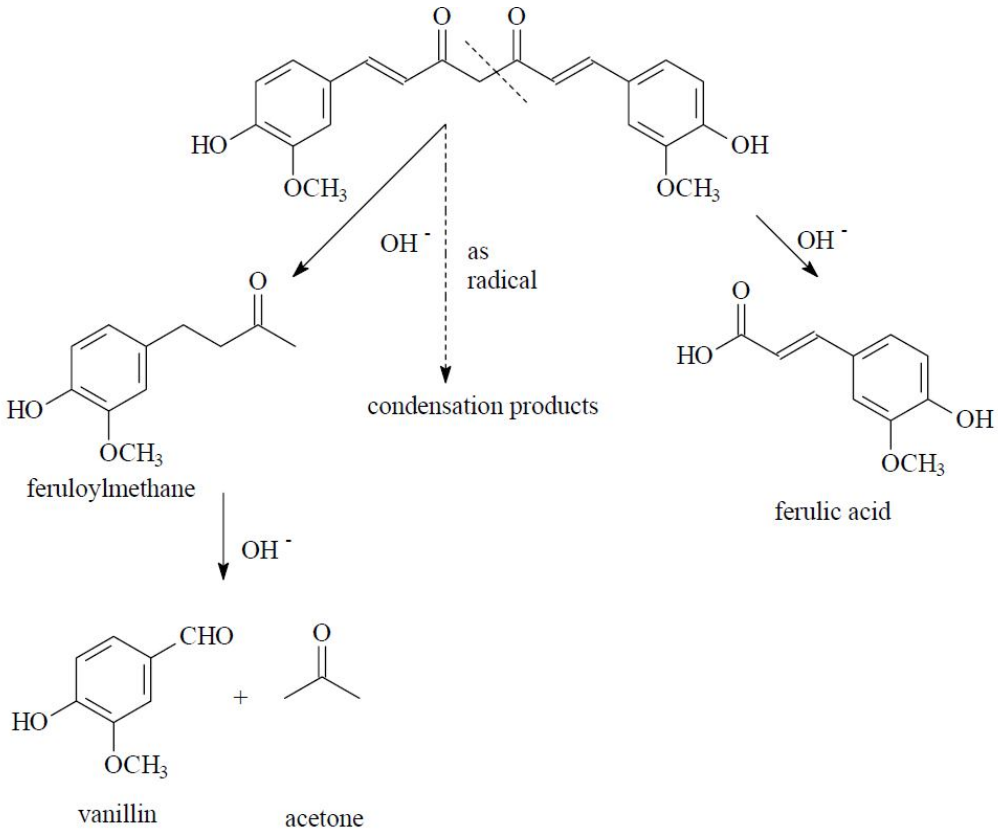


Figure 14: Physico-chemical of Curcumin

In another study [22] curcumin was incubated in 0.1 M phosphate buffer, pH 7.2 at 37 °C and 90 percent was decomposed within 30 min. trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal was predicted as major degradation product and vanillin, feruloyl methane were identified as minor degradation products.

The authors of another study observed that compound 3 was less susceptible to degradation at pH 10.2 than compound 1 or compound 2.

The objective of the present study can be summaries as this points :

- Curcumin extraction from turmeric.
- Characterize the yield using infrared red (IR), thin layer chromatography (TLC), melting point(M.P) , and also ash content.
- finally Applying curcumin as coloring agent in the pharmaceutical field in coating Metronidazole tablets.

Chapter II

Experimental

Chapter Two

Experimental

2.1 Preparation of Samples :

Samples were collected from Sudanese local market.

2.2 Glass Ware:

All glasses used were of class A.

2.3 Instruments :

Electronic Balance (Adam PW124), Reflux Condenser, Heating Mental(Sunbim), Water Bath(TORRE PICENARDI(CR)ITALY), Muffle Furnace(OSWORLD Model JRIC-12), Melting point, TLC Tank , Fourier Transform Infrared Spectroscopy (FT - I R) (SHIMADZU Model IRAffinity-1), Coating Machines, Disintegration Test(ERWEKA Model ZT3-2), Magnetic Stirrer, Colorimeter (KONICA MINOLTA model CR-410)

2.4 Chemicals:

All chemicals used were of analytical grade type, and it includes the following chemicals:

Dichloride methane(CH_2Cl_2), Hexane(C_6H_{14}), Potassium Bromide (KBr), Methanol (CH_3OH), Ethanol (C_2H_6O), Deionizer water.

2.5 Extraction Procedure of curcumin:

20 g of ground turmeric in 50 mL of dichloromethane was magnetically stirred and heated at reflux condenser for 1 h.
The mixture was suction-filtered1 and the filtrate was concentrated in a hot-water bath maintained at 50°C. The reddish yellow oily residue was treated with20 mL of hexane and the resulting solid was collected by suction filtration.[77]

The obtained yield was calculated as flow:

Wpr/WtheoX100

Where :

Wpr = Practical weight
Wtheo = Theoretical weight

2.6 Characterization of curcmin

2.6.1 Fourier Transform Infrared Spectroscopy (FT -I R):

2 mg of the curcumin was Triturated with 300 mg of dried potassium bromide *R*. These quantities were usually sufficient to given a suitable intensity of spectrum when use a disc with diameter (10-15 mm). Carefully the mixture was grinded, was spread it uniformly in a suitable die, and submitted to a pressure of about 800 MPa (8 $t \cdot cm^{-2}$). For substances that were unstable under normal atmospheric conditions, the disc had been pressed in vacuo. Several factors might be caused the formation of faulty discs, such as insufficient or excessive grinding, humidity or other impurities in the dispersion medium or an insufficient reduction of particle size. A disc was rejected if visual examination had been show lack of uniform transparency or when transmittance at about 2000 cm^{-1} (5 μm) in the absence of a specific absorption band was less than 60 per cent without compensation, unless otherwise prescribed.

Samples have been prepared by the same procedure and the spectrum was recorded the spectra between 4000- 400 cm^{-1} (2.5-15.4 μm) under the same operational conditions. The transmission minima (absorption maxima) in the spectrum was obtained with the substance to be examined correspond in position and relative size to those in the spectrum obtained with the reference substance [80].

2.6.2 Thin Layer Chromatography (TLC):

solvents system used of characterization of curcumin powder were prepared by mixed of (3% methanol and 97% dichloromethane).

The diameter of TLC plate was 10X20 cm.[77]

2.6.3 Melting point (M.P):

Sufficient quantity of curcumin had been introduced into a capillary tube to give a compact column 4 mm to 6 mm in height. The temperature of the bath was raised to bout 10 °C below the presumed melting point and then the rate of heating was adjusted to about 1 °C/min. When the temperature was reach to 5 °C below the presumed melting point, the capillary tube was introduce into the instrument. For the apparatus described above, the capillary tube was immersed so that the closed end is near the centre of the bulb of the thermometer, the immersion mark of which is at the level of the surface of the liquid. The temperature was recorded at which the last particle was passed into the liquid phase.[80]

2.6.4 Ash

A porcelain crucible was ignited at 600 ± 50 °C for 30 min, then was allowed to cooled in a desiccators over silica gel. The prescribed amount of the curcumin was placed in the crucible and weighed. Was gently heated at as low a temperature as practicable until the sample is thoroughly was charred. Until white fumes were no longer were evolved and ignited at 600 ± 50 °C until the residue was completely incinerated. The crucible was allowed to cool in a desiccators over silica gel, was weighed again and the percentage yield was calculated [80].

2.6.5 Percentage of Whiteness:

The samples powder was putted in dishes, distributed regularly highlighted and then the color was measured against white color standard and finally the percentage was recorded.

2.7 Coating of Tablets with Curcumin:

Į- Preparation of Solution :

25g of opadry white has been dissolved in 500 ml of ethanol with stirring during 30 min .then 1g curcmin powder was added during 10 min.

Įį- Operation of Machine:

Operating screen menu were opened. And Tablets were placed into coating chamber, then the main unit button were pressed .

The exhaust were operated until the powder totally removed from the tablets .after that the hot air were operated until the tablet temperature reached 50°C. The temperature control key were operated with a hot air in order to control the temperature during coating. Then the Spray was operated turn sprayers were opened until the tablets totally coated. And Spray was turn off after coating will the others keys were working until the tablets dried. The coating machine were stopped, the tablets were took out. [80]

Chapter Three

RESULTS& DISCUSSION

CHAPTER Three

RESULTS& DISCASSION

3.1 Yield Percentage of Extracted Curcumin:-

The obtained yields of curcumin for the different samples were shown in Table 3.1 below and the obtained compounds were finely soft clear yellow powder. The yield of samples were agreed with the method described by (Andrew et.al) [77], and the appearance of powder was clear as standard color.

Table 3.1: Percentage of Extracted samples.

Samples Number	1	2	3
Percentage	86.36%	81.81%	82.72%

The turmeric plant is identifiable by both its characteristic tuberous root and the leaves that extend upward from erect, thick stems arising from the root. Turmeric root is actually a fleshy oblong tuber 2–3 inch (5–10 cm) in length, and close to 1 inch (2.54 cm) wide. It is tapered at each end, and its exterior can be yellow, tan, or olive-green in color. The interior of the root is hard, firm, and either orange-brown or deeply rust-colored, with transverse resinous parallel rings. , in A Modern Herbal, states that the root is dense and breaks into a powder that is lemon yellow in color. Turmeric root has a fragrant aroma and a somewhat bitter, peppery, biting taste reminiscent of ginger. When eaten, it colors the saliva yellow and leaves a warm sensation in the mouth.

3.2 Description

The appearance of powder is reddish yellow and it was clear as standard color. Curcumin is a member of the *Curcuma* botanical group, which is part of the ginger family of herbs, the Zingiberaceae. Its botanical name is Curcuma longa. Turmeric is widely grown both as a kitchen spice and for its medicinal uses. Two closely related plants, Curcuma petolata and Curcuma roscoeana, are natives

of Cambodia and are grown for their decorative foliage and blossoms. All curcumas are perennial plants native to southern Asia.

3.3 IR Spectroscopy

Figures 15,16 and 17 shown the absorption of the three sample. These Figures contain the following; Broad band of three OH group at 3250-3500 cm^{-1}, sharp peak Stretch of C- H aliphatic system less than 3000cm^{-1}, weak peak of C-H aromatic system around 3050cm, shape peak of c═o at 1510 cm^{-1}, medium peak of c—o at 1300cm^{-1}, and sharp peak of c≃c of aromatic system at about 1510cm^{-1}.

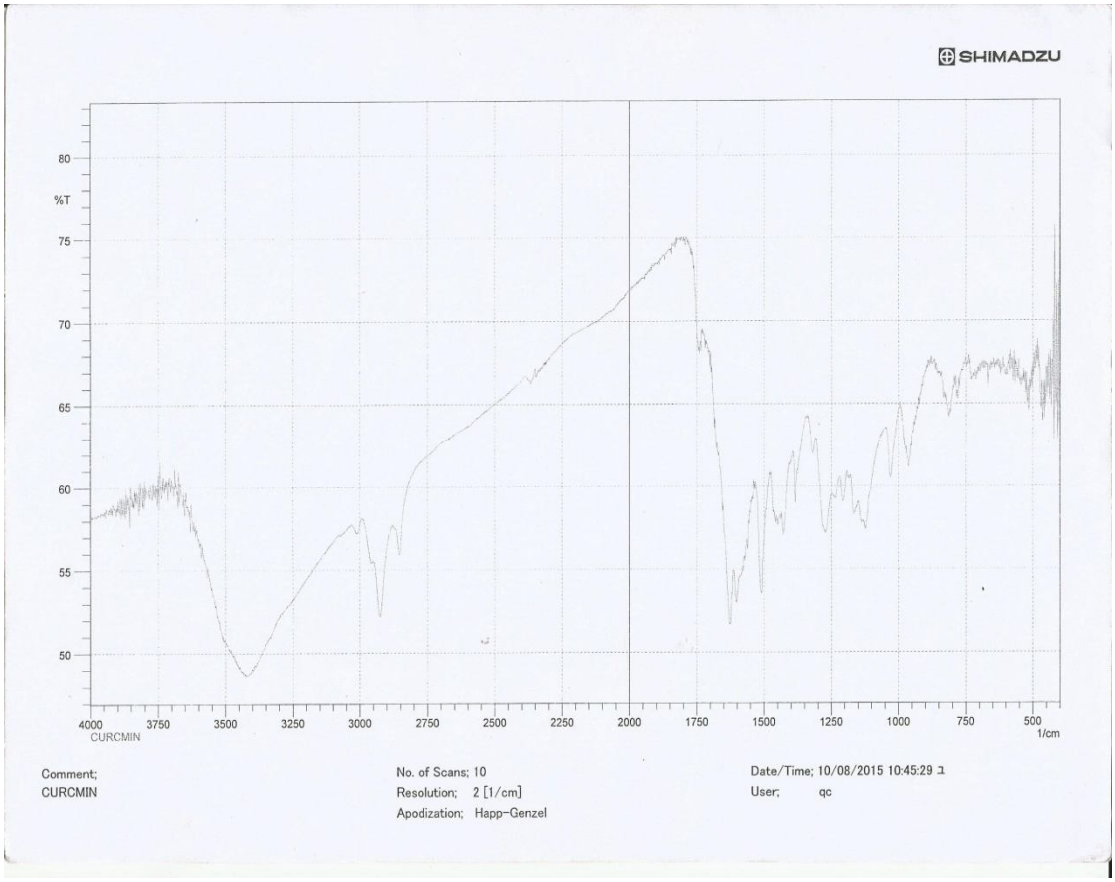


Figure 15: IR spectrum of curcmin sample 1

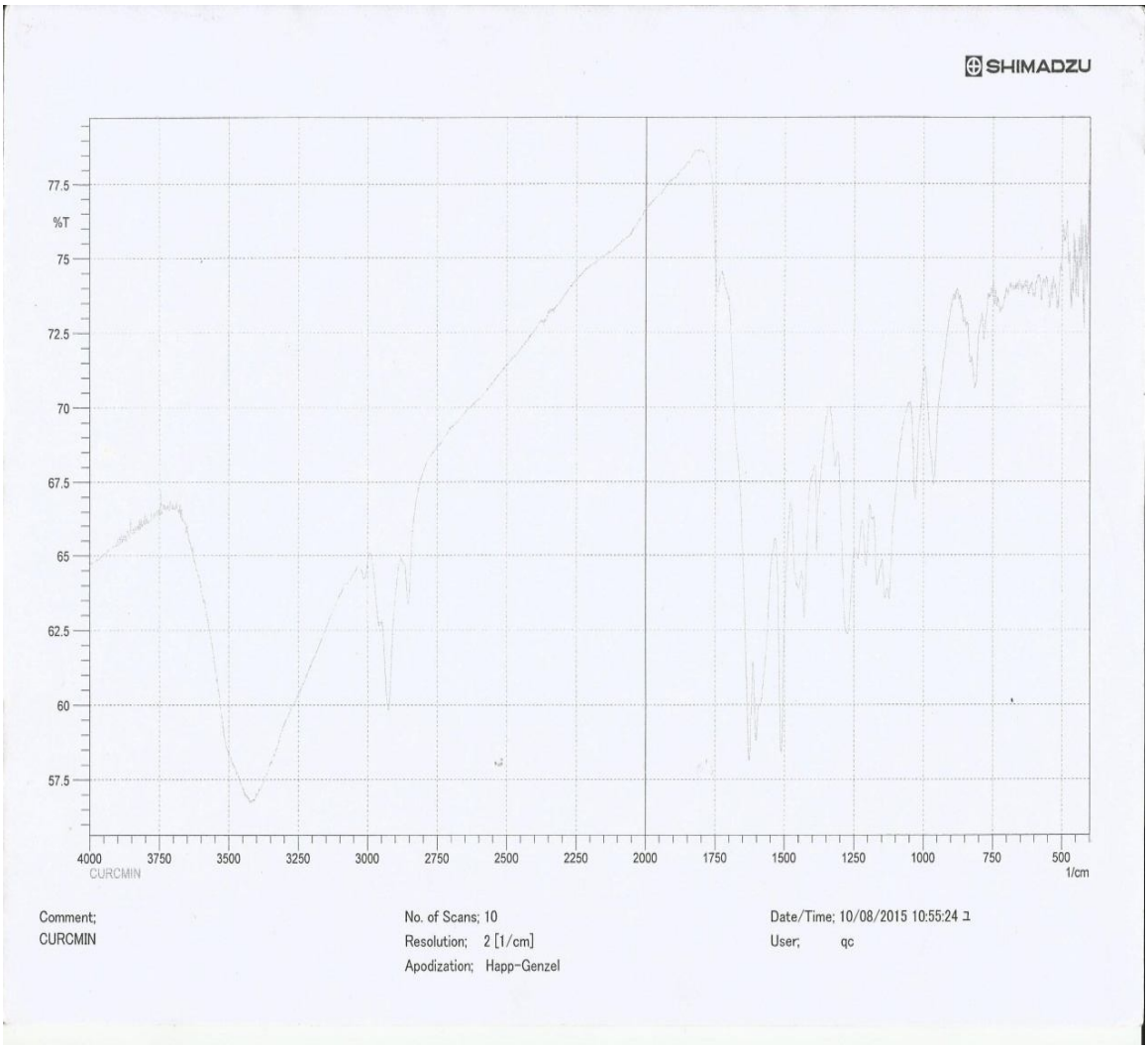


Figure 16: IR spectrum of curcmin sample 2

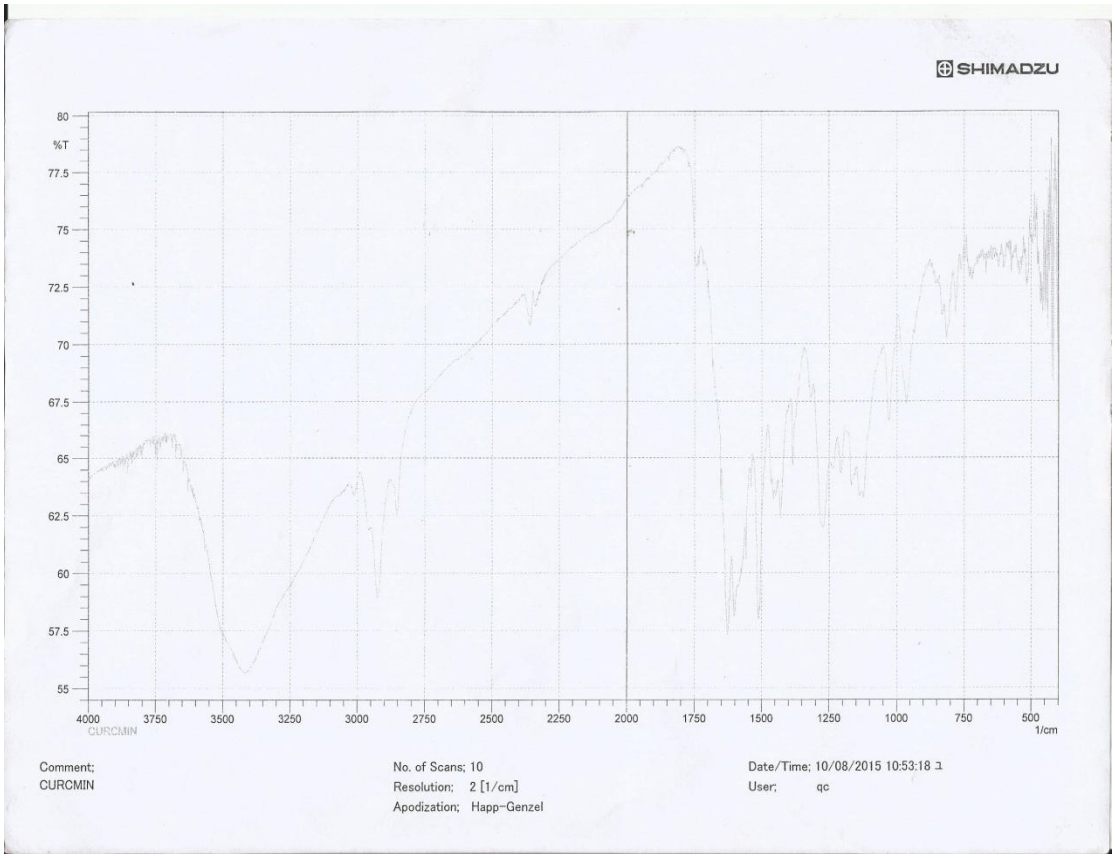


Figure 17: IR spectrum of curcmin sample 3

3.4 TLC Chromatography :

The TLC plate of the compound was shown in Figure 18. The obtained chromatogram showed that the curcmin was separated to three distinguished compound and it's clearly that result agreed with almost the previous studies (Andrew et.al). [77]

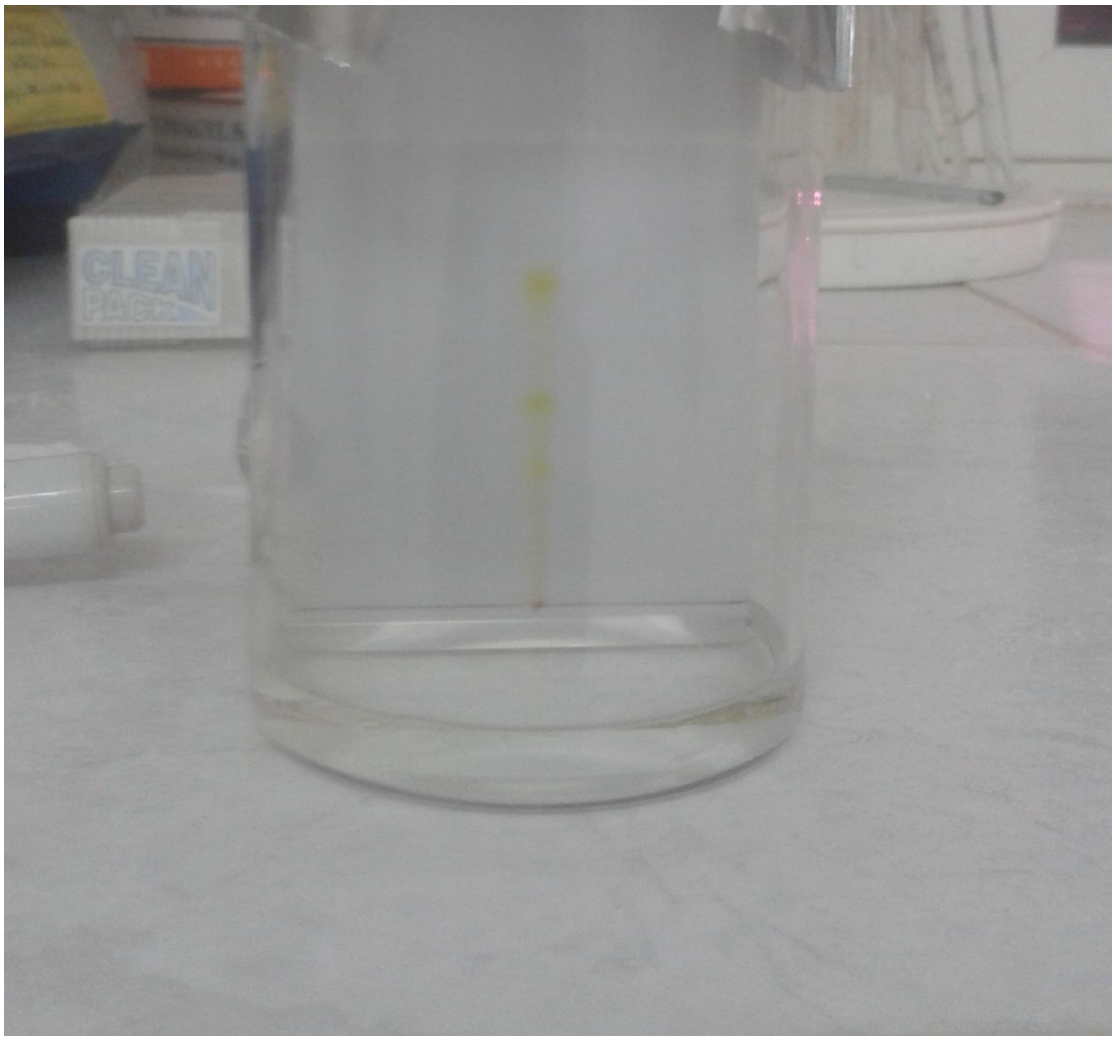


Figure 18: TLC of Curcmin sample

3.5 Melting Point:

The melting point determined by the capillary method and it is the temperature at which the last solid particle of a compact column of a substance in a tube passes into the liquid phase which shown in Table 3.2 of the three samples.

The melting point of curcumin is 183°C (BP2012) [80]. However, this result was in agreement with most of the results found in literature [80].

Table 3.2: Temperature of the three samples

Samples NO	**1**	**2**	**2**
Result	183°C	184°C	182°C

3.6 Ash :

The ash content obtained of the samples were shown in table 3.3

Table 3.3: Percentage of Ash of the three samples

Samples NO	**1**	**2**	**2**
Result	1.5%	3.09%	3.17%

In summary, from the three samples above, the percentage of Ash in curcumin was good [80].

3.7 Percentage of Whiteness :

The percentage of whiteness of the three samples obtained was shown in Table 3.3.

Table 3.4: Percentage whiteness

Samples NO	1	2	3
Result	52.04%	53.03%	51.08%

3.8 Physical Properties of Metronidazole Tablet

The physical properties of metronidazole tablets before coating were as the follow;

1. Average Weight=323.0mg
2. Disintegration time= 23 sec, and 22 sec.

However for the physical properties of metronidazole tablet after coating was found as the following:

1. Average Weight=324.74mg
2. Disintegration time= 120 sec, 93 sec, and 114 sec.

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums, gelatin, inactive and insoluble fillers, sugars, plasticisers, polyols, waxes, coloring matter authorised by the competent authority and sometimes flavoring substances and active substances. The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is a very thin polymeric coating, the tablets are known as film-coated tablets.

Coated tablets have a smooth surface, which is colored and may be polished; a broken section, when examined under a lens, shows a core surrounded by one or more continuous layers with a different texture.

The quality of this product is considered to be acceptable when used in accordance with the conditions. The yellow and the red dyes used in the film –coating are both ferric oxides and are generally acceptable dyes for use in the manufacture of medicinal. The color of curcumin is stable, used as food and more medical activates.

Coated tablets other than film-coated tablets comply with the test. Use purified water as the liquid. Add a disc to each tube. Operate the apparatus for 60 min, unless otherwise justified and authorized, and examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing purified water with 0.1 M hydrochloric acid.

Film-coated tablets comply with the disintegration test prescribed above except that the apparatus is operated for 30 min, unless otherwise justified and authorized.

Curcmin as color agent is more advance and safety because it is neutral material, stable, and in this application was disappeared the bitter taste of metronidazole.

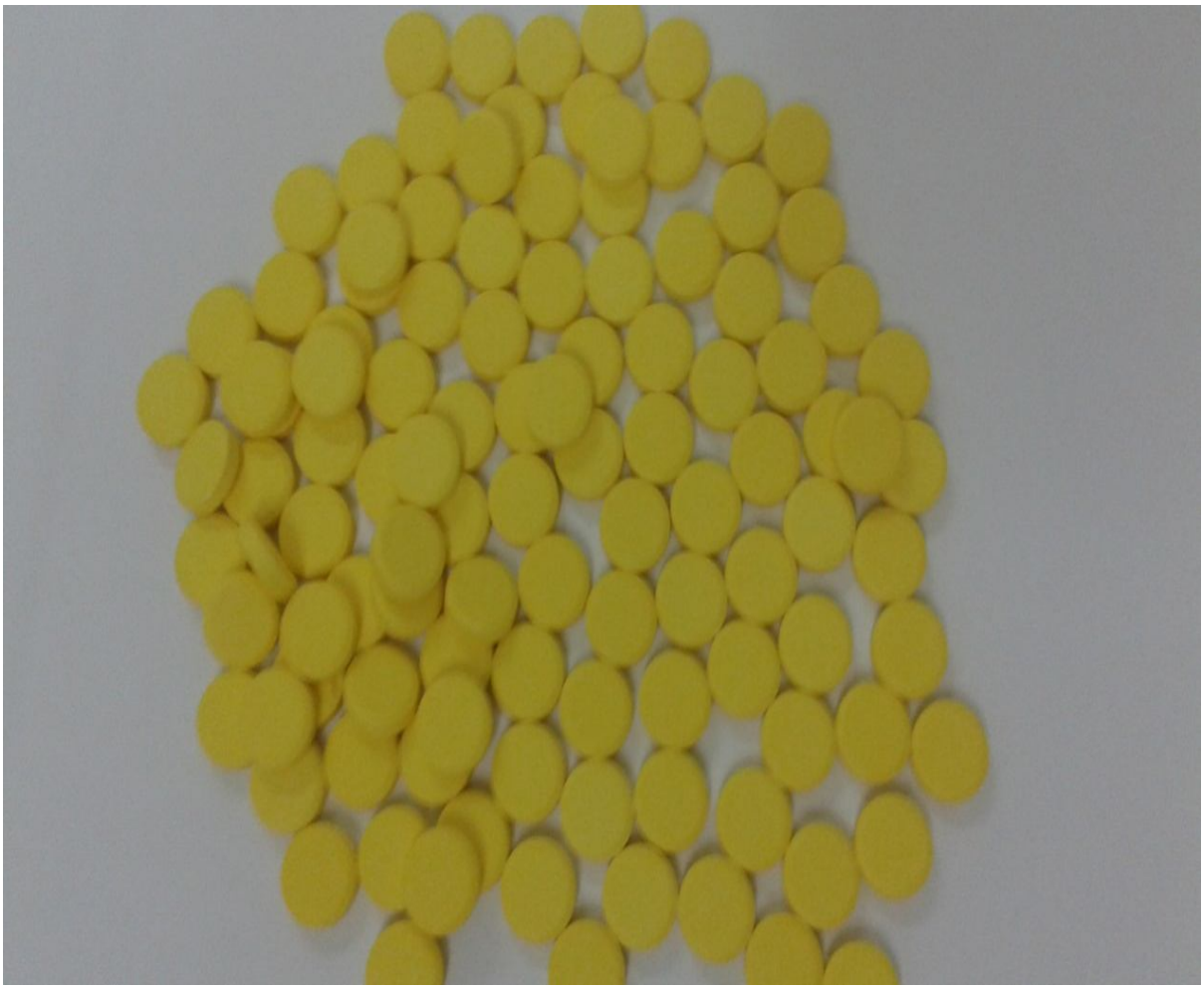


Figure 19: Metronidazole Tablet after Coating

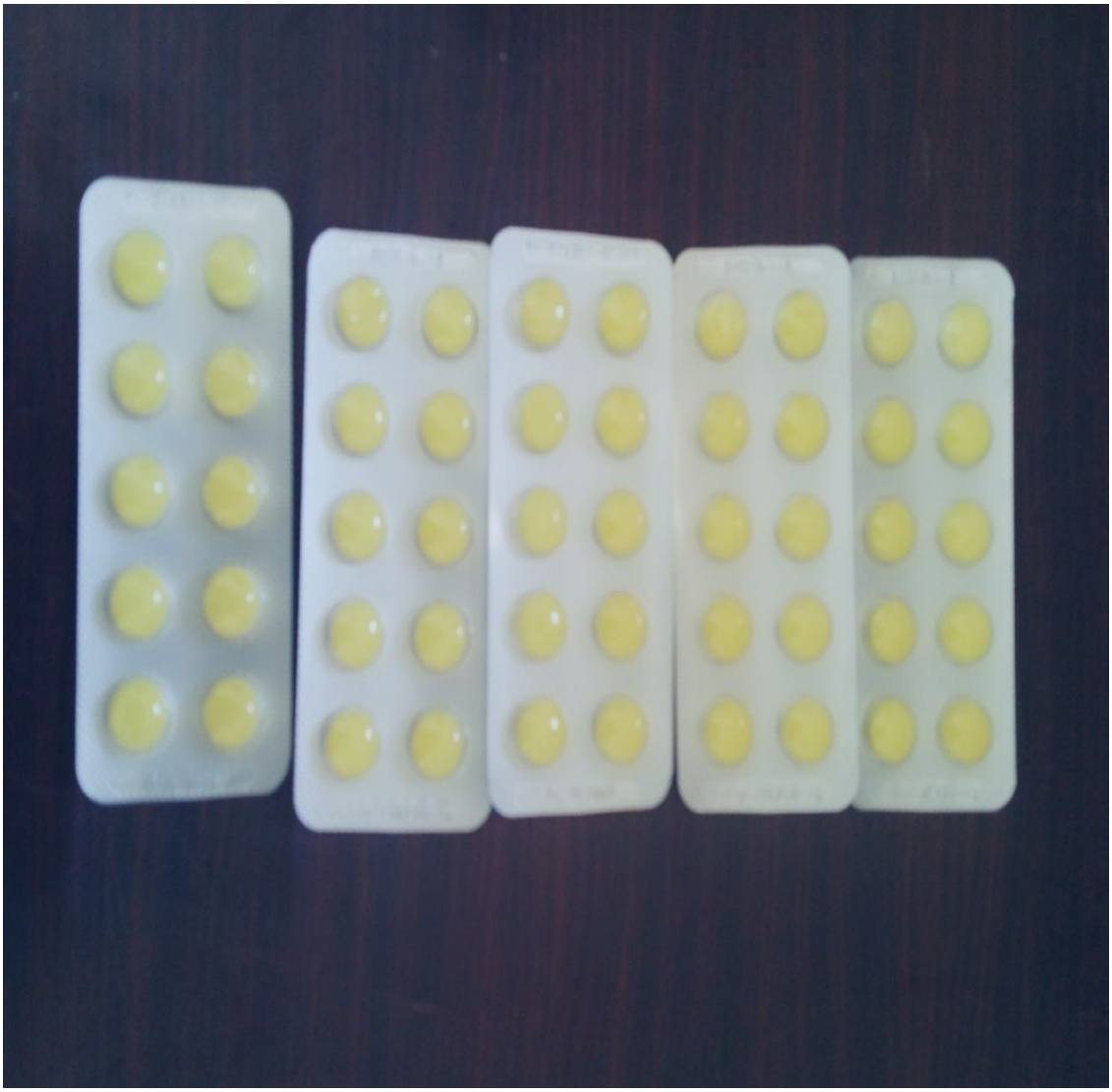


Figure 20: Metronidazole Tablet after Blistering

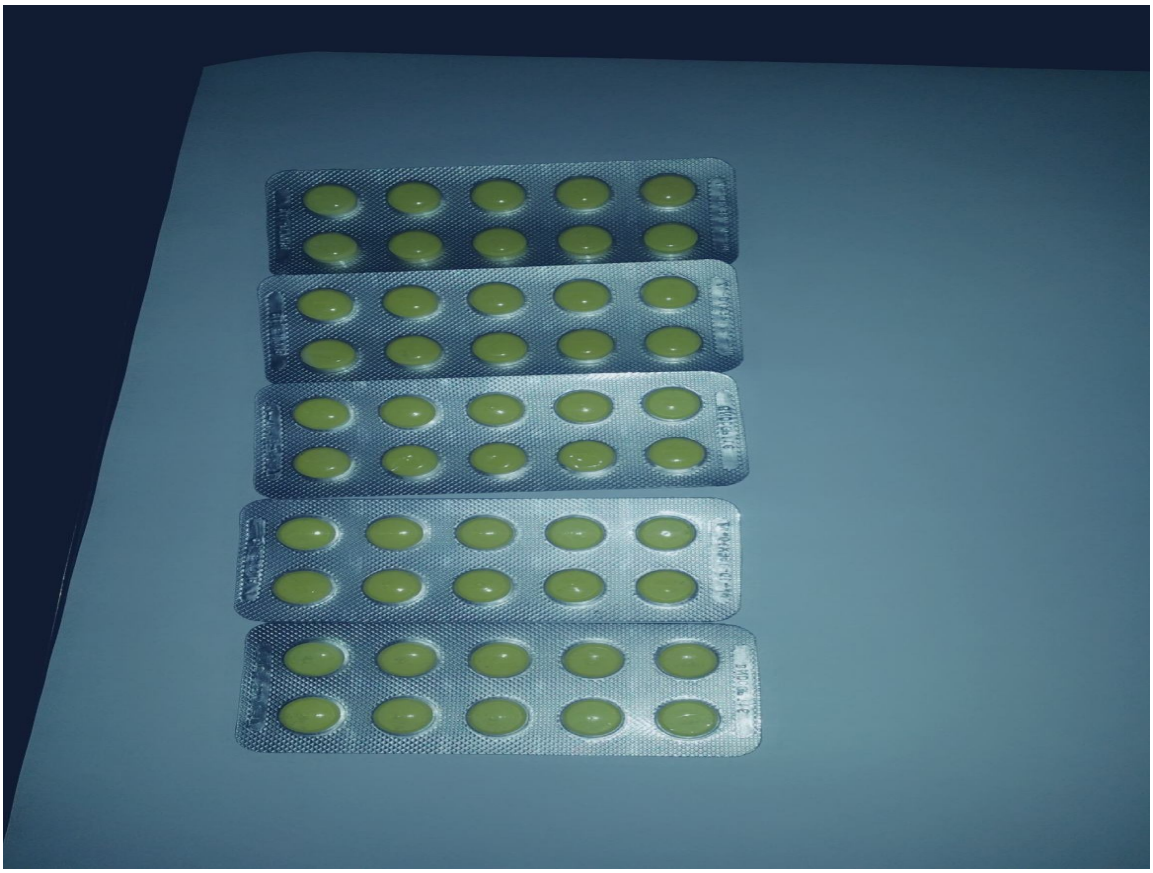


Figure 21: Metronidazole Tablet after Blistering

3.9 Conclusion:-

The primary purpose of the study in this dissertation is to look into the study of the isolation of curcumin from turmeric and also to characterize curcumin as color coating martial for metronidazole tablet in pharmaceutical. However, to achieve this goal solution of extraction methods was applied.

As it is know that curcumin has several medicinal characteristics, such as an anti-oxidant, anti-cancers, anti- inflammatory, and also has many benefits in cosmetics field. The turmeric plant is known in India and East Asia is medical tree.

In this research curcumin was extracted from turmeric in a simple and easy way by used dichloromethane and triturated with hexane and the yield of extraction was finely soft clear yellow powder.

In this research, curcumin material was used in the pharmaceutical field as a colorful agent that used in coating Metronidazole tablet and before that curcumin was characterized using IR Spectroscopy, TLC Chromatography, Melting Point, Percentage of Whiteness, and stability in high temperature during the coating .

The IR Spectroscopy were showed main group of curcumin and TLC Chromatography was showed three compound, and also The melting point of curcumin is 183°C. the result of samples were average 183°C, However, this result was agreed with the standard melting point of curcumin.

The experimental results showed that the coating Metronidazole tablets curcumin pills have no side effects.The experimental results of this research also showed stability of color even at high temperature .finally The taste of bitterness ratio in Metronidazole pills reduced or disappeared due to the impact of using curcumin material.

3.10 Recommendations :-

-We suggest to cultivate Curcmin trees in Sudan for its different medical benefits.

- To use curcumin as drugs.

- to use curcumin as color material in formula of drugs .

-To use curcumin as oxidizing agent with other ingredients which have side effects as an oxidants.

- Researching and developing studies about curcumin as drugs of anti-cancer.

Reference :

[1]-Indian Spice Board:http://www.indianspices.com

[2]- Robbins, P. **1995**. Tropical Commodities and their markets (A Guide and Directory), **229-230**.

[3]- Narayana, D.B.A., Brindavanam, N.B., Dobriyal, R.M., and Katuyar, K.C. **2000**. Indian Spices: An overview with special reference to neutraceuticals. *Journal of Medicinal andAromatic Plant Sciences*. **22 (1B):236-246**.

[4]- ASTA **2002**. A concise guide to Spices, Herbs, Seeds, and Extractives. American Spice Trade Association,**48-50**.

[5]- Dahal, K.R., and Idris, S. **1999**. Curcuma longa L. In : de Guzman, C.C. and Siemonsma,J.S. (Editors) : Plant Resources of South-East Asia No 13. Spices. Backhuys Publishers,Leiden, The Netherlands, **111-116**.

[6]- Weiss, E.A. **2002**. Spice Crops. CAB International publishing, Oxon, UK.

[7]- Govindarajan, V.S. **1980**. Turmeric - chemistry, technology, and quality. Crit. Rev. in FoodSci. & Nutr. T.E. Furia, Ed., CRC Press, Boca Raton, FL. **12:199-301.**

[8]-ASTA **2002**. A concise guide to Spices, Herbs, Seeds, and Extractives. American Spice Trade Association, **48-50**.

[9]- Aggarwal, B. B., & Harikumar, K. B. (**2009).** Potential therapeutic effects of

curcumin, the anti-inflammatory agent, against neurodegenerative,

cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases.

The International Journal of Biochemistry & Cell Biology, ***41*****(1), 40–59**.

[10]- Leong-Skornicková, J., Sída, O., et al. (**2007**). Chromosome numbers and
genome size variation in Indian species of Curcuma (Zingiberaceae). *Annals*
of Botany, ***100*****(3), 505–26**.
[11]- Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (**2008**). Curcumin as
"Curecumin": From kitchen to clinic. *Biochemical Pharmacology*, ***75*****(4),**
787–
809.
[12]- Chandran, B., & Goel, A. (**2012**). A randomized, pilot study to assess the efficacy and safety of curcumin in patients with active rheumatoid arthritis.
Phytotherapy Research : PTR, ***26*****(11), 1719–25**.
[13]- Balasubramanian, K. (**2006**). Molecular Orbital Basis for Yellow Curry SpiceCurcumin's Prevention of Alzheimer's Disease. *Journal of Agricultural and*
Food Chemistry, ***54*****(10), 3512–3520**.
[14]-Grynkiewicz, G., & Ślifirski, P. (**2012**). Curcumin and curcuminoids in quest for medicinal status. *Acta Biochimica Polonica*, ***59*****(2), 201–12.**
[15]-Chignell, C. F., Bilskj, P., et al. (**1994**). Spectral and photochemical properties of curcumin. *Photochemistry and Photobiology*, ***59*****(3), 295–**
302.
[16]- Sun, Y., Wang, R., Yuan, S., Lin, X., & Liu, C. (**2010**). Theoretical study on the antioxidant activity of curcumin. *Chinese Journal of Chemistry*, ***22*****(8), 827–**
[17]- Sun, Y., Zhang, H., Chen, D., & Liu, C. (**2002**). Theoretical Elucidation on the Antioxidant Mechanism of Curcumin: A DFT Study. *Organic Letters*, ***4*****(17),**

2909–2911.

[19]- Litwinienko, G., & Ingold, K. U. (**2004**). Abnormal solvent effects on hydrogen atom abstraction. 2. Resolution of the curcumin antioxidant controversy. The role of sequential proton loss electron transfer. *The Journal of Organic*

Chemistry, ***69*(18), 5888–96**.

[20]- Strimpakos, A. S., & Sharma, R. A. **(2008**). Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxidants & Redox Signaling*, ***4*(14), 2909–2911**.

[21]-Griesser, M., Pistis, V., et al. (**2011**). Autoxidative and Cyclooxygenase-2

Catalyzed Transformation of the Dietary Chemopreventive Agent Curcumin.

Journal of Biological Chemistry, ***286*(2), 1114–1124.**

[22]-Wang, Y., Pan, M., Cheng, A. et al. (**1997**). Stability of curcumin in buffer

solutions and characterization of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis*, ***15*(12), 1867–1876**.

[23]- Pandya, A., Goswami, H., et al. (**2012**). A novel nanoaggregation detection

technique of TNT using selective and ultrasensitive nanocurcumin as a probe. *The Analyst*, ***137*(8), 1771–4**.

[24]-Jacob, A., Wu, R., Zhou, M., & Wang, P. (**2007**). Mechanism of the Antiinflammatory Effect of Curcumin: PPAR-gamma Activation. *PPAR Research*,

***2007*, 89369.**

[25]-Sanphui, P., Goud, N. R., et al. (**2011**). New polymorphs of curcumin. *ChemicalCommunications, **47*(17),**

[26]-Wilken, R.,Veena, M. S.,Wang, M. B., & Srivatsan, E. S. (**2011**). Curcumin: Areview of anti-cancer properties and therapeutic activity in head and neck
squamous cell carcinoma. *Molecular Cancer*, ***10*, 12.**
[27]- Weisberg, S. P., Leibel, R., & Tortoriello, D. V. (**2008**). Dietary curcumin
significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. *Endocrinology*, ***149*(7), 3549–58**.
[28]-Weber, W. M., Hunsaker, L. A. et al. (**2006**). Activation of NFkB is inhibited by curcumin and related enones. *Bioorganic Medicinal Chemistry*, ***14*(7), 2450–**
2461.
[29]-Hong, D., Zeng, X., et al. (**2010**). Altered profiles of gene expression in curcumintreated rats with experimentally induced myocardial infarction.
Pharmacological Research, ***61*(2), 142–8.**
[30]- Chanpoo, M., Petchpiboonthai, H., et al. (**2010**). Effect of curcumin in the
amelioration of pancreatic islets in streptozotocin-induced diabetic mice.
Journal of the Medical Association of Thailand, **S152–9.**
[31]-Quitschke, W. W., Steinhauff, N., & Rooney, J. (**2013**). The effect of cyclodextrinsolubilized curcuminoids on amyloid plaques in Alzheimer transgenic mice: brain uptake and metabolism after intravenous and subcutaneous injection.
Alzheimer's Research & Therapy, ***5*(2), 16**.
[32]-Suzuki, M., Betsuyaku, T., et al. (**2009**). Curcumin attenuates elastase- and
cigarette smoke-induced pulmonary emphysema in mice. *American Journal*

of Physiology. Lung Cellular and Molecular Physiology, ***296*(4), L614–23**.

[33]-Valacchi, G., Pagnin, E. et al (**2004**). Inhibition of NFkappaB activation and IL-8 expression in human bronchial epithelial cells by acrolein. *Antioxidants &*

Redox Signaling, *7***(1-2), 25–31**.

[34]-Marczylo, T. H., Verschoyle, R. D., et al. (**2007**). Comparison of systemic

availability of curcumin with that of curcumin formulated with

phosphatidylcholine. *Cancer Chemotherapy and Pharmacology*, ***60*(2), 171–7**.

[35]-Liu, Y., Yang, Z., et al. (**2008**). Interaction of curcumin with intravenous

immunoglobulin: a fluorescence quenching and Fourier transformation

infrared spectroscopy study. *Immunobiology*, ***213*(8), 651–61**.

[36]- Chen, Y., Shu, W., et al. (**2007**). Curcumin, both histone deacetylase and

p300/CBP-specific inhibitor, represses the activity of nuclear factor kappa B

and Notch 1 in Raji cells. Basic & Clinical Pharmacology & Toxicology, **101(6), 427–33**.

[37]- Sinha, R., Anderson, D. E., McDonald, S. S., & Greenwald, P. (**2003**). Cancer risk and diet in India. *Journal of Postgraduate Medicine*, ***49*(3), 222–8**.

[38]- Singh, S., & Khar, A. (**2006**). Biological effects of curcumin and its role in cancer chemoprevention and therapy. *Anticancer Agents in Medicinal Chemistry*,

***6*(3), 259–270**.

[39]-Singh, M., Ramos, I., Asafu-Adjei, D., et al. (**2013**). Curcumin improves the
therapeutic efficacy of Listeria(at)-Mage-b vaccine in correlation with improved T-cell responses in blood of a triple-negative breast cancer model
4T1. *Cancer Medicine*, ***2*****(4), 571–82**.
[40]-Ranjan, A. P., Mukerjee, A., et al. (**2013**). Efficacy of Liposomal Curcumin in a Human Pancreatic Tumor Xenograft Model: Inhibition of Tumor Growth and
Angiogenesis. *Anticancer Res*, ***33*****(9), 3603–3609**.
[41]-Perry, M., Demeule, M., et al. (**2010**). Curcumin inhibits tumor growth and
angiogenesis in glioblastoma xenografts. *Molecular Nutrition & Food Research*, ***54*****(8), 1192–201**.
[42]-Johnson, J. J., & Mukhtar, H. (**2007**). Curcumin for chemoprevention of colon
cancer. *Cancer Letters*, ***255*****(2), 170–181**.
[43]-Mahmoud, N. N., Carothers, A. M., et al. (**2000**). Plant phenolics decrease
intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis*, ***21*****(5), 921–7**.
[44]-Huang, M. T., Newmark, H. L., & Frenkel, K. (**1997**). Inhibitory effects of
curcumin on tumorigenesis in mice. *Journal of Cellular Biochemistry. Supplement*, ***27*****, 26–34**.
[45]-Huang, M. T., Lou, Y. R., et al. (**1998**). Effect of dietary curcumin and
dibenzoylmethane on formation of 7,12-dimethylbenz[a]anthracene-induced

mammary tumors and lymphomas/leukemias in Sencar mice.
Carcinogenesis, ***19*****(9), 1697–700**.
[46]-Aoki, H., Takada, Y., et al. (**2007**). Evidence that curcumin suppresses the
growth of malignant gliomas in vitro and in vivo through induction of autophagy: role of Akt and extracellular signal-regulated kinase signaling pathways. *Molecular Pharmacology*, ***72*****(1), 29–39**.
[47]- Li, Z., Ouyang, K., et al. (**2009**). Curcumin induces apoptosis and inhibits growth of human Burkitt's lymphoma in xenograft mouse model. *Molecules and*
Cells, ***27*****(3), 283–9**.
[48]-Olszanecki, R., Jawień, J., et al. (**2005**). Effect of curcumin on atherosclerosis in apoE/LDLR-double knockout mice. *Journal of Physiology and*
Pharmacology, ***56*****(4), 627–35**.
[49]-Zhao, X., C. Wang, et al. (**2013**). "Chronic curcumin treatment normalizes
depression-like behaviors in mice with mononeuropathy: involvement of supraspinal serotonergic system and GABAA receptor."
Psychopharmacology: **1-17**.
[50]- Hishikawa, N., Takahashi, Y., et al. (**2012**). Effects of turmeric on Alzheimer's disease with behavioral and psychological symptoms of dementia. *Ayu*, ***33*****(4), 499–504**.
[51]-(Ahmed & Gilani, 2009),
[52]-Belcaro, G., Cesarone, M. R., et al. (**2010**). Efficacy and safety of Meriva®, a
curcumin-phosphatidylcholine complex, during extended administration in
osteoarthritis patients. *Alternative Medicine Review*, ***15*****(4), 337–44**.

[53]-Hanai, H., Iida, T., et al. (**2006**). Curcumin maintenance therapy for ulcerative
colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clinical Gastroenterology and Hepatology, **4*****(12), 1502–6**.
[54]-Chuengsamarn, S., Rattanamongkolgul, S., et al. (**2014**). Reduction of
atherogenic risk in patients with type 2 diabetes by curcuminoid extract: a randomized controlled trial. *The Journal of Nutritional Biochemistry*, ***25*****(2),**
144–50.
[55]-He, Z., Shi, C., et al. (**2011**). Upregulation of p53 expression in patients with
colorectal cancer by administration of curcumin. *Cancer Investigation*, ***29*****(3),**
208–13.
[56]- Jurrmann, N., Brigelius-Flohe, R., & Bol, G. (**2005**). Curcumin Blocks Interleukin-1 (IL-1) Signaling by Inhibiting the Recruitment of the IL-1 Receptor-
Associated Kinase IRAK in Murine Thymoma EL-4 Cells. *J. Nutr.*, ***135*****(8),**
1859–1864.
[57]-Al-Amiery, A. A., Kadhum, A. A. H., et al. (**2013**). Synthesis and antioxidant
activities of novel 5-chlorocurcumin, complemented by semiempirical calculations. *Bioinorganic Chemistry and Applications*, ***2013*****, 354982.**
[58]- Barzegar, A., & Moosavi-Movahedi, A. A. (**2011**). Intracellular ROS protection efficiency and free radical-scavenging activity of curcumin. *PloS One*, ***6*****(10),e26012**.

[59]-Jat, D., Parihar, P., et al.. (**2013).** Curcumin reduces oxidative damage by
increasing reduced glutathione and preventing membrane permeability transition in isolated brain mitochondria. *Cellular and Molecular Biology, 59*
Suppl, OL**1899–905**.
[60]-Barclay, L. R., Vinqvist, M. R., et al. (**2000**). On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant
mechanism and activity. *Organic Letters*, ***2*****(18), 2841–3.**
[61]- Priyadarsini, K. I., Maity, D. K., et al. (**2003**). Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. *Free Radical Biology & Medicine*, ***35*****(5), 475–84.**
[62]- Gordon, O. N., & Schneider, C. (**2012**). Vanillin and ferulic acid: not the major degradation products of curcumin. *Trends in Molecular Medicine*, ***18*****(7),**
361–363.
[63]- Nafisi, S., Adelzadeh, M., Norouzi, Z., & Sarbolouki, M. N. (**2009**). Curcumin binding to DNA and RNA. *DNA and Cell Biology*, ***28*****(4), 201–8.**
[64]-Ravindran, J., Prasad, S., & Aggarwal, B. B. (**2009**). Curcumin and cancer cells: how many ways can curry kill tumor cells selectively? *AAPS J*, ***11*****(3), 495–**
510.
[65]-Awasthi, S., Pandya, U., et al. (**2000**). Curcumin–glutathione interactions and the role of human glutathione S-transferase P1-1. *Chemico-Biological*
Interactions, ***128*****(1), 19–38**.

[66]-Heger, M., van Golen, R. F., et al. (**2014**). The molecular basis for the
pharmacokinetics and pharmacodynamics of curcumin and its metabolites in
relation to cancer. *Pharmacological Reviews*, ***66*****(1), 222–307.**
[67]-Selvam, C., Jachak, S. M., Thilagavathi, R., & Chakraborti, A. K. (**2005**). Design, synthesis, biological evaluation and molecular docking of curcumin
analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory
agents. *Bioorganic & Medicinal Chemistry Letters*, ***15*****(7), 1793–7**.
[68]-Bera, R., Sahoo, B. K., Ghosh, K. S., & Dasgupta, S. (**2008**). Studies on the
interaction of isoxazolcurcumin with calf thymus DNA. *International Journal
of Biological Macromolecules*, ***42*****(1), 14–21**.
[69]- Ketterer, B. (**1988**). Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. *Mutation Research*, ***202*****(2), 343–361**.
[70]- Holder, G. M., Plummer, J. L., & Ryan, A. J. (**1978**). The Metabolism and
Excretion of Curcumin (1,7-Bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in the Rat. *Xenobiotica*, ***8*****(12), 761–768**.
[71]-Wahlström, B., & Blennow, G. (**1978**). A study on the fate of curcumin in the rat. *Acta Pharmacologica et Toxicologica*, ***43*****(2), 86–92**.
[72]-Hoehle, S. I., Pfeiffer, E., Sólyom, A. M., & Metzler, M. (**2006**). Metabolism of curcuminoids in tissue slices and subcellular fractions from rat liver. *Journal
of Agricultural and Food Chemistry*, ***54*****(3), 756–64**.

[73]- Pan, M., Huang, T., & Lin, J. (**1999**). Biotransformation of Curcumin Through Reduction and Glucuronidation in Mice. *Drug Metab. Dispos.*, ***27*****(4), 486–494**.

[74]-Strimpakos, A. S., & Sharma, R. A. (**2008**). Curcumin: preventive and therapeuticproperties in laboratory studies and clinical trials. *Antioxidants & Redox*
Signaling, ***4*****(14), 2909–2911**.

[75]- Zebib, B., Mouloungui, Z., & Noirot, V. (**2010**). Stabilization of curcumin by
complexation with divalent cations in glycerol/water system. *Bioinorganic*
Chemistry and Applications, **292760**.

[76]- European Food Saftey Authority (EFSA), Parma, Italy EFSA Jouranal **2010**; **8(9) : 1676**

[77]- Andrew M. Anderson, Matthew S. Mitchell,and Ram S.Mohan(**2000**)Journal of Chemical Education . Vol .77. NO.3 March 2000 JChemEd.chem.wisc.edu

[78]-Srinivasan, K.R. **1952**. The colouring matter in turmeric. *Current Science*, **311-313**

[79]-Humphrey, A.M. **1980**. Chlorophyll. *Food. Chem.* ***5: 57***

[80]- British Pharmacopoeia **2012**