Review on Analytical Methodologies, Chemical and Therapeutic Perspectives of Curcumin: A Ubiquitous Natural Molecule

Umang Shah[1*], Deep Patel[1], Drashti Dalsania[1], Mehul Patel[1], Meghana Patel[1], Ashish Patel[1], Nilay Solanki[1], Swayamprakash Patel[1], Samir Patel[1]

[1]*Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology (CHARUSAT), CHARUSAT Campus, Changa 388421, India*

*Corresponding Author
Umang Shah
Department of Pharmaceutical Chemistry
Ramanbhai Patel College of Pharmacy
Charotar University of Science and Technology
Email: umangshah.ph@gmail.com
Mob: +91-9537554414

Abstract

Curcumin is a lipophilic polyphenolic yellow compound extracted from *Curcuma longa* L. (turmeric) rhizome with a broad spectrum of pharmacological and medicinal properties as propounded by several *in vivo, in vitro* and clinical studies**.** Considerable research over the past century has been extensively studied on chemical, biological, and analytical perspectives of curcumin. Nowadays, curcumin is widely used in food and pharmaceutical formulation due to excellent health benefit. Therefore, characterization and quantification of curcuminoids in nutraceuticals and pharmaceuticals are required to measure their quality control parameters to address issues related to processing and storage. This review article precis current exploration on analytical methodologies used to extract and quantify curcuminoids in different matrices.

Moreover, this review offers phytochemistry, synthetic and biosynthetic pathways, extraction methodologies, degradation and metabolism pathways. and health benefits of the curcumin, scurrying from the kitchen shelf toward the clinic.

Keywords: Curcumin, Turmeric, Analytical methods, Phytochemistry, Biological activity

1.INTRODUCTION

Turmeric's main component, curcumin, has been used for medicinal purposes since ancient times. The discovery of curcumin dates to around two centuries ago when Vogel and Pelletier reported the isolation of yellow coloring matter from the rhizomes of *Curcuma longa* and named it curcumin, and its structure was determined in 1910. [1, 2] Three major curcuminoids such as curcumin (60–70%), dimethoxy curcumin (20–27%), and bisdemethoxycurcumin (10–15%) are present in the extract of turmeric. Curcumin has two aromatic o-methoxy phenolic groups, a β-dicarbonyl moiety and a seven-carbon linker containing two enone moieties.[2] Curcumin belongs to the family of Zingiberaceae family.[1] It is cultivated in India, Indonesia, China, Thailand, and other tropical regions, including Africa. [3]

Curcumins showed anticancer effects[4], anti-inflammatory effects[5], antidiabetic [6], anti-arthritis[7], and antioxidant effects[8]. They were also protective against multiple sclerosis[9], inflammatory bowel disease[10], cystic fibrosis[11], and alzheimer's[12]. Also showed results against tropical pancreatitis, peptic and gastric ulcer, oral lichen planus, gastric inflammation, idiopathic orbital inflammatory pseudotumor, psoriasis, vitiligo, atherosclerosis, acute coronary syndrome, diabetic nephropathy, diabetic microangiopathy, diabetes, lupus nephritis, acquired immunodeficiency syndrome, renal conditions, β-thalassemia, Dejerine-Sottas disease, biliary dyskinesia, cholecystitis, and chronic bacterial prostatitis. [2, 13]

Another essential property of curcumin is that no severe toxicity has been reported despite being consumed for several years in Asian countries [14]. In combination with piperine, curcumin increases oral bioavailability in humans and effectively targets breast cancer stem cells. [15–17]

2. PHYTOCHEMISTRY OF CURCUMIN

Curcumin was isolated from turmeric for the first time in 1815. Only a few reports on its chemistry, structure, synthesis, and biochemical activity had been published prior to 1970. Following that, some research on curcumin's antioxidant properties was published. Curcumin research has now drawn researchers from all branches of chemistry, including analytical, inorganic, organic, and physical chemists.[18] Curcumin is a polyphenol extracted from the rhizome of the plant turmeric.[19] Commercially marketed curcumin (turmeric extracts), a mixture of three curcuminoids containing 77% pure curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin.[20] IUPAC name of Curcumin is (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), with a molecular formula of $C_{21}H_{20}O_6$ and molecular weight of 368.38 g/mol.[1, 21]The melting point for curcumin at 182-184^{o}C.[22]

Curcumin is freely soluble in organic solvents such as methanol, ethanol, acetone, and chloroform due to its lipophilic nature with a log P of 3.0, but practically insoluble in water and ether.[23, 24] Also, in the presence of light and aqueous media, curcumin suffers rapid degradation due to its extreme lipophilic nature. [21]

All three curcuminoids vary in chemical structures, as illustrated in Fig. **1**. So, it is possible to have altered chemical, functional, and color characteristics. Pure curcumin is expensive and scarce, whereas desmethoxycurcumin and bisdemethoxycurcumin are not commercially available.[25]

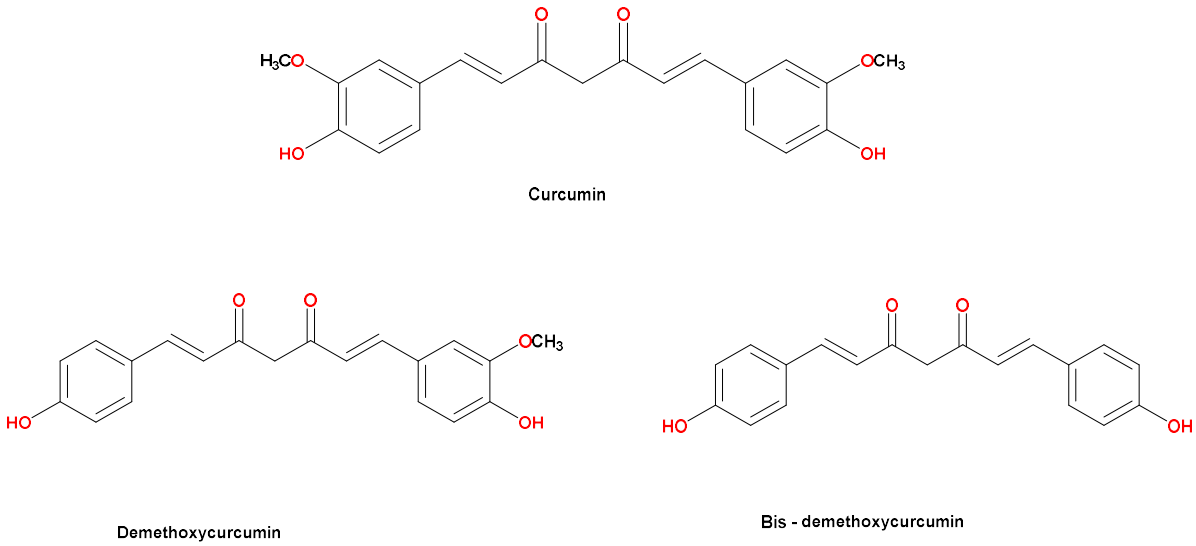

Fig.(1). Chemical structures of curcuminoids

Curcumin has two tautomeric forms, keto, and enol tautomers, shown in Fig. **2**. [23]

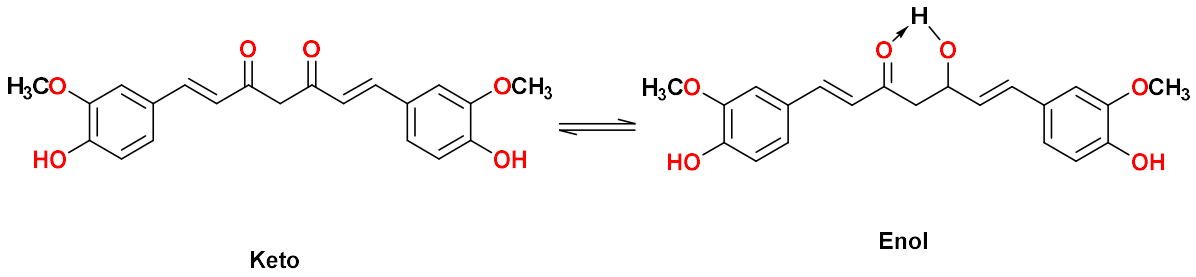

Fig.(2). Keto-enol tautomers of curcumin

3. ANALYTICAL PROFILING OF CURCUMIN

As curcumin shows incredible pharmacological action, its qualitative and quantitative determination becomes more critical. Literature review (LR) reveals that there are many methods developed for such determination of curcumin. For instance, spectroscopic methods like UV Visible spectrophotometry[26–28] and Fluorescence spectroscopy[29, 30], and chromatographic methods like High-performance liquid chromatography (HPLC)[31–40], Liquid Chromatography with tandem mass spectrometry (LC-MS/MS)[35, 39], Gas chromatography-mass spectrometry (GC-MS)[37], High-speed counter-current chromatography (HSCCC)[35] , High-performance Thin Layer Chromatography (HPTLC)[41], Thin Layer Chromatography (TLC)[21, 36, 42] and Ultra-performance liquid chromatography (UPLC)[43].

UV spectroscopy requires alcohol (either methanol or ethanol) as a solvent to dissolve curcumin API in spectroscopic techniques. The majority of developed methods have used linearity range between 2 to 10 µg/ml. Although it contains higher numbers of chromophoric groups, it has a detection wavelength of more than 420 nm[26–28]. As Curcumin is fluorescent active compound fluorometric methods for its quantification was also possible. By doing LR, it was found that either curcumin dissolved in alcohol or human serum containing curcumin was used for detection. When detected in human serum, detection wavelength was kept 298 nm[29]; otherwise, more than 420 was observed (Table 1).

For separation and identification, chromatographic methods exhibit the most success. For example, curcumin can be separated and identified using chromatographic techniques. HPLC is one of those. Powder of turmeric, extracts, blood plasma was used to separate curcumin with a different column as stationary phase, among which C_{18} was frequently used column. A mobile phase mixture of various solvents with all over-acidic pH was used. Chiefly used acidic components were citric acid and formic acid, and glacial acetic acid of rare bases. Acetonitrile was the common solvent observed in all the HPLC methods that came across during literature review. In some cases, phosphate buffers were also used. Although most mobile phases were made a pass-through column with a flow rate of 1 $mL.min^{-1}$ and detection wavelength of more than 420 nm, exceptions were observed with mobile phase containing water with a flow rate of 0.2 to 0.4 $mL.min^{-1}$ and detection wavelength of 254 nm.[35] The LC-MS/MS mobile phase containing acetonitrile, water, and formic acid in different compositions was spotted while doing literature review. For example, one of the LC-MS/MS methods with TSK-GEL ODS 80Ts column and a mobile phase of 0.1% formic acid in water and acetonitrile were seen with the detection wavelength range of 200 to 500 nm. [35]

GC-MS method for detection of curcumin from extract using 5% phenyl methyl silox fused-silica capillary column as stationary phase and nitrogen as mobile phase with a flow rate of 1.0 mL/min was mentioned.[37]

HSCCC is a two-stage dissolvable framework, without stationary phase, rather than with liquid stationary phase, to determine target compounds depending on the differential dividing of solutes between two immiscible solvents, making it a compelling device for the preparative division. While doing literature review, one of such HSCCC method for curcumin was found, in which 0.1% formic acid in water/0.1% formic acid in acetonitrile (50/50, %v/v) was used as stationary phase and as a mobile phase hexane: chloroform: methanol: water (5/10/7.5/2.5, %v/v/v, total 2 L) were used with a flow rate of 1.0 L/min. The detection wavelength in that developed method was uttered as 405 nm.[35]

HPTLC is one of the chromatographic methods most commonly used for the separation and identification of herbal compounds. For example, Curcumin is such an herbal compound that its separation and identification method mentioned in various literature used pre-coated silica gel 60 F_{254} TLC plates as stationary and mobile phases containing several solvents. Specific solvents observed in those articles were citric acid and acetic acid used in mobile phase composition to provide required acidity to the solvent system.[41] (Table 2).

One specific UPLC method coupled with Q-ToF MS detection system was found in which powder was used to separate curcumin from it using C_{18} as stationary phase and formic acid in the water, and ACN was passed through a stationary phase with 0.4 mL/min flow rate. [43]

In the course of LR of analytical methods available for detection and quantification of curcumin, we came to know that an acidic solvent system with optimum compotation and flow rate (if relevant) is required to separate and quantify curcumin on the selected stationary phase and higher wavelength for detection.

Table 1. Reported spectroscopic methods

Method	**Matrix**	**Experimental conditions**			**References**
		Solvent	**Linearity range**	**Detection wavelength**	
UV Visible Spectrophotometer	NLC formumation	Methanol	1-11 µg/ml	425 nm	[26]

	API	Ethanol	2-10 µg/ml	429 nm	[27]
	API	Ethanol	2-10 µg/ml	430 nm	[28]
Fluorescence Spectroscopy	Curcumin powder	Human Serum Albumin solution	1.0×10^{-5} mol/L	295 nm	[29]
	Curcumin powder	Methanol	-	420 nm	[30]

Table 2. Reported chromatographic methods

Method	Matrix	Stationary Phase	Expe
HPLC	Plasma	C_{18}	
HPLC	Plasma	C_{18}	
HPLC	Plasma	C_{18}	
HPLC	Powder	C_{18}	
HPLC	Powder	C_8	
HPLC	Powder	TSK-GEL ODS 100 V, 100 Z, and 80 Ts; 4.6 mm × 150 mm, 5.0 μm, Tosoh Co., Tokyo, Japan	
HPL	Powder	C_{18}	

Method	Matrix	Stationary Phase	Expe
C			
HPLC	Extract	C_{18}	
HPLC	Extract	C_{18}	
HPLC	Extract	C_{18}	
HPLC	Human urine	RP-18 analytical column (Shim-Pack CLCC18)	
LC-MS/MS	Powder	TSK-GEL ODS 80Ts (4.6 mm × 150 mm, 5 μm: Tosoh Co., Tokyo, Japan)	
LC MS	Extract	C_{18}	
GC-MS	Extract	5% Phenyl Methyl Silox fused-silica capillary column	
HSCCC	Powder	s 0.1% FA in water/0.1% FA in acetonitrile (50/50, v/v)	

Method	Matrix	Stationary Phase	Expe
HPTLC	Powder	silica gel HPTLC plate $60F_{254}$, 10x10 cm	
TLC	Polyherbal capsule	TLC plates precoated with 0.2-mm layer of 10 cm)	
TLC	Liquid sample	Silica Gel	
TLC	Liquid sample	silica gel 60 F_{254} plates	
UPLC–Q-ToF MS	Powder	C_{18}	

4. BIOSYNTHESIS OF CURCUMIN

Curcumin is a biphenolic compound with seven carbon unsaturated aliphatic chains. It has two metabolites in keto form and enol form. The diatonic form is present in its molecule, and it permits them to convert into their keto and enol forms interchangeably. Curcumin biosynthesis occurs from two molecules Malonyl CoA, and Feruloyl CoA, in enzymes curcumin synthase (CURS) and diketide CoA synthase (DCS), and both the enzymes belong to the type III polyketide family. Malonyl CoA in the presence of coenzyme A and DCS enzyme reacts with feruloyl CoA and gives the diatonic compound feruloyldiketide – CoA. Then again reacts with the enzyme DCS and CURS, and dehydration occurs, and we get curcumin in its two enantiomeric forms like keto and enol form, which are rapidly interconverted. The schematic representation of curcumin biosynthesis is represented in Fig.**3**. [44]

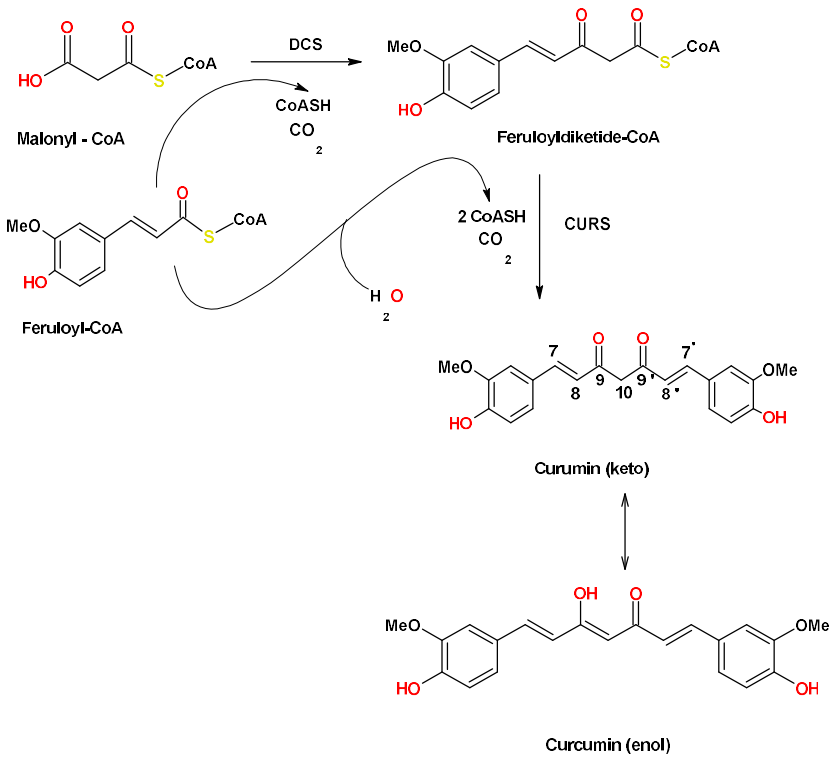

Fig. (3). Biosynthesis of curcumin

5. SYNTHETIC TECHNIQUES OF CURCUMIN

Ciamician and Silber first suggested the molecular formula of curcumin which is $C_{21}H_{20}O_6$. [45] Pelletier and Vogel reported the first synthetic method of curcumin in 1815, then a century after, Polish Chemists Lampe and Milobedzka 1913 synthesized curcumin from carboxyferuloyl chloride and ethyl acetoacetate**.** [46, 47]

Then Pavolini, in 1937, curcumin was obtained from vanillin and acetylacetone using boric anhydride as the condensing agent[18]. Then Pavolini, with Pavolini et al. in 1950, synthesized curcumin from acetylacetone, vanillin, and boric anhydride by heating over a free flame for 30 minutes (Fig.**4**).[48]

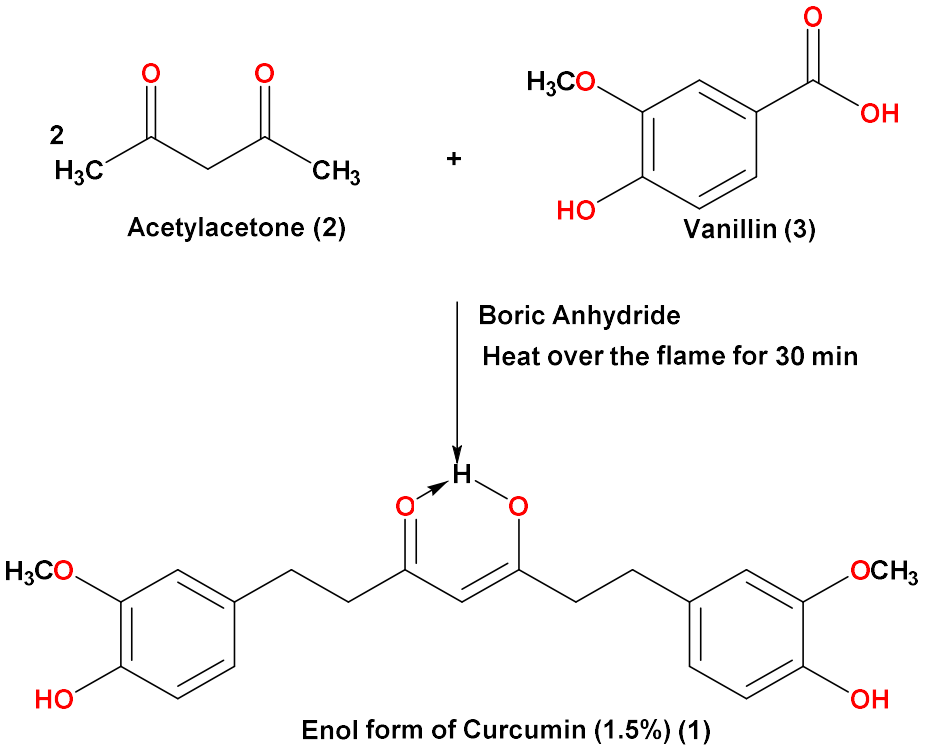

Fig. (4). Synthesis of curcumin by Pavolini et al.[48]

Then after Pavolini, in 1964, Pabon gave a simple method for synthesizing curcumin from trialkyl borate, butylamine, and boron trioxide. In this type of reaction, 2,4 – diketones react with aromatic aldehydes. The complexation with boron is favored to prevent knoevenagel condensations, and polar aprotic solvents and anhydrous conditions are needed where curcumin can be easily separated (Fig.**5**). An alkali medium is required to deprotonate the alkyl groups of ketone, which is provided by catalysts acting as primary and secondary amines. Although alkyl borates are used to remove produced water during the condensation reaction, any remaining moisture can react with the diketone complex, reducing curcumin yield. Furthermore, under slightly acidic conditions, the boron complex dissociates into curcumin. Curcumin from the reaction mixture can be separated using column chromatography through washing and precipitation.

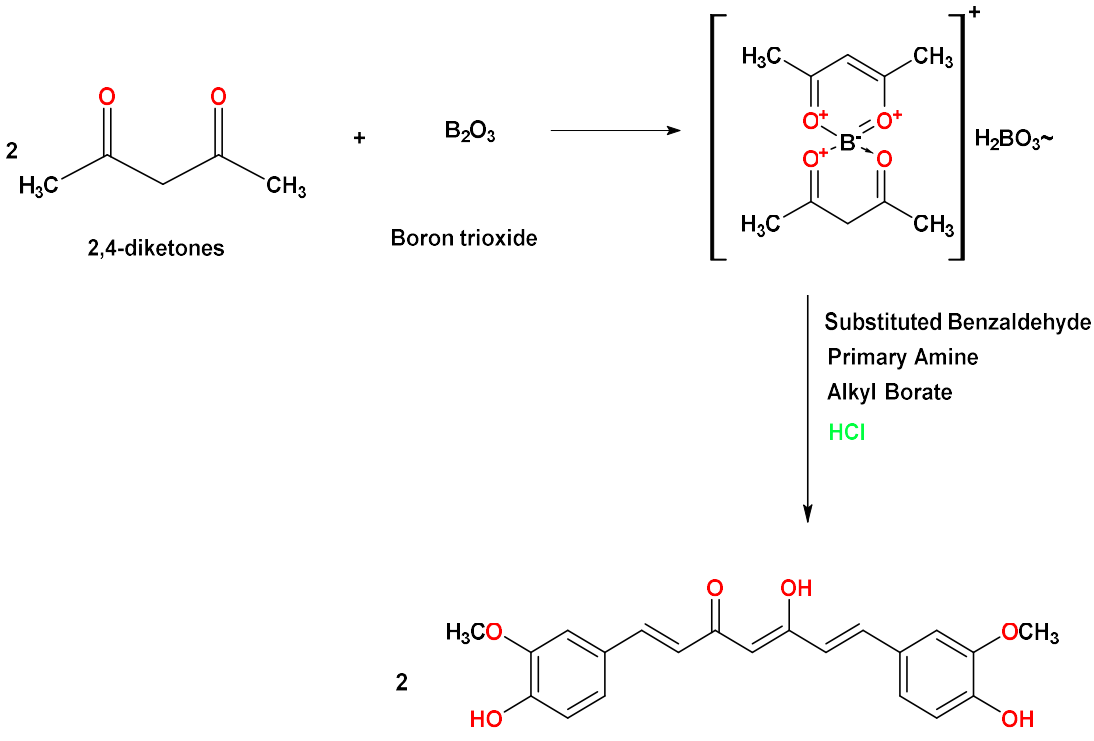

Fig.(5). Synthesis of curcumin by Pabon[18, 46]

Pabon also synthesized curcumin from vanillin (4-hydroxy-3-methoxybenzaldehyde) and acetylacetone (pentane-2,4-dione). Acetylacetone was complexed with boron trioxide in this reaction to prevent the methyl groups from knoevenagel condensation. As a result, benzaldehyde is used to perform a nucleophilic attack on both terminal methyl groups, and n-butylamine is added stepwise. Simultaneously, n-tributyl borate was used to remove water resulting from benzaldehyde condensation (Fig.**6**). Finally, in the slightly acidic environment, boron dissociates into two curcuminoids.

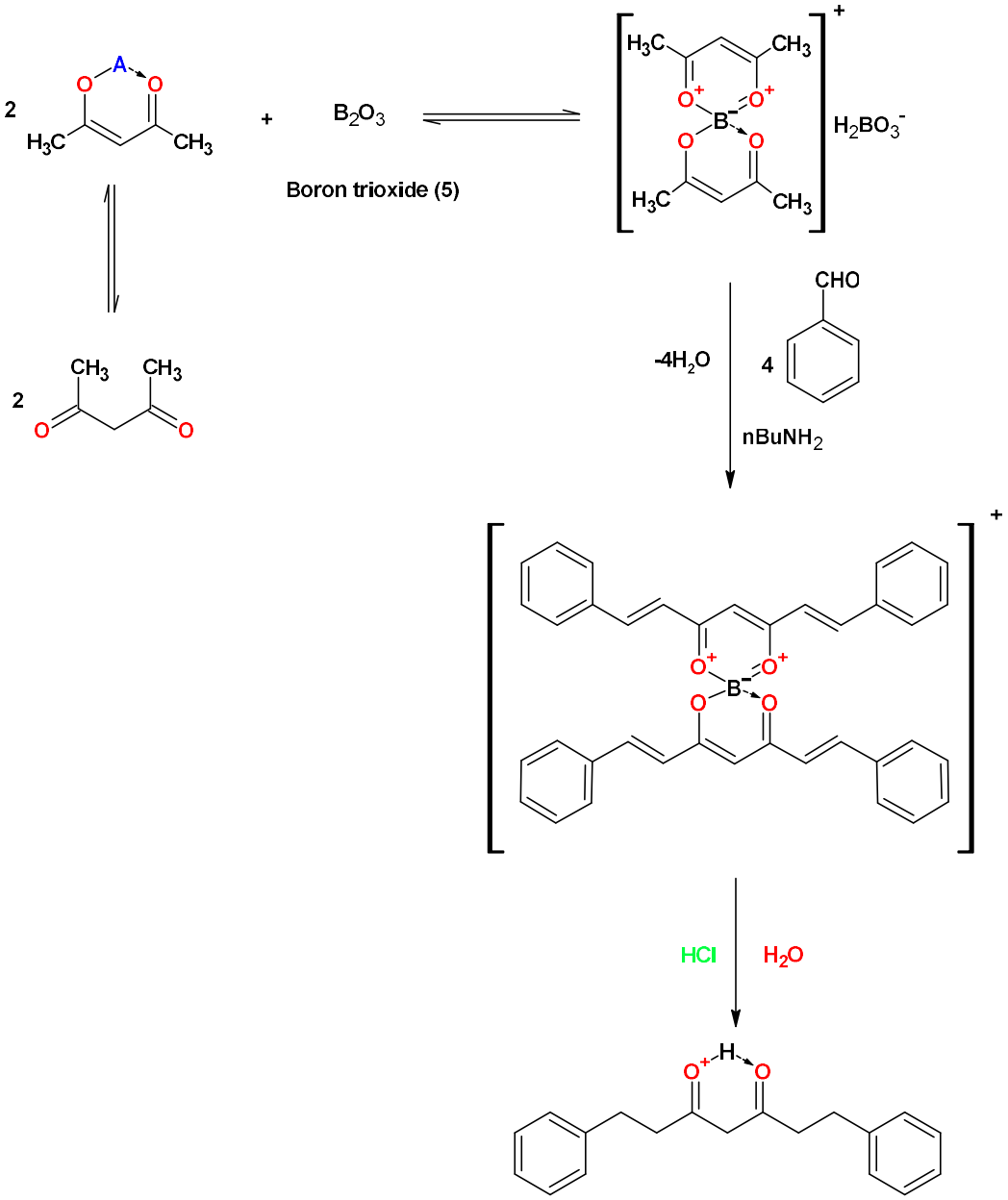

Fig. (6). Synthesis of curcumin by Pabon

After Pabon, Lin and co-workers reported the synthesis of asymmetric curcumin analogs using 4-acetyl-5-oxo-hexanoate instead of pentadienone. The symmetric curcumin analogs were first synthesized by Pederson's method, which involves synthesizing symmetric curcuminoids with the help of 2,4 - pentanediones and benzaldehyde in the presence of boron complex(Fig.**7**). [49]

Aldol condensation can occur at both dione terminals, and two equivalents of aldehyde derivative are required. For the preparation of asymmetric compounds having aryl rings, monoaryl termination was designed and subsequently condensed with an appropriate second aldehyde to give the target compounds. The yield obtained by this method is nearly 24-55%. [49]

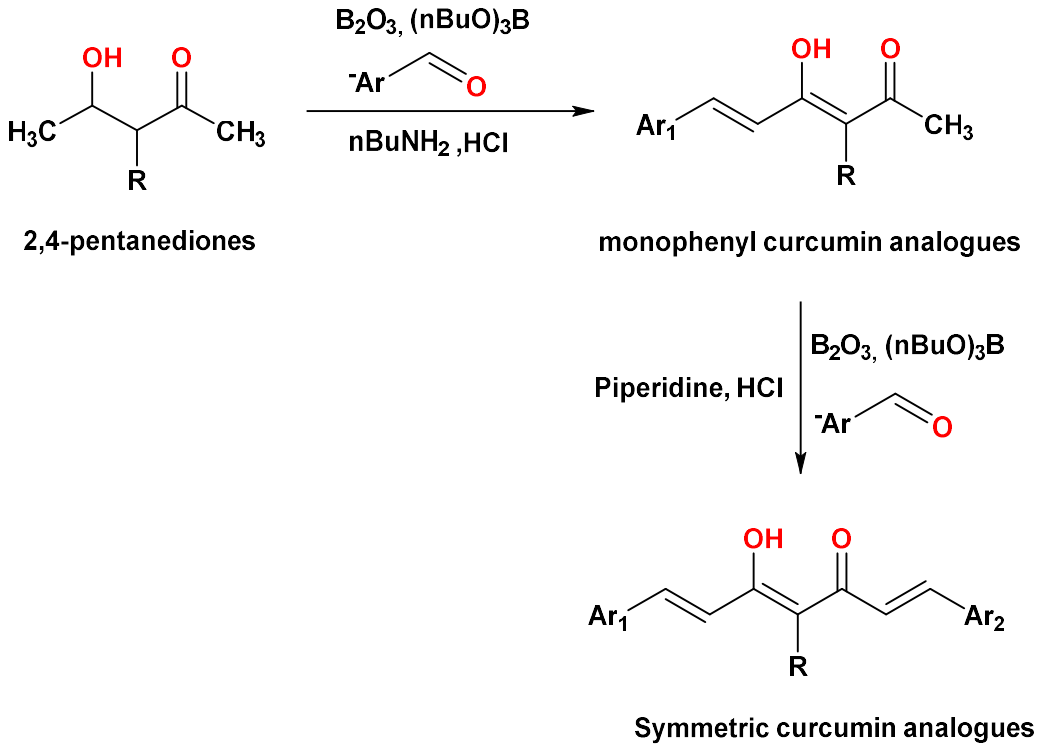

Fig.(7). Synthesis of asymmetric curcumin by Lin *et al.*

6. REPORTED EXTRACTION METHODS OF CURCUMIN

In 1815, curcumin extraction was reported first-time-ever; curcumin has always been a part of the discussion because it has many extraction methods from turmeric. Soxhlet, ultrasonic, microwave, and zone-refining extraction techniques have been tried, and among them, microwave, ultrasonic, and soxhlets extractions are the most widely used. [50]

6.1 Soxhlet Extraction Method

From time immemorial, the most usual method used for extraction is the soxhlet extraction method followed by column chromatography with a heating range of time as long as up to 12h.[51, 52] This method is time-consuming and involves using a high number of organic solvents, and the risk of thermal decomposition is high as it involves 12h of heating. This type usually involves solubilizing the active constituents by extracting solvent, which serves to be more tedious. [51]

In this extraction method, 25g dried rhizomes of turmeric were dissolved in 1L n-hexane, and formed suspension was stirred for three days and placed in a thimble in a soxhlet apparatus. Then 310 ml of methanol is heated in the flask, and the vapors are condensed in the condenser. The condenser drips the vapor in the thimble, and that marks the beginning of extraction. In the chamber, the liquid level ascends to the top of the siphon tube, and the liquid contents flood into the flask. After three days, the solvent is evaporated by a rotary evaporator. To this, 100 ml toluene is added, and the solution is poured into a separating funnel and basify the extract with 100 ml 0.2M NaOH and shaken. After collecting the aqueous phase, it will acidify to pH 3 by 0.2M HCl. The brown extract obtained at the end further purifies the undergoes illumination in this step, turning yellow. With the help of diethyl ether, the filtrate is extracted, and ether alters to pale yellow, suggesting the end of extraction. Ether is wholly removed, and crude

curcuminoid products are produced as yellow solid. Crude curcumin is purified with column chromatography technique. [53]

6.2 Ultrasonic Extraction Method

The most acceptable extraction method of curcumin is ultrasonic extraction, and it is performed with the help of a sonicator and solvents used such as ethanol and water in the process.

6.2.1 Pulsed ultrasonic extraction method

This method is environmentally friendly and is helpful as it utilizes less solvent for the extraction process. As Chemat and colleagues explained the six principles of green extraction and the USA's Environmental Protection Agency(EPA) set the twelve principles of green chemistry, this eco-compatible extraction technique, concerning standard methods, aims to lessen environmental impact in terms of energy and time. [54] In this method, dried turmeric powder (0.1g) was measured and transferred into different concentrations (%v/v) of 20 ml ethanol. Pulsed ultrasonication was carried out using different ratios of pulsed duration/interval (s/s) time in the ultrasonic processor (Sonics VCX 500, Connecticut, 74 USA) and different amplitudes 76 (AMP). After ultrasonication, centrifugation of the 77 samples was conducted for 5 min at 3000 rpm (5415R, Eppendorf, USA), after that 1 mL 78 supernatant was taken out, and before analysis, 10-fold dilution in ethanol was followed. [55]

6.2.2 Ultrasonic extraction method

Extraction experiments of the ultrasonic method were performed with a QSonica Q55 sonicator with the following specifications: 20 kHz, power 55 W, converter fabricated of aluminum alloy with 15cm×3 cm dimension, and 1/8 microchip made of titanium alloy with 13.8 cm×1.3 cm

dimension. Two-gram dry powder of turmeric was placed into a round bottom flask (150-mL) with a customized thermometer for temperature measurement. In a suitable ratio, the sample (2 g) was added into water and preached (soaking a matrix with a solvent before irradiation) for 10 min at room temperature before extraction in Ultrasonic-assisted extraction (UAE). With the help of ultrasound, the suspension was irradiated, and the extract of crude was carefully poured out and centrifuged for 15 min at 3,800 rpm, and filtration of the supernatant was carried out with a 0.45 μm membrane nylon syringe filter. Before the HPLC analysis, the sample was spiked with Internal standard biphenyl (10 ppm). [56]

All the extraction product of curcuminoid was analyzed with the help of HPLC coupled with UV detector. Hypersilgold pentafluorophenyl (PFP) column (4.6× 100 mm, 5 μm) from Agilent was used for the chromatographic separation of curcuminoids. The separation was performed using isocratic mobile phase ACN-water (30:70 v/v) at a column temperature of 40^0C. The detection wavelength, injection volume, and flow rate were fixed at 254 nm, 20 μL, and 1 mL min^{-1}, respectively. Agilent chemstation software was used for the handling of chromatographic data. The curcumin and curcuminoids are determined by comparison with the standard sample of curcuminoids. [56]

6.3 Microwave Extraction Method

This extraction method involves a higher extraction rate, less solvent, shorter extraction time, and lower cost and works on heating the plant tissues and the solvents with microwaves help, thereby increasing the kinetics of extraction. This method has solvent-free microwave-assisted extraction (SFMAE) and pressurized microwave-assisted extraction (PMAE). [57]

Microwave-assisted extraction (MAE) system was used for the treatment of turmeric. In which the series of twelve Teflon closed vessels including an automatic fiber optic temperature control terminal used under the various set of conditions for temperature (30 and 130 ^{0}C), time (0-20 min), and solvent mixture (MeOH and EtOH in water). The 0-1500W power was applied to quickly heat the materials inside the oven to the designated temperatures. In a typical run (e.g., at 80°C for 2 min), a sample of turmeric (0.1 g) was submerged in 20 mL of 80% MeOH. After that, with continuous stirring, the mixture was placed in the microwave oven, and the temperature was increased to 80 ^{0}C for 3 min, and maintain at 80 ^{0}C for 2 min. After extraction, the mixture was cooled to room temperature. For the conventional heat-assisted extraction technique, a sample (0.25 g) was weighed and submerged to 50 mL of 50% MeOH. After that, this mixture was transferred to a distillation flask, added a few pieces of boiling stone, and then refluxed at 80°C for 1/2 h. The flask was cooled to room temperature under running tap water. In the filtration process, methanolic extract was filtered through a filter paper and then through 0.45 µm PTFE (polytetrafluoroethylene) syringe filters (Whatman) and maintain at +4 °C in a refrigerator before analysis. [58]

With the help of HPLC analysis, the extraction of curcumin was known easily. In the reverse-phase HPLC analysis, an isocratic elution program was used. The mobile phase consisted of acetonitrile: acetic acid (40:60, v/v) mixture, at a flow rate of 1.2 mL/min and a total run time of 30 min per sample. The column temperature and a detection wavelength are 33°C and 425 nm, respectively. The calibration curve was obtained for curcumin in linear equations of peak area versus concentration using the above working mode. [58]

6.4 Zone Refining Method

The zone refining method of extraction of curcumin was the most efficient. It had obtained the crude curcumin from turmeric powder, maintaining the highest purity, and this method is the chromatographic partitioning of the solutes between two immiscible liquids. A two-phase solvent system was used for extracting the target components in these techniques. [59] As per the standard Counter-current chromatography (CCC), 1L of a solvent mixture consisting of ethanol, hexane, water, and ethyl acetate (1:1:1:1) was prepared. After that, it was added to the separatory funnel for the separation of two phases. In the pH-zone refining CCC, two phases were separated from the prepared mixture of Methyl-tert-butyl ether, acetonitrile, and water (4:1:5). Then sodium hydroxide was added to the lower phase, and the concentration of 30mM was maintained, which served as the eluter. To the upper stationary phase, trifluoracetic acid was added to keep the concentration of 20mM, which served as the retainer. The molar concentration of the eluter determines the length of the pH zone. [60, 61] The sample solution was prepared by dissolving the crude curcumin 5mg to 2g in the equal volume of each solvent phase, having a volume of 5-50 ml. The turmeric powder 100mg to 2g was extracted, and the upper organic phase was 10-50 ml, and the particulates were eliminated by centrifugation. The upper stationary phase was loaded into the multilayer coil, and the sample was injected through the sample port. The lower mobile phase was flowed into the column with the rate of 3ml/min and rotated then with the speed of 800 rpm. The continuous monitoring of the effluent was done at 280 nm and then collected into test tubes in 1-2 min intervals. [60] For further analysis, the pH of each fraction was measured by a portable pH meter, and then the curcuminoid fractions were extracted with 2ml ethyl acetate. The study of peak fractions was analyzed by NMR, TLC, and

Matrix-Assisted Laser Desorption / Ionization (MALDI). The TLC was performed with Hexane: Ethyl acetate: Ethyl alcohol (8:4:1) as the mobile phase and silica gel as the stationary phase.[60]

7. DEGRADATION OF CURCUMIN

In late 1900, Wang and co-workers are worked on the degradation of heptadienedione, and as a result, they found that it degrades into ferulic acid, vanillin, ferulic methane, ferulic aldehyde, and Trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal. After revisiting the degradation reaction by Wang and co-workers, it was found that it was only a minor pathway [62]**.** The primary product was Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal, and the minor products were ferulic acid, vanillin, and feruloyl methane(Fig.**8**). The amount of vanillin was increased with time. [59] All the identified degradation products were found due to non-enzymatic degradation and under the neutral-alkaline pH conditions. The degradation products of curcumin were further confirmed by RP-HPLC analyses of the reaction of curcumin with 0.1M phosphate buffer with pH 7.2 at 37 ^{0}C for 2h. [63]

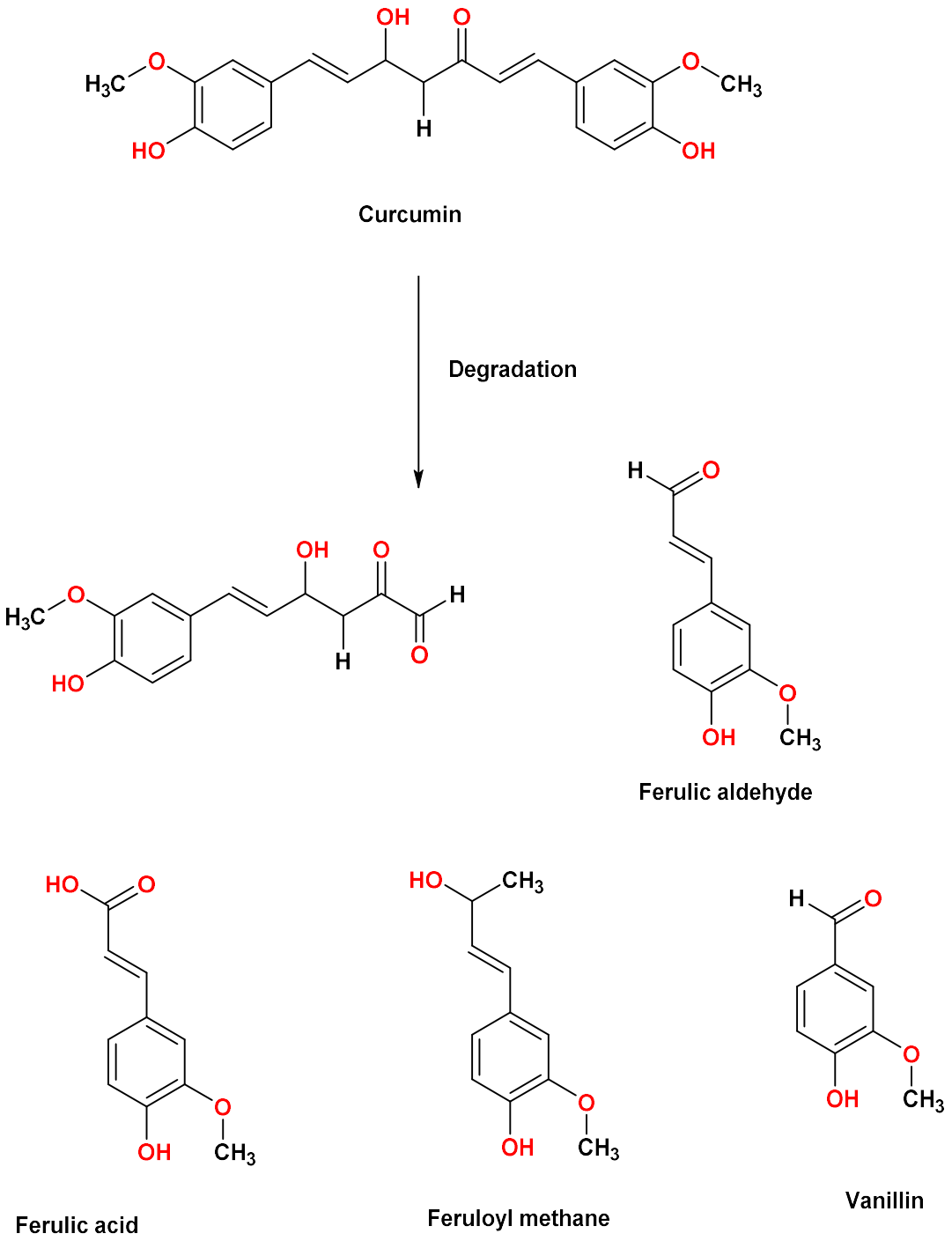

Fig. (8). Degradation of curcumin by wang *et al.*

Autoxidation is the degradation of curcumin at physiological pH in an aqueous buffer, and it is the primary pathway of degradation. [63, 64] Autoxidative transformation of curcumin was

highest at pH 8 and associated with stoichiometric uptake of O_2. COX-2 The degradation rate of curcumin increased by 3.5-fold and 10-fold, respectively, from the addition of 50 nm COX-2 and 300 μM H_2O_2. [65] The novel transformation of curcumin involves an autoxidation reaction involving reaction, which leads to oxygen incorporation and the formation of a dicyclopentadiene derivative of curcumin. Autoxidation of Curcumin was conducted in 500μl of 10mM NH_4OAc buffer, pH 7.4 at room temperature. After 45 min, 250μL of the reaction mixture were analyzed without extraction using RP-HPLC.[62]

A recent study was conducted which depicted the degradation rate of curcumin, and it was found that the rate was maximum in the presence of surfactants and salts. The findings of the study were conducted by a photodiode-array(PDA) detector of UV spectroscopy. The research showed that curcumin degradation in the presence of NaCl at neutral pH was negligible for 16h. Maximum degradation was seen in the presence of 28.5mM Na_2CO_4 at pH ~ 10.8, and the minimum was found in cationic surfactants. [66]

8. METABOLISM OF CURCUMIN

Along with gut microbiota and intestine, the liver is the primary site of metabolism for curcumins. Curcumin undergoes two types of metabolism, Phase 1 and Phase 2 [67]. Reduction of Curcumin mainly occurs in the enterocytes and hepatocytes, where curcumin is subjected to oxidative degradation. [67, 68] Phase 1 biotransformation of curcumin takes place where the formation of dihydrocurcumin, tetrahydrocurcumin, hexahydrocurcumin, and octa-hydro curcumin. [68] This phase 1 biotransformation results from double bonds reduction of the heptadienedione system with the help of the alcohol dehydrogenase enzyme. [69] Secondary biliary metabolites were in minor proportion, and they were found to be ferulic acid and dihydro-ferulic acid**.** [67] Curcumin and phase 1 metabolites undergo glucuronic acid and sulfate at the

phenolic site. This type of conjugation is the Phase 2 metabolism which is more active and occurs in intestinal and hepatic cytosol. [67]Curcumin glucuronide and sulfate are the two primary metabolites found in the rat plasma during the study of enzymatic hydrolysis. [70]The process continues, and so, in the cytosol, curcumin is sulfated by SULT1A1 (Sulphonyltransferase Family 1A Member 1) and SULT1A3 (Sulphonyltransferase Family 1A Member 3). In the intestine and hepatic microsomes, UGTs help through catalysis in the glucuronidation of curcumin. [67]

The study found that curcumin can also be metabolized by another significant pathway which involves *E.Coli (Escherichia Coli)* acting on the curcumin. The metabolism which occurs this way starts with the purification of Curcumin Converting Enzyme (Cura) by *E.Coli*. After that, the reduction process begins in the presence of the purified enzyme. The process is divided into two steps: the curcumin is first converted into NADPH dependent product, Dihydrocurcumin is the first intermediate, and then Tetrahydrocurcumin is formed as the end product. [71]

The same metabolism of curcumin takes place in the presence of *Blautia. sp*. The successive demethylation of curcumin converts it to the demethylcurcumin and finally bis-demethylcurcumin. [72]

The excretion of curcumin metabolites depends on the route of administration that is used. [73] When the rats were administered orally with 1g/kg of curcumin, it was found that 75% of curcumin was excreted in the feces and very trace amounts were found in urine. The gut absorption was deficient, and it wasn't found toxic up to the dose of 5g/kg. In the intravenous route, a significant part of curcumin was found to be metabolized. [74]In the intraperitoneal

route, up to 73% of the curcumin was excreted, and 11% of metabolites were found in the urine. [73]**.** The whole metabolism pathway of curcumin is shown in Fig.**9**.

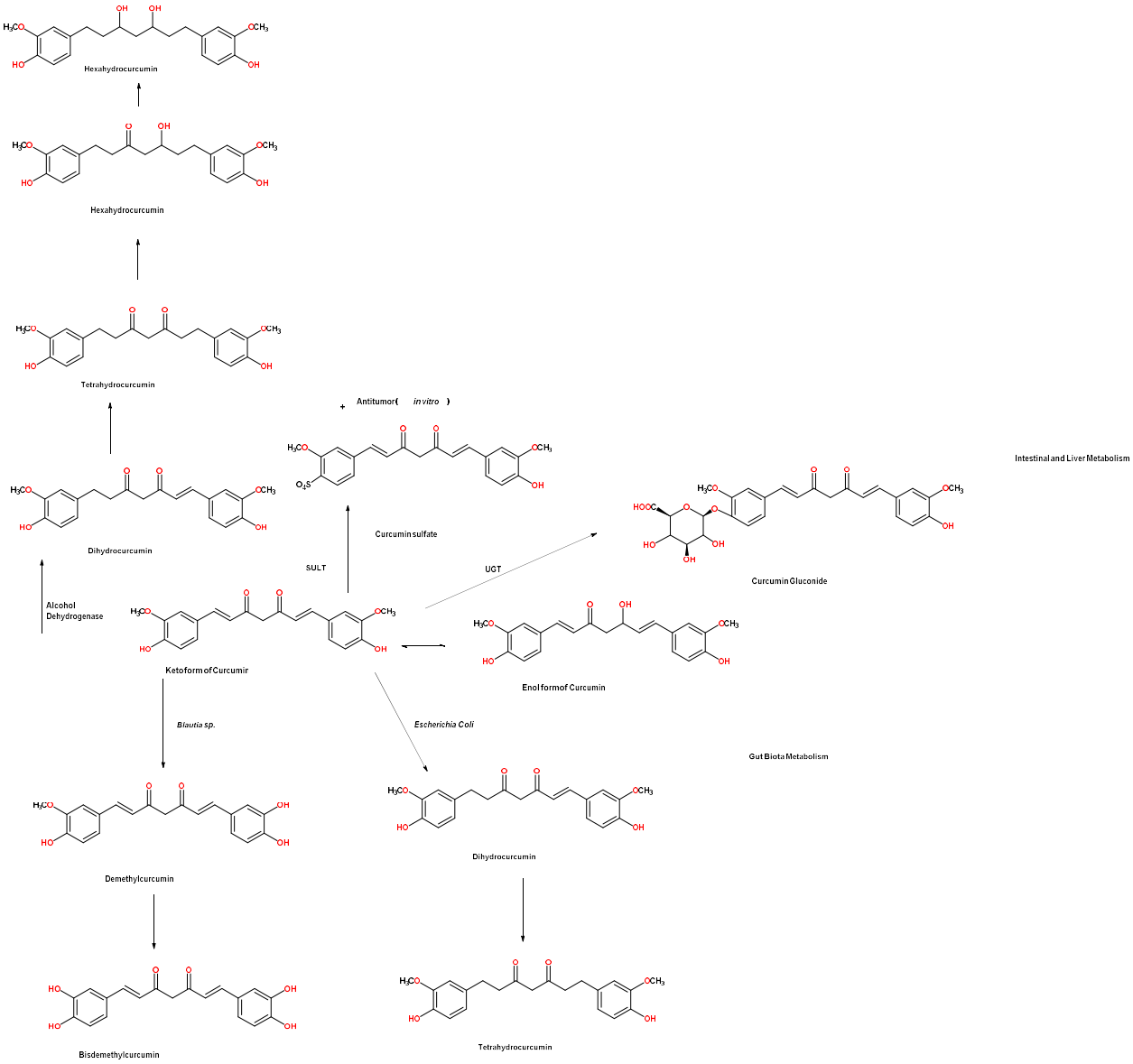

Fig.(9). Metabolism of curcumin[75]

9. PHARMACOLOGICAL EFFECTS OF CURCUMIN

9.1 Melanoma

Melanoma is one of the deadliest cancer of all cancers as it doesn't have any promising treatment. [76] The studies on curcumin in vivo as well as in vitro have shown the effect on various targets like mPTP, JAK-2/STAT-3, Mcl-1, Bcl-2, Bax, caspase-8, Caspase-3, NF-κB, p38, p53, caspase-3/7, ERK /Akt, MST1, JNK, Foxo3, Bim-1, MRP1, GSTM1, PDE1A, NFκB, MT1-MMP, MMP-2, PI3K/Akt/ GSK 3β, ERK, p38 MAPK, EphA2, PI3K, MMP9, Bcl-XL, X-IA, PRL-3, eIF2α, GADD 153, COX-I, COX-II, OPN, Nm23, E-cadherin, iNOS, cyclin D1, BclXL, Erk, VEGF – such targets were studied. Different properties were determined where curcumin was found to have multiple biological effects in giving the improved therapeutic response to Melanoma. In the mouse model, the curcumin is showing reaction in 147 miRNAs. [76] Topical Curcumin administration on mouse skin cancer model showed inhibition of pS6, pAKT, pSTAT3, pERK1/2, and p-4EBP1 in SRB12-p9 cells.[77]

9.2 HIV

Curcumin effects on purified HIV type 1 integrase virus have been determined. The inhibitory concentrations of Curcumin IC50 for standard transfer are 40μM. A mutant of integrase deletion mutant containing only amino acids 50-212 shows that the integrase catalytic center interacts with curcumin. Methyl cinnamate and chlorogenic acid, two structural analogs, were inactive. Studies of energy minimization indicate that curcumin's anti-integrase activity may be due to an intramolecular stacking of two phenyl rings that brings the hydroxyl groups nearby. Curcumin interacts with integrase catalytic core, and this integration of HIV-1 has a notable anti-viral effect. [78]

9.3 Liver damage

The curcumin's ability to inhibit several factors, including nuclear factor-kB, which modulates several pro-inflammatory and profibrotic cytokines and its antioxidant properties, provides a reasonable molecular basis to use it in hepatic disorders. Curcumin attenuates ethanol-induced liver damage, thioacetamide, iron overdose, cholestasis, and acute, subchronic, and chronic carbon tetrachloride (CCl4) poisoning; also, it somewhat reverses CCl4 cirrhosis. [79] The hepatoprotective effect of curcumin was investigated using a dimethylnitrosamine (DMN)-induced model of rat liver cirrhosis. Curcumin-treated or lactulose-treated cirrhotic liver tissues showed more excellent conductivity than tissues in the DMN-only community. Specifically, cirrhotic liver conductivity after treatment with curcumin was close to that of normal liver tissue. Histological staining and immune-histo-chemical analysis showed considerable levels of attenuated fibrosis and reduced inflammatory response compared with weakened liver tissue by DMN after treatment with both curcumin and lactulose. [80]

9.4 Lung cancer

Curcumin is a highly promising viable treatment strategy for lung cancer with fewer adverse effects. Curcumin demonstrates anticancer effects in lung cancer *via* numerous mechanisms, including inhibition of cell proliferation, invasion, metastasis, apoptosis activation, epigenetic alterations, and regulation of microRNA expression. Many in vitro and in vivo studies have shown that molecular targets include EGFR, STAT3, TGF-β, FOXO3a, COX-2, eIF2a, Bcl-2, etc. ROS, PI3KAkt / mTOR, Cdc42, Fas / FasL, MMPs, E-cadherin, and adiponectin modulate these pathways. [81]

9.5 Antidepressant effect

The antidepressant effect of curcumin was studied in an animal model's amygdala by measuring the Brain-Derived Neurotrophic Factor (BDNF). The findings revealed that curcumin therapy (40 mg/kg, i.p.) significantly decreased depressive-like activities in mice during the forced swim test. Chronic curcumin administration (40 mg/kg, i.p., 21 days) raised the levels of BDNF protein in the amygdala, and this change was suppressed by pretreatment with SL3277 inhibitor extracellular signal-regulated kinase (ERK). Besides, the elevated levels of ERK curcumin phosphorylation in the amygdala were inhibited by the ERK inhibitor, and the inhibition of this kinase avoided the antidepressant effects of curcumin. Both the symptoms of curcumin were nearly similar to those found with fluoxetine, the therapeutic antidepressant. [82]

9.6 Ischaemia/Reperfusion Insult

The model adopted was forebrain ischemia induced by surgery, done for 1h by bilateral typical carotid artery occlusion (BCCAO), followed by reperfusion for another 1h. The repercussions of a specific i.p. The CUR (50, 100, or 200 mg kg^{-1}) dose, given 0.5 h after the onset of ischemia, was assessed by evaluating biochemical parameters linked to oxidative stress in the forebrain of rats. CUR lowered the I/R-induced elevated activity of xanthine oxidase (XO), superoxide anion (O2radical dot−) growth, malondialdehyde (MDA) level and glutathione peroxidase (GPx), superoxide dismutase (SOD), and lactate dehydrogenase (LDH) activities at the highest dose level 200 mg kg−1). On the other hand, due to the I/R insult, the reduced glutathione (GSH) content was not impacted by CUR. CUR has been found to shield the forebrain of rats from I/R abuse. These defensive results can be due to its antioxidant properties and inhibitory effects on the conversion of xanthine dehydrogenase/xanthine oxidase (XD/XO) and the subsequent development of O2radical spots. [83]

9.7 Chronic asthma

Curcumin is indicated to block the release of histamine by mast cells and inhibit lipoxygenase activity, potentially hindering the production of leukotrienes. It also inhibits the synthesis of inflammatory cytokines, such as IL-5 and IL-8, which are involved in inflammation formation and have also been found to inhibit transcription factors such as the kappa-B (NF-kB) nuclear factor the activating protein 1 (AP1). Recently, the anti-adhesion property of curcumin has been shown. These properties of curcumin may be due to the discovery of decreased airway constriction in BALB/c mice. [84]

9.8 Mucosal injury

Oxidative and nitrosative stress, leucocyte infiltration, and upregulation of pro-inflammatory cytokines describe inflammatory bowel disease (IBD). The beneficial effects of curcumin, an anti-inflammatory and antioxidant food derivative, on 2,4,6-trinitrobenzene sulphonic acid-induced colitis in mice, a model for IBD, have been explored in this review. [85]

9.9 Antihyperalgesic effect

The vanilloid curcumin moiety is essential for activating vanilloid 1 (TRPV1) transient receptor potential, which plays a significant role in nociception. Thermal hyperalgesia was caused by subcutaneous capsaicin injection in the vibrissal pad region of rats. Intraperitoneally administered curcumin blocked thermal hyperalgesia induced by capsaicin in a dose-dependent manner. Whereas curcumin decreased capsaicin-induced currents in trigeminal ganglion neurons and TRPV1-expressing HEK 293 cells in a dose-dependent way, curcumin did not impact TRPV1 heat-induced currents. Although, our findings suggested that curcumin prevents the activation of TRPV1 caused by capsaicin and thus reduces hypersensitivity to pain mediated by TRPV1. [86]

9.10 Leukemia

The effects of curcumin on apoptosis have been tested in acute human promyelocytic leukemia (HL-60) cells. MTT determined the cytotoxic effects of Curcumin on HL-60 cells. Increased annexin V-binding potential and caspase-3 activation with flow cytometric examination showed that HL-60 cells underwent apoptosis when treated with curcumin. Curcumin concentrations of 15, 20, and 40 μMsignificantly decreased the proliferation of cells; curcumin has been shown to have significant cytotoxic and apoptotic effects on HL-60 cells. Curcumin has been suggested to have a possible therapeutic role in human leukemia. [87]

9.11 Hepatic fibrosis

Curcumin controlled the dissemination of HSCs in a dose-dependent way. The PPARγ nuclear expression level decreased as HSCs underwent incremental activation with culture prolongation. PPARγ expression was up-regulated by curcumin and significantly inhibited the development of alpha-SMA and collagen I. PPARγ is expressed in the cytoplasm and nucleus and is uniformly distributed in the HSCs, but accumulates in the HSC nucleus following curcumin therapy and disappears from the cytoplasm. Hoechst 33258 staining revealed that curcumin-induced apoptosis of culture-activated HSCs significantly increased pro-apoptotic Bax expression and decreased anti-apoptotic Bcl-2 expression. Cyclin D1 gene activated NF add Bp65 protein, and TGFβR-I protein expression has been dramatically reduced by curcumin. Curcumin significantly improved the activities of MMP-2 and MMP-9.[88]

9.12. Liver cirrhosis

The hepatoprotective function of curcumin was studied using a rat liver cirrhosis model that was induced with dimethylnitrosamine (DMN). A magnetic resonance-based electrical conductivity imaging approach was used to assess tissue conditions consistent with a protective effect

combined with a biochemical study. The impact of curcumin therapy and lactulose treatment on cirrhosis of the liver has been compared. Electrical conductivity images revealed that liver tissues affected by DMN had reduced conductivity relative to normal liver tissues. In comparison, cirrhotic liver tissues treated with curcumin or lactulose demonstrated improved conductivity relative to tissues in the DMN-only community. Specifically, the conductivity of the cirrhotic liver after curcumin therapy was close to that of normal liver tissues.[76]

9.13 Antioxidant effects

Most experiments have shown that curcumin, or its variants, effectively against oxidative and nitrosative stress in different cell and animal models. Overall, curcumin is safe against lipid and protein degradation with lower amounts of malondialdehyde, protein carbonyls, thiols, and nitrotyrosine. Curcumin is also showed to trigger the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. [77] Among bioconjugates (Di-demethylated piperoyl, di-valinoyl, and di-glutamyl esters), the curcumin glutamyl diester showed improved safety compared to other conjugates against PN-dependent CI inhibition and protein nitration. Di-glutamyl Curcumin shielded dopaminergic neurons from 1-methyl-4-phenylpyridinium (MPP+)-mediated neuronal death. Compared to curcumin alone, these results were increased, meaning that di-glutamyl curcumin may be a safer neuroprotective agent in neurodegenerative disorders such as Parkinson's disease. [80]

9.14 Scleroderma

In organ pathologies such as scleroderma and others, curcumin is considered to be helpful. By precisely modulating the protein kinase C (PKC) pathway, curcumin exerts its defensive effect. In fibroblasts involved in the growth of fibrosis, PKC-dependent signal transduction pathways have been shown to control several intracellular events. In scleroderma, two of the recent PKCs,

ε and δ, play significant roles. In scleroderma dermal fibroblasts, PKC δ is present at high levels that may be implicated in the dysregulation of collagen gene expression typical of systemic sclerosis. In both DNA synthesis and cell survival, thrombin-induced p21Cip1/WAF1 provides additional evidence for the essential roles of thrombin-induced signaling in the growth, proliferation, and maintenance of myofibroblast phenotype crucial to the formation and progression of pulmonary fibrosis. Thrombin-induced p21Cip1/WAF1 provides additional evidence for the essential functions of thrombin-induced signaling in the development, proliferation, and maintenance of the myofibroblast phenotype indispensable for the formation and progression of pulmonary fibrosis in both cell survival and DNA synthesis. [89]

9.15 Corneal neovascularization

In the presence and absence of an essential fibroblast growth factor (bFGF), curcumin has been tested for its ability to inhibit the proliferation of primary endothelial cells as well as its ability to inhibit the proliferation of an immortalized endothelial cell line. Subsequently, curcumin and its derivatives were tested for their ability to suppress corneal neovascularization caused by bFGF in the mouse cornea. Curcumin was eventually evaluated for its ability to inhibit mRNA development of phorbol ester-stimulated vascular endothelial growth factor (VEGF). Curcumin blocked the proliferation of endothelial cells successfully in a dose-dependent way. The significant inhibition of bFGF-mediated corneal neovascularization in the mouse was shown by curcumin and its derivatives. Curcumin did not affect the development of VEGF induced by phorbol ester. [90]

10. Conclusion and future prospects

Herein, an attempt has been made to present an overview of the phytochemical, analytical, and pharmacological review of curcumin that will be helpful for researchers and reviewers to

consider in their respective roles. Numerous analytical methods were applied to determine curcumin in various matrices using spectroscopic methods like UV and spectroflurimetry with chromatographic methods such as HPLC, LC-MS/MS, GC-MS, and HSCCC, TLC, and UPLC. Apart from these analytical methodologies, the biosynthesis curcumin is described in this review with specific laboratory techniques to synthesize curcumin on a laboratory scale, where boron trioxide is used as a standard laboratory reagent for curcumin synthesis. For curcumin extraction from their plant materials, various extraction techniques, i.e., such as soxhlet microwave, zone refining, and ultrasonic extraction, are discussed, and microwave extraction involves a higher extraction rate, low solvent consumption, shorter extraction time. Therefore, it is considered the most economical method. Degradation of curcumin under applied stress conditions yields ferulic acid, vanillin, ferulic methane, and ferulic aldehyde as minor degradants and Trans-6-(4′-hydroxy-3′- methoxyphenyl)-2,4-dioxo-5-hexenal as a major degradation product. Curcumin has diversified therapeutic potency, but the low oral bioavailability of curcumin is the main obstacle for developing formulation from laboratory to clinical stage.

Further, it can be enhanced with the co-administration of piperine as it serves as a bio enhancer. Curcumin itself is a nutraceutical phytoconstituent which shown synergistic with other nutraceuticals such as genistein, catechins, piperine, etc. However, curcumin and its kindred analogs are not applicable to possess the properties required for a superior medicament (high water solubility, low toxicity, chemical stability, high bioavailability). Moreover, due to its bioavailability, some functional antagonistic activity may be appraised for further research to enhance therapeutic efficacy by upgraded technology such as the design of curcumin analogs and its formulations, including liposomes adjuvants nanoparticles, phospholipid complexes, and micelles.

Consent for Publication

Not applicable.

Funding

None.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgements

The authors thank Ramanbhai Patel College of Pharmacy, a constituent college of Charotar University of Science and Technology (CHARUSAT) for providing the necessary facilities to carry out the review work.

Abbreviation:

- **IUPAC:** International Union of Pure and Applied Chemistry
- **LR:** Literature review
- **HPLC:** High Performance Liquid Chromatography
- **TLC:** Thin Layer Chromatography
- **HPTLC:** High Performance Thin Layer Chromatography
- **UV:** Ultra Violet
- **LC-MS:** Liquid Chromatography Mass Spectrometry
- **GC:** Gas Chromatography
- **MS:** Mass Spectrometry
- **UPLC:** Ultra Performance Liquid Chromatography
- **HSCCC:** High Speed Centrifugal Countercurrent Chromatography
- **API:** Active Pharmaceutical Ingredient
- **mL:** Mililiter
- **ODS:** Octadecyl-silica
- **FA:** Formic Acid
- **Q-TOF:** Quadrupole Time of Flight
- **ACN:** Acetonitrile
- **NLC: Nanostructured Lipid Carrier**
- **v/v:** Volume/Volume
- **CURS:** Curcumin Synthase
- **DCS:** Diketide CoA Synthase
- **EPA:** Environmental Protection Agency
- **AMP:** Amplitudes
- **UAE:** Ultrasonic Assisted Extraction
- **PPM:** Parts Per Million

- **PFP:** Pentafluorophenyl
- **nm:** Nanometer
- **MeOH:** Methyl Alcohol
- **EtOH:** Ethyl Alcohol
- **PTFE:** Polytetrafluoroethylene
- **CCC:** Countercurrent Chromatography
- **mM:** Milimetre
- **µM:** Micrometre
- **NMR:** Nuclear Magnetic Resonance
- **MALDI:** Matrix Assisted Laser Desorption/ Ionization
- **COX-2:** Cyclooxygenase-2
- **RP-HPLC:** Reverse Phase High Performance Liquid Chromatography
- **PDA:** Photodiode-array
- **SULT:** Sulphonyltransferase
- **UGT:** Uridine Glucuronyl Transferases
- **NADPH:** Nicotinamide Adenine Dinucleotide Phosphate
- **mPTP:** (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
- **JAK:** Janus Activated Kinase
- **MCL:** Myeloid-cell leukemia
- **BCL:** β-Cell Leukemia
- **NF-kB:** Nuclear Factor kappa B
- **ERK:** Extracellular signal regulated kinase
- **MST:** Macrophage Stimulating
- **JNK:** c-Jun N-terminal kinase
- **BIM:** Bisindolylmaleimide
- **GSTM:** Glutathione S-Transferase Mu
- **PDE1A:** Phosphodiesterase 1A
- **MMP:** Matrix Metallopeptidase
- **GADD:** Growth arrest and DNA damage inducible
- **iNOS:** Inducible Nitric oxide synthase
- **HIV:** Human Immunodeficiency Virus
- **EGFR:** Epidermal Growth Factor Receptor
- **STAT3:** Signal transducer and activator of transcription 3
- **TGF-β:** Transforming growth factor beta
- **FOXO:** Forehead box transcription factors
- **ROS:** Reactive Oxygen Species
- **m-TOR:** Mammalian target of Rapamycin
- **BDNF:** Brain derived neurotrophic factor
- **BCCAO:** Bilateral common carotid artery occlusion
- **XO:** Xanthine oxidase
- **MDA:** Malondialdehyde
- **GPx:** Glutathione peroxidise
- **GSH:** Glutathione
- **SOD:** Superoxide dismutase

- **XD:** Xanthine dehydrogenase
- **IBD:** Inflammatory bowel disease
- **TRPV1:** Transient receptor potential vanilloid type 1
- **HSCs:** Hematopoietic stem cells
- **PPAR-γ:** Peroxisome proliferator-activated receptor gamma
- **PKC:** Protein kinase C
- **bFGF:** Fibroblast Growth Factor
- **VEGF:** Vascular Endothelial Growth Factor

11. REFERENCES:

[1] Hewlings, S. J.; Kalman, D. S. Curcumin: A Review of Its Effects on Human Health. *Foods*, **2017**, *6* (10), 1–11. https://doi.org/10.3390/foods6100092.

[2] Gupta, S. C.; Patchva, S.; Koh, W.; Aggarwal, B. B. Discovery of Curcumin, a Component of Golden Spice, and Its Miraculous Biological Activities. *Clin. Exp. Pharmacol. Physiol.*, **2012**, *39* (3), 283–299. https://doi.org/10.1111/j.1440-1681.2011.05648.x.

[3] Xu, X. Y.; Meng, X.; Li, S.; Gan, R. Y.; Li, Y.; Li, H. Bin. Bioactivity, Health Benefits, and Related Molecular Mechanisms of Curcumin: Current Progress, Challenges, and Perspectives. *Nutrients*, **2018**, *10* (10). https://doi.org/10.3390/nu10101553.

[4] Tomeh, M. A.; Hadianamrei, R.; Zhao, X. A Review of Curcumin and Its Derivatives as Anticancer Agents. *Int. J. Mol. Sci.*, **2019**, *20* (5), 1033. https://doi.org/10.3390/ijms20051033.

[5] Fadus, M. C.; Lau, C.; Bikhchandani, J.; Lynch, H. T. Curcumin: An Age-Old Anti-Inflammatory and Anti-Neoplastic Agent. *J. Tradit. Complement. Med.*, **2017**, *7* (3), 339–346. https://doi.org/https://doi.org/10.1016/j.jtcme.2016.08.002.

[6] Marton, L. T.; Pescinini-e-Salzedas, L. M.; Camargo, M. E. C.; Barbalho, S. M.; Haber, J.

F. dos S.; Sinatora, R. V.; Detregiachi, C. R. P.; Girio, R. J. S.; Buchaim, D. V.; Cincotto dos Santos Bueno, P. The Effects of Curcumin on Diabetes Mellitus: A Systematic Review . *Frontiers in Endocrinology* . **2021**, p 443.

[7] Rinkunaite, I.; Simoliunas, E.; Alksne, M.; Dapkute, D.; Bukelskiene, V. Anti-Inflammatory Effect of Different Curcumin Preparations on Adjuvant-Induced Arthritis in Rats. *BMC Complement. Med. Ther.*, **2021**, *21* (1), 39. https://doi.org/10.1186/s12906-021-03207-3.

[8] Hewlings, S. J.; Kalman, D. S. Curcumin: A Review of Its Effects on Human Health. *Foods (Basel, Switzerland)*, **2017**, *6* (10), 92. https://doi.org/10.3390/foods6100092.

[9] Qureshi, M.; Al-Suhaimi, E.; Shehzad, A. Chapter 20 - Curcumin Impact on Multiple Sclerosis; Farooqui, T., Farooqui, A. A. B. T.-C. for N. and P. D., Eds.; Academic Press, 2019; pp 365–380. https://doi.org/https://doi.org/10.1016/B978-0-12-815461-8.00020-7.

[10] Fallahi, F.; Borran, S.; Ashrafizadeh, M.; Zarrabi, A.; Pourhanifeh, M. H.; Khaksary Mahabady, M.; Sahebkar, A.; Mirzaei, H. Curcumin and Inflammatory Bowel Diseases: From in Vitro Studies to Clinical Trials. *Mol. Immunol.*, **2021**, *130*, 20–30. https://doi.org/https://doi.org/10.1016/j.molimm.2020.11.016.

[11] S, P.; AK, T. Curcumin and Cystic Fibrosis Defects: A Spicy Treatment. *Hered. Genet.*, **2015**, *s7*, 1–2. https://doi.org/10.4172/2161-1041.s7-e001.

[12] Chen, M.; Du, Z.-Y.; Zheng, X.; Li, D.-L.; Zhou, R.-P.; Zhang, K. Use of Curcumin in Diagnosis, Prevention, and Treatment of Alzheimer's Disease. *Neural Regen. Res.*, **2018**, *13* (4), 742–752. https://doi.org/10.4103/1673-5374.230303.

[13] Prasad, S.; Gupta, S. C.; Tyagi, A. K.; Aggarwal, B. B. Curcumin, a Component of Golden Spice: From Bedside to Bench and Back. *Biotechnol. Adv.*, **2014**, *32* (6), 1053–

1064. https://doi.org/10.1016/j.biotechadv.2014.04.004.

[14] Maheshwari, R. K.; Singh, A. K.; Gaddipati, J.; Srimal, R. C. Multiple Biological Activities of Curcumin: A Short Review. *Life Sci.*, **2006**, *78* (18), 2081–2087. https://doi.org/10.1016/j.lfs.2005.12.007.

[15] Shoba, G.; Joy, D.; Joseph, T.; Majeed, M.; Rajendran, R.; Srinivas, P. S. S. R. Influence of Piperine on the Pharmacokinetics of Curcumin in Animals and Human Volunteers. *Planta Med.*, **1998**, *64* (4), 353–356. https://doi.org/10.1055/s-2006-957450.

[16] Shah, U.; Jasani, A. UV Spectrophotometric and RP-HPLC Methods for Simultaneous Estimation of Isoniazid, Rifampicin and Piperine in Pharmaceutical Dosage Form. *Int J Pharm Pharm Sci*, **2014**, *6* (10), 274–280.

[17] Shah, U.; Patel, S.; Raval, M. Stability Indicating Reverse Phase HPLC Method for Estimation of Rifampicin and Piperine in Pharmaceutical Dosage Form. *Curr. Drug Discov. Technol.*, **2018**, *15* (1), 54–64.

[18] Priyadarsini, K. I. The Chemistry of Curcumin: From Extraction to Therapeutic Agent. *Molecules*, **2014**, *19* (12), 20091–20112. https://doi.org/10.3390/molecules191220091.

[19] Mazzarino, L.; Bellettini, I. C.; Minatti, E.; Lemos-Senna, E. Development and Validation of a Fluorimetric Method to Determine Curcumin in Lipid and Polymeric Nanocapsule Suspensions. *Brazilian J. Pharm. Sci.*, **2010**, *46* (2), 219–226. https://doi.org/10.1590/S1984-82502010000200008.

[20] Singh, A.; Avupati, V. R. Development and Validation of UV-Spectrophotometric Method for the Estimation of Curcumin in Standardised Polyherbal Formulations. *J. Young Pharm.*, **2017**, *9* (4), 491–495. https://doi.org/10.5530/jyp.2017.9.96.

[21] Carolina Alves, R.; Perosa Fernandes, R.; Fonseca-Santos, B.; Damiani Victorelli, F.;

Chorilli, M. A Critical Review of the Properties and Analytical Methods for the Determination of Curcumin in Biological and Pharmaceutical Matrices. *Crit. Rev. Anal. Chem.*, **2019**, *49* (2), 138–149. https://doi.org/10.1080/10408347.2018.1489216.

[22] Chahar, M. Y.; Rajashree, M. Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Curcumin and Piperine in Bulk Mixture. *World J. Pharm. Res.*, **2016**, *5* (5), 1262–1276.

[23] Kotha, R. R.; Luthria, D. L. Curcumin: Biological, Pharmaceutical, Nutraceutical, and Analytical Aspects. *Molecules*, **2019**, *24* (16), 1–27. https://doi.org/10.3390/molecules24162930.

[24] Analysis, H.; Hazra, K.; Bagh, M. Hazra et Al.,. **2015**, *2* (3), 127–130. https://doi.org/10.13040/IJPSR.0975-8232.IJP.2(3).127-30.

[25] Paramasivam, M.; Poi, R.; Banerjee, H.; Bandyopadhyay, A. High-Performance Thin Layer Chromatographic Method for Quantitative Determination of Curcuminoids in Curcuma Longa Germplasm. *Food Chem.*, **2009**, *113* (2), 640–644. https://doi.org/10.1016/j.foodchem.2008.07.051.

[26] Rapalli, V. K.; Kaul, V.; Gorantla, S.; Waghule, T.; Dubey, S. K.; Pandey, M. M.; Singhvi, G. UV Spectrophotometric Method for Characterization of Curcumin Loaded Nanostructured Lipid Nanocarriers in Simulated Conditions: Method Development, in-Vitro and Ex-Vivo Applications in Topical Delivery. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, **2020**, *224*, 117392. https://doi.org/10.1016/j.saa.2019.117392.

[27] Majumder, K. K.; Sharma, J. B.; Kumar, M.; Bhatt, S.; Saini, V. Development and Validation of UV-Visible Spectrophotometric Method for The Estimation of Curcumin in Bulk and Pharmaceutical Formulation. *Pharmacophores*, **2020**, *10* (1), 115–121.

[28] Mohan, P. R. K.; Sreelakshmi, G.; Muraleedharan, C. V.; Joseph, R. Water Soluble Complexes of Curcumin with Cyclodextrins: Characterization by FT-Raman Spectroscopy. *Vib. Spectrosc.*, **2012**, *62*, 77–84. https://doi.org/10.1016/j.vibspec.2012.05.002.

[29] Ge, Y. S.; Jin, C.; Song, Z.; Zhang, J. Q.; Jiang, F. L.; Liu, Y. Multi-Spectroscopic Analysis and Molecular Modeling on the Interaction of Curcumin and Its Derivatives with Human Serum Albumin: A Comparative Study. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, **2014**, *124*, 265–276. https://doi.org/10.1016/j.saa.2014.01.009.

[30] Sahu, A.; Kasoju, N.; Bora, U. Fluorescence Study of the Curcumin-Casein Micelle Complexation and Its Application as a Drug Nanocarrier to Cancer Cells. *Biomacromolecules*, **2008**, *9* (10), 2905–2912. https://doi.org/10.1021/bm800683f.

[31] Ma, Z.; Shayeganpour, A.; Brocks, D. R.; Lavasanifar, A.; Samuel, J. High-Performance Liquid Chromatography Analysis of Curcumin in Rat Plasma: Application to Pharmacokinetics of Polymeric Micellar Formulation of Curcumin. *Biomed. Chromatogr.*, **2007**, *21* (5), 546–552. https://doi.org/10.1002/bmc.795.

[32] Heath, D. D.; Pruitt, M. A.; Brenner, D. E.; Rock, C. L. Curcumin in Plasma and Urine: Quantitation by High-Performance Liquid Chromatography. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, **2003**, *783* (1), 287–295. https://doi.org/10.1016/S1570-0232(02)00714-6.

[33] Schiborr, C.; Eckert, G. P.; Rimbach, G.; Frank, J. A Validated Method for the Quantification of Curcumin in Plasma and Brain Tissue by Fast Narrow-Bore High-Performance Liquid Chromatography with Fluorescence Detection. *Anal. Bioanal. Chem.*, **2010**, *397* (5), 1917–1925. https://doi.org/10.1007/s00216-010-3719-3.

[34] Zheng, X. H.; Shao, Y. X.; Li, Z.; Liu, M.; Bu, X.; Luo, H. Bin; Hu, X. Quantitative Structure-Retention Relationship of Curcumin and Its Analogues. *J. Sep. Sci.*, **2012**, *35* (4), 505–512. https://doi.org/10.1002/jssc.201100903.

[35] Inoue, K.; Nomura, C.; Ito, S.; Nagatsu, A.; Hino, T.; Oka, H. Purification of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin by High-Speed Countercurrent Chromatography. *J. Agric. Food Chem.*, **2008**, *56* (20), 9328–9336. https://doi.org/10.1021/jf801815n.

[36] Revathy, S.; Elumalai, S.; Benny, M.; Antony, B. Isolation , Purification and Identification of Curcuminoids from Turmeric (Curcuma Longa L .) by Column Chromatography. *J. Exp. Sci.*, **2011**, *2* (7), 21–25.

[37] Chen, Z.; Quan, L.; Zhou, H.; Zhao, Y.; Chen, P.; Hu, L.; Yang, Z.; Hu, C.; Cao, D. Screening of Active Fractions from Curcuma Longa Radix Isolated by HPLC and GC-MS for Promotion of Blood Circulation and Relief of Pain. *J. Ethnopharmacol.*, **2019**, *234*, 68–75. https://doi.org/10.1016/j.jep.2018.09.035.

[38] Xu, H.; Paxton, J.; Lim, J.; Li, Y.; Wu, Z. Development of a Gradient High Performance Liquid Chromatography Assay for Simultaneous Analysis of Hydrophilic Gemcitabine and Lipophilic Curcumin Using a Central Composite Design and Its Application in Liposome Development. *J. Pharm. Biomed. Anal.*, **2014**, *98*, 371–378. https://doi.org/10.1016/j.jpba.2014.06.022.

[39] Heffernan, C.; Ukrainczyk, M.; Gamidi, R. K.; Hodnett, B. K.; Rasmuson, Å. C. Extraction and Purification of Curcuminoids from Crude Curcumin by a Combination of Crystallization and Chromatography. *Org. Process Res. Dev.*, **2017**, *21* (6), 821–826. https://doi.org/10.1021/acs.oprd.6b00347.

[40] Rahimi, M.; Hashemi, P.; Nazari, F. Cold Column Trapping-Cloud Point Extraction Coupled to High Performance Liquid Chromatography for Preconcentration and Determination of Curcumin in Human Urine. *Anal. Chim. Acta*, **2014**, *826* (1), 35–42. https://doi.org/10.1016/j.aca.2014.04.012.

[41] Gupta, A. P.; Gupta, M. M.; Kumar, S. SIMULTANEOUS DETERMINATION OF CURCUMINOIDS IN CURCUMA SAMPLES USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY. *J. Liq. Chromatogr. Relat. Technol.*, **1999**, *22* (10), 1561–1569. https://doi.org/10.1081/JLC-100101751.

[42] Abdul Zahar, Z.; Mohsin, H. F.; Ibtisam, A. W. The Study on Curcuminoids in Chromatography, Spectroscopy and Regioisomerism. *J. Phys. Conf. Ser.*, **2020**, *1529* (2). https://doi.org/10.1088/1742-6596/1529/2/022035.

[43] Lou, Y.; Zheng, J.; Hu, H.; Lee, J.; Zeng, S. Application of Ultra-Performance Liquid Chromatography Coupled with Quadrupole Time-of-Flight Mass Spectrometry to Identify Curcumin Metabolites Produced by Human Intestinal Bacteria. *J. Chromatogr. B, Anal. Technol. Biomed. life Sci.*, **2015**, *985*, 38–47. https://doi.org/10.1016/j.jchromb.2015.01.014.

[44] Oyarce, P.; De Meester, B.; Fonseca, F.; de Vries, L.; Goeminne, G.; Pallidis, A.; De Rycke, R.; Tsuji, Y.; Li, Y.; Van den Bosch, S.; et al. Introducing Curcumin Biosynthesis in Arabidopsis Enhances Lignocellulosic Biomass Processing. *Nat. Plants*, **2019**, *5* (2), 225–237. https://doi.org/10.1038/s41477-018-0350-3.

[45] Srinivasan, K. R. A Chromatographic Study of the Curcuminoids in Curcuma Longa, L. *J. Pharm. Pharmacol.*, **1953**, *5* (1), 448–457. https://doi.org/10.1111/j.2042-7158.1953.tb14007.x.

[46] Lampe, V.; Milobedzka, J. Studien Über Curcumin. *Berichte der Dtsch. Chem. Gesellschaft*, **1913**, *46* (2), 2235–2240. https://doi.org/10.1002/cber.191304602149.

[47] Grynkiewicz, G.; Ślifirski, P. Curcumin and Curcuminoids in Quest for Medicinal Status. *Acta Biochim. Pol.*, **2012**, *59* (2), 201–212. https://doi.org/10.18388/abp.2012_2139.

[48] Agarwal, S.; Mishra, R.; Gupta, A. K.; Gupta, A. *Turmeric: Isolation and Synthesis of Important Biological Molecules*; Elsevier Ltd, 2018. https://doi.org/10.1016/b978-0-08-102071-5.00005-2.

[49] Lin, L.; Shi, Q.; Nyarko, A. K.; Bastow, K. F.; Wu, C. C.; Su, C. Y.; Shih, C. C. Y.; Lee, K. H. Antitumor Agents. 250. Design and Synthesis of New Curcumin Analogues as Potential Anti-Prostate Cancer Agents. *J. Med. Chem.*, **2006**, *49* (13), 3963–3972. https://doi.org/10.1021/jm051043z.

[50] Wakte, P. S.; Sachin, B. S.; Patil, A. A.; Mohato, D. M.; Band, T. H.; Shinde, D. B. Optimization of Microwave, Ultra-Sonic and Supercritical Carbon Dioxide Assisted Extraction Techniques for Curcumin from Curcuma Longa. *Sep. Purif. Technol.*, **2011**, *79* (1), 50–55. https://doi.org/10.1016/j.seppur.2011.03.010.

[51] Mandal, V.; Mohan, Y.; Hemalatha, S. Microwave Assisted Extraction of Curcumin by Sample-Solvent Dual Heating Mechanism Using Taguchi L9 Orthogonal Design. *J. Pharm. Biomed. Anal.*, **2008**, *46* (2), 322–327. https://doi.org/10.1016/j.jpba.2007.10.020.

[52] Andrew Anderson, W. M.; Mitchell, M. S.; Mohan, R. S.; Och, C. O. In the Laboratory Isolation of Curcumin from Turmeric. *J. Chem. Educ.*, **2000**, *77* (359), 359–360.

[53] Communication, I. C.; Nabati, M.; Mahkam, M.; Heidari, H. Isolation and Characterization of Curcumin from Powdered Rhizomes of Turmeric Plant Marketed in Maragheh City of Iran with Soxhlet Technique. *Iran. Chem. Commun.*, **2014**, *2* (4), 236–

243.

[54] Turrini, F.; Donno, D.; Beccaro, G. L.; Zunin, P.; Pittaluga, A.; Boggia, R. Pulsed Ultrasound-Assisted Extraction as an Alternative Method to Conventional Maceration for the Extraction of the Polyphenolic Fraction of Ribes Nigrum Buds: A New Category of Food Supplements Proposed by the Finnover Project. *Foods*, **2019**, *8* (10), 1–14. https://doi.org/10.3390/foods8100466.

[55] Li, M.; Ngadi, M. O.; Ma, Y. Optimisation of Pulsed Ultrasonic and Microwave-Assisted Extraction for Curcuminoids by Response Surface Methodology and Kinetic Study. *Food Chem.*, **2014**, *165*, 29–34. https://doi.org/10.1016/j.foodchem.2014.03.115.

[56] Hadi, B. J.; Sanagi, M. M.; Wan Ibrahim, W. A.; Jamil, S.; AbdullahiMu'azu, M.; Aboul-Enein, H. Y. Ultrasonic-Assisted Extraction of Curcumin Complexed with Methyl-β-Cyclodextrin. *Food Anal. Methods*, **2015**, *8* (6), 1373–1381. https://doi.org/10.1007/s12161-014-0016-3.

[57] Delazar, A.; Nahar, L.; Hamedeyazdan, S.; Sarker, S. D. Microwave-Assisted Extraction in Natural Products Isolation. *Methods Mol. Biol.*, **2012**, *864*, 89–115. https://doi.org/10.1007/978-1-61779-624-1_5.

[58] Bener, M.; Özyürek, M.; Güçlü, K.; Apak, R. Optimization of Microwave-Assisted Extraction of Curcumin from Curcuma Longa L. (Turmeric) and Evaluation of Antioxidant Activity in Multi-Test Systems. *Rec. Nat. Prod.*, **2016**, *10* (5), 542–554.

[59] Ito, Y.; Ma, Y. PH-Zone-Refining Countercurrent Chromatography. *J. Chromatogr. A*, **1996**, *753* (1), 1–36. https://doi.org/10.1016/S0021-9673(96)00565-1.

[60] Patel, K.; Krishna, G.; Sokoloski, E.; Ito, Y. Preparative Separation of Curcuminoids from Crude Curcumin and Turmeric Powder by PH-Zone-Refining Countercurrent

Chromatography. *J. Liq. Chromatogr. Relat. Technol.*, **2000**, *23* (14), 2209–2218. https://doi.org/10.1081/JLC-100100482.

[61] Ito, Y. PH-Zone-Refining Counter-Current Chromatography: Origin, Mechanism, Procedure and Applications. *J. Chromatogr. A*, **2013**, *1271* (1), 71–85. https://doi.org/10.1016/j.chroma.2012.11.024.

[62] Schneider, C.; Gordon, O. N.; Edwards, R. L.; Luis, P. B. Degradation of Curcumin: From Mechanism to Biological Implications. *J. Agric. Food Chem.*, **2015**, *63* (35), 7606–7614. https://doi.org/10.1021/acs.jafc.5b00244.

[63] Gordon, O. N.; Schneider, C. Vanillin and Ferulic Acid: Not the Major Degradation Products of Curcumin. *Trends Mol. Med.*, **2012**, *18* (7), 361–363. https://doi.org/10.1016/j.molmed.2012.04.011.

[64] Gordon, O. N.; Luis, P. B.; Sintim, H. O.; Schneider, C. Unraveling Curcumin Degradation: Autoxidation Proceeds through Spiroepoxide and Vinylether Intermediates En Route to the Main Bicyclopentadione. *J. Biol. Chem.*, **2015**, *290* (8), 4817–4828. https://doi.org/10.1074/jbc.M114.618785.

[65] Griesser, M.; Pistis, V.; Suzuki, T.; Tejera, N.; Pratt, D. A.; Schneider, C. Autoxidative and Cyclooxygenase-2 Catalyzed Transformation of the Dietary Chemopreventive Agent Curcumin. *J. Biol. Chem.*, **2011**, *286* (2), 1114–1124. https://doi.org/10.1074/jbc.M110.178806.

[66] Aboudiab, B.; Tehrani-Bagha, A. R.; Patra, D. Curcumin Degradation Kinetics in Micellar Solutions: Enhanced Stability in the Presence of Cationic Surfactants. *Colloids Surfaces A Physicochem. Eng. Asp.*, **2020**, *592* (December 2019), 124602. https://doi.org/10.1016/j.colsurfa.2020.124602.

[67] Cas, M. D.; Ghidoni, R. Dietary Curcumin: Correlation between Bioavailability and Health Potential. *Nutrients*, **2019**, *11* (9), 1–14. https://doi.org/10.3390/nu11092147.

[68] Stohs, S. J.; Chen, O.; Ray, S. D.; Ji, J.; Bucci, L. R.; Preuss, H. G. Highly Bioavailable Forms of Curcumin and Promising Avenues for Curcumin-Based Research and Application: A Review. *Molecules*, **2020**, *25* (6), 1–12. https://doi.org/10.3390/molecules25061397.

[69] Nelson, K. M.; Dahlin, J. L.; Bisson, J.; Graham, J.; Pauli, G. F.; Walters, M. A. The Essential Medicinal Chemistry of Curcumin. *J. Med. Chem.*, **2017**, *60* (5), 1620–1637. https://doi.org/10.1021/acs.jmedchem.6b00975.

[70] Vareed, S. K.; Kakarala, M.; Ruffin, M. T.; Crowell, J. A.; Normolle, D. P.; Djuric, Z.; Brenner, D. E. Pharmacokinetics of Curcumin Conjugate Metabolites in Healthy Human Subjects. *Cancer Epidemiol. Biomarkers Prev.*, **2008**, *17* (6), 1411–1417. https://doi.org/10.1158/1055-9965.EPI-07-2693.

[71] Hassaninasab, A.; Hashimoto, Y.; Tomita-Yokotani, K.; Kobayashi, M. Discovery of the Curcumin Metabolic Pathway Involving a Unique Enzyme in an Intestinal Microorganism. *Proc. Natl. Acad. Sci. U. S. A.*, **2011**, *108* (16), 6615–6620. https://doi.org/10.1073/pnas.1016217108.

[72] Burapan, S.; Kim, M.; Han, J. Curcuminoid Demethylation as an Alternative Metabolism by Human Intestinal Microbiota. *J. Agric. Food Chem.*, **2017**, *65* (16), 3305–3310. https://doi.org/10.1021/acs.jafc.7b00943.

[73] Pulido-Moran, M.; Moreno-Fernandez, J.; Ramirez-Tortosa, C.; Ramirez-Tortosa, M. C. Curcumin and Health. *Molecules*, **2016**, *21* (3). https://doi.org/10.3390/molecules21030264.

[74] Wahlstrom, B.; Blennow, G. Study on Fate of Curcumin in Rat. *Acta Pharmacol. Toxicol. (Copenh).*, **1978**, *43* (2), 86–92.

[75] Luca, S. V.; Macovei, I.; Bujor, A.; Miron, A.; Skalicka-Woźniak, K.; Aprotosoaie, A. C.; Trifan, A. Bioactivity of Dietary Polyphenols: The Role of Metabolites. *Crit. Rev. Food Sci. Nutr.*, **2020**, *60* (4), 626–659. https://doi.org/10.1080/10408398.2018.1546669.

[76] Kyung, E. J.; Kim, H. B.; Hwang, E. S.; Lee, S.; Choi, B. K.; Kim, J. W.; Kim, H. J.; Lim, S. M.; Kwon, O. I.; Woo, E. J. Evaluation of Hepatoprotective Effect of Curcumin on Liver Cirrhosis Using a Combination of Biochemical Analysis and Magnetic Resonance-Based Electrical Conductivity Imaging. *Mediators Inflamm.*, **2018**, *2018*. https://doi.org/10.1155/2018/5491797.

[77] Abrahams, S.; Haylett, W. L.; Johnson, G.; Carr, J. A.; Bardien, S. Antioxidant Effects of Curcumin in Models of Neurodegeneration, Aging, Oxidative and Nitrosative Stress: A Review. *Neuroscience*, **2019**, *406*, 1–21. https://doi.org/10.1016/j.neuroscience.2019.02.020.

[78] Mazumder, A.; Raghavan, K.; Weinstein, J.; Kohn, K. W.; Pommier, Y. Inhibition of Human Immunodeficiency Virus Type-1 Integrase by Curcumin. *Biochem. Pharmacol.*, **1995**, *49* (8), 1165–1170. https://doi.org/10.1016/0006-2952(95)98514-A.

[79] Rivera-Espinoza, Y.; Muriel, P. Pharmacological Actions of Curcumin in Liver Diseases or Damage. *Liver Int.*, **2009**, *29* (10), 1457–1466. https://doi.org/10.1111/j.1478-3231.2009.02086.x.

[80] Mythri, R. B.; Harish, G.; Dubey, S. K.; Misra, K.; Srinivas Bharath, M. M. Glutamoyl Diester of the Dietary Polyphenol Curcumin Offers Improved Protection against Peroxynitrite-Mediated Nitrosative Stress and Damage of Brain Mitochondria in Vitro:

Implications for Parkinson's Disease. *Mol. Cell. Biochem.*, **2011**, *347* (1–2), 135–143. https://doi.org/10.1007/s11010-010-0621-4.

[81] Tajuddin, W. N. B. W. M.; Lajis, N. H.; Abas, F.; Othman, I.; Naidu, R. Mechanistic Understanding of Curcumin's Therapeutic Effects in Lung Cancer. *Nutrients*, **2019**, *11* (12), 1–28. https://doi.org/10.3390/nu11122989.

[82] Zhang, L.; Xu, T.; Wang, S.; Yu, L.; Liu, D.; Zhan, R.; Yu, S. Y. Curcumin Produces Antidepressant Effects via Activating MAPK/ERK-Dependent Brain-Derived Neurotrophic Factor Expression in the Amygdala of Mice. *Behav. Brain Res.*, **2012**, *235* (1), 67–72. https://doi.org/10.1016/j.bbr.2012.07.019.

[83] Ghoneim, A. I.; Abdel-Naim, A. B.; Khalifa, A. E.; El-Denshary, E. S. Protective Effects of Curcumin against Ischaemia/Reperfusion Insult in Rat Forebrain. *Pharmacol. Res.*, **2002**, *46* (3), 273–279. https://doi.org/10.1016/S1043-6618(02)00123-8.

[84] Karaman, M.; Firinci, F.; Cilaker, S.; Uysal, P.; Tugyan, K.; Yilmaz, O.; Uzuner, N.; Karaman, O. Anti-Inflammatory Effects of Curcumin in a Murine Model of Chronic Asthma. *Allergol. Immunopathol. (Madr).*, **2012**, *40* (4), 210–214. https://doi.org/10.1016/j.aller.2011.04.006.

[85] Ukil, A.; Maity, S.; Karmakar, S.; Datta, N.; Vedasiromoni, J. R.; Das, P. K. Curcumin, the Major Component of Food Flavour Turmeric, Reduces Mucosal Injury in Trinitrobenzene Sulphonic Acid-Induced Colitis. *Br. J. Pharmacol.*, **2003**, *139* (2), 209–218. https://doi.org/10.1038/sj.bjp.0705241.

[86] Yeon, K. Y.; Kim, S. A.; Kim, Y. H.; Lee, M. K.; Ahn, D. K.; Kim, H. J.; Kim, J. S.; Jung, S. J.; Oh, S. B. Curcumin Produces an Antihyperalgesic Effect via Antagonism of TRPV1. *J. Dent. Res.*, **2010**, *89* (2), 170–174. https://doi.org/10.1177/0022034509356169.

[87] Dikmen, M.; Canturk, Z.; Ozturk, Y.; Tunali, Y. Investigation of the Apoptotic Effect of Curcumin in Human Leukemia HL-60 Cells by Using Flow Cytometry. *Cancer Biother. Radiopharm.*, **2010**, *25* (6), 749–755. https://doi.org/10.1089/cbr.2010.0822.

[88] Cheng, Y.; Ping, J.; Xu, L.-M. Effects of Curcumin on Peroxisome Proliferator-Activated Receptor Gamma Expression and Nuclear Translocation/Redistribution in Culture-Activated Rat Hepatic Stellate Cells. *Chin. Med. J. (Engl).*, **2007**, *120* (9), 794–801.

[89] Thangapazham, R. L.; Sharma, A.; Maheshwari, R. K. Beneficial Role of Curcumin in Skin Diseases. *Adv. Exp. Med. Biol.*, **2007**, *595*, 343–357. https://doi.org/10.1007/978-0-387-46401-5_15.

[90] Arbiser, J. L.; Klauber, N.; Rohan, R.; Van Leeuwen, R.; Huang, M. T.; Fisher, C.; Flynn, E.; Byers, H. R. Curcumin Is an in Vivo Inhibitor of Angiogenesis. *Mol. Med.*, **1998**, *4* (6), 376–383. https://doi.org/10.1007/bf03401744.