A comprehensive review on *Curcuma longa* Linn.: Phytochemical, pharmacological, and molecular study

Kamran Ashraf[1,2], Sadia Sultan[1,2]

[1]Department Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia, [2]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

Abstract

Curcuma longa Linn. is well-known and valued medicinal plant. It has a long history of traditional uses ranging from folk medicine to several culinary preparations. The phytochemical, pharmacological, and molecular studies of *C. longa* are reviewed. The rhizome is rich in essential oils, and various numbers of chemical constituents with biomedical significance have been isolated from it. The management of indigenous knowledge by appropriate documentation is recommended. This review was compiled to provide recent consolidated information covering different aspects of the plant, phytochemical, pharmacological, and molecular study to provide a basis on which to plan future studies and to promote sustainable use of *C. longa*.

Key words: Antidiabetic, *Curcuma longa*, curcuminoids, random amplification polymorphism DNA

INTRODUCTION

Turmeric (*Curcuma longa* L.) belongs to the family Zingiberaceae includes more than 80 species of rhizomatous perennial herbs and has widespread existence in the tropics of Asia, Africa, and Australia.[1] It is a perennial herbaceous plant, which reaches a stature of up to 1 m. There are highly branched, yellow-to-orange, cylindrical, aromatic rhizomes. *C. longa*, commonly known as turmeric (Haldi), is a well-known plant which is used as a drug in Ayurvedic and Unani system of medicine.[2] Others various common name includes *Curcuma* (Sp. It. Fr.), acafrao da India (port.), geelwortel (Dutch), kurkum (Arab), Manjano (East Africa [KiSwahili]), manjal (Tamil), kunyit (Indonesia), temukunyit (Malaysian), and iyu-chin (Chin.). The most important chemical components of turmeric are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin.[3-5] India is the largest producer, consumer, and exporter of turmeric in the world and contains highest diversity (40 species) of *C. longa*.[2] The World Health Organization has suggested the use of turmeric as a spice.[6] Comprehensively, *Curcuma* is

attainment importance as a growing source of new drug (s) to fight a variety of ailments as the species contain molecules validated with anti-fungal properties,[7] anti-inflammatory, hepatoprotective, antitumor, antiviral,[8] and anticancer activities.[9] The structure of curcuminoids are shown in Figure 1.

In recent year, several studies have come out in the literature about this plant, and this may imitate the status of the subject and its common use as a spice and a medicinal plant. From the literature, it was found that very few or no review is available which correlates the data of phytochemical, pharmacological, and molecular properties of turmeric together. Here, the aim was to recapitulate the more recent and common actions including the phytochemical, pharmacological, and molecular aspect of *C. longa*.
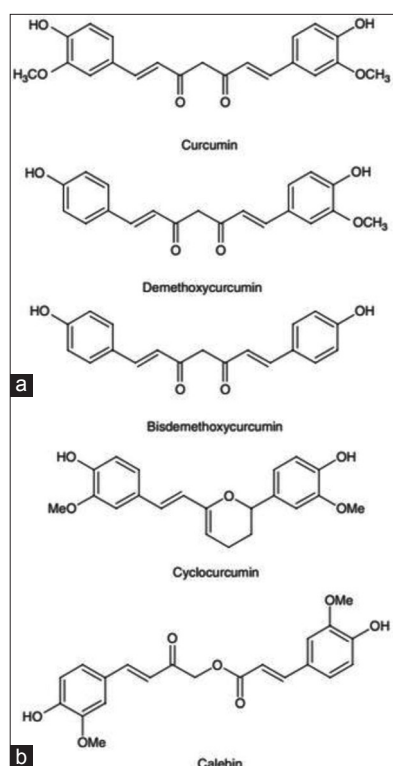
Address for correspondence:
Dr. Kamran Ashraf, Department Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia. Phone: +60183238786, Fax: +603 3258 4602.
E-mail: kamran1368@puncakalam.uitm.edu.my

Figure 1: (a and b) Structure of curcumin, demethoxycurcumin, bisdemethoxycurcumin, cyclocurcumin, and calebin in *Curcuma longa*

Chemical Constituents of Turmeric

Phytochemical investigations carried out on *C. longa* revealed the presence of many rich sources of polyphenolic curcuminoids, i.e., curcumin, demethoxycurcumin, and bisdemethoxycurcumin.[10] Curcuminoids contain curcumin the principal curcuminoids (about 80%), and other two curcuminoids are demethoxycurcumin (about 12%) and bisdemethoxycurcumin[11,12] along with other one's protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%), and sesquiterpenes (53%).[13]

Medicinal Uses

As part of the ancient Indian medical system, Ayurveda, a poultice of turmeric paste is used to treat common eye infections and to dress wounds, treat bites, burns, acne, and various skin diseases.[14] In northern India, women are given a tonic of fresh turmeric paste with powder of dried ginger roots and honey in a glass of hot milk to drink twice daily after childbirth. Johnson and Johnson (An American Pharma Company) makes turmeric band-aids for the Indian market.[15] A poultice of turmeric is also applied to the perineum to aid in the healing of any lacerations in the birth canal.[16] Powdered turmeric when taken with boiled milk is helpful in curing cough and related respiratory ailment, and roasted turmeric

is an ingredient used as an antidysenteric for children.[14] Turmeric is also used in the treatment of dental diseases, digestive disorders such as dyspepsia and acidity, indigestion, flatulence, ulcers, antioxidant, antifertility as well to alleviate the hallucinatory effects of hashish, and other psychotropic drugs.[16-18] In food and manufacturing, curcumin is currently used in perfumes and as a natural yellow-coloring agent, as well as an approved food additive to flavor various types of curries and mustards.[19,20] Recent emphasis on the use of natural and complementary medicines in western medicine has drawn the notice of the scientific community to this ancient remedy. Current researches have revealed that curcumin has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, and chemopreventive and chemotherapeutic activity. These activities have been demonstrated both in cultured cells and animal models and have paved the way for ongoing human clinical trials.

Adulteration

Turmeric and other spices are commonly the potential exist for powders of toxic, cheaper agents with a similar color to be added, such as lead (II, IV) oxide, giving turmeric an orange-red color in its place of its native gold-yellow.[21] Metanil yellow (also known as acid yellow 36) is another common adulterant in turmeric. It is believed to be an illegal dye for use in foods by the British Food Standards Agency.[22]

PHYTOCHEMICAL ANALYSIS

A curcuminoids are a linear diarylheptanoid, with molecules such as curcumin or derivatives of curcumin with different chemical groups. These compounds are natural phenols and produce a pronounced yellow color. Many curcumin characters are incompatible for use as drugs by themselves. They have a meager solubility in water at acidic and physiological pH and also hydrolyze rapidly in alkaline solutions. Therefore, curcumin derivatives are synthesized to increase their solubility and hence bioavailability.[23] Curcuminoids are soluble in dimethyl sulfoxide, acetone, and ethanol but are poorly soluble in lipids.[24] The solubility of curcuminoids is enhanced in the aqueous phase with the application of surfactants or cosurfactants.[25] Several studies have been reported to separate curcuminoids pigments by thin-layer chromatography (TLC), high-performance TLC (HPTLC), and column chromatography. Silica gel is used as a most common stationary phase with different solvent systems including benzene, ethyl acetate, ethanol, chloroform, acetic acid, hexane, and methanol for chromatographic separations.[26,27] A HPTLC method was developed for the simultaneous determination of curcuminoids in turmeric plant extract using chloroform:methanol (95:5) as the mobile phase. They separated at the R_F value 0.69, 0.44, and 0.29 for curcumin, demethoxycurcumin, and bisdemethoxycurcumin,

respectively.[27] In another experiment, analysis of curcuminoids in Chinese herbal medicine was carried out using MEEKC. They are separated using uncoated fused-silica capillary column with a buffer consisting of 25mM hydroxypropyl-β-CD, 10% MeOH, 0.04M sodium borate, and 0.04 M sodium dodesylsulfate at pH 9.50 <10 min. The recoveries obtained were in the range of 95.7–106.3%. The calibration curves exhibited good linearity in the range of 90–1220 µg/mL with r of 0.9996 for C, 80–1120 µg/mL with r of 0.9998 (DMC), and 80–1200 µg/mL with r of 0.9998 (BDMC).[28] Analysis of curcuminoids in some *Curcuma* genus which are indigenous to Indonesia, namely, *C. manga* Val. and *v. Zijp*, *C. heyneana* Val. and *v. Zijp*, *C. aeruginosa* Roxb., and *C. soloensis* Val. was carried out using high-performance liquid chromatography (HPLC) with PDA detector at 425 nm. The separation was achieved using Zorbax Eclipse XDBC18 (250 × 4.6 mm i.d.; 5 µm) with mobile phase consisted of a mixture of MeOH-H$_2$O (containing 0.1% trifluoroacetic acid)-acetonitrile (39.5:350:468, v/v/v). The developed method gives the accuracy of 100.4 ± 0.922% (C), 99.8 ± 0.806% (DMC), and 99.9 ± 0.574% (BDMC), with limit of detection of 0.044 µg for C, 0.048 µg for DMC, and 0.058 µg for BDMC.[29] Payton *et al*.[30] have carried out experiment on nuclear magnetic resonance (NMR) study of the solution structure of curcumin. The solution structure of curcumin was determined on the basis of NMR techniques including DEPT, HMQC, HMBC, and COSY. The results of the NMR studies showed that curcumin exists in solution as keto–enol tautomers. In another experiment, a HPTLC method for the determination of curcuminoids in turmeric plant extract has been reported by Paramasivam *et al*.[31] In this method, different solvents were tried for the extraction of curcuminoids, and ultimately, methanol gave the maximum extraction of the compound. The separations of the curcuminoids were performed with chloroform:methanol (48:2, v/v) as the mobile phase. The assay combined the separation and quantification of analysts on silica gel 60GF254 HPTLC plates with visualization under ultraviolet (UV) and scanned at 425 nm. Zhang *et al*.[32] have achieved successful separation of curcuminoids within 30 min by a Cadenza CD-C18 column (250 ¥ 4.6 mm; i.d., 3 mm) with a mixture of 0.1 M of acetate buffer (pH 4.0) and CH3CN (= 57:43, v/v) as a mobile phase. The retention times of BDMC, DMC, and C were 19, 22, and 25 min, respectively. The developed method was also validated to determine its accuracy and precision by carrying out linearity and recovery experiment. In most cases, HPLC methods with UV/visible detection at around 260 or 450 nm were used, and these methods require simple instruments and are sufficiently sensitive to determine curcuminoids in rhizomes of *Curcuma* species.[33] Paramapojn and Gritsanapan[34] have carried out the determination of curcuminoids in turmeric plant extract by HPLC method and were found to be sensitive, precise, and accurate in the extract of rhizome *C. longa*. In another study, Herebian *et al*.[35] showed for the first time a gas chromatography–mass spectrometry-based method for metabolic profiling of the hydrophilic extract. They also

identified 61 polar metabolites as TMS derivatives. In another study, HPLC–UV–MS experiment was performed on an Agilent series 1100 HPLC system including a quaternary pump, a variable wavelength detector, an autosampler, and a column compartment. The separation was performed on a YMC ODS-A C18 reversed column (250 4.6 mm i.d., 5 lm) equipped with an Agilent Zorbax SB-C18 guard column (12.5 4.6 mm i.d., 5 lm) at 30°C. The mobile phase consisted of acetonitrile (CH3CN, A) and 0.1% formic acid in water (B) using a gradient program of 40–50% (A) in 0–30 min, 50–65% (A) in 30–35 min, 65–70% (A) in 35–42 min, 70% (A) in 42–55 min, and 70–100% (A) in 55–60 min. The mobile phase flow rate was 1.0 mL/min. The UV detector was monitored at 270 nm for fingerprinting analysis and 380 nm for quantitative analysis.[36] Ashraf *et al*. reported variations in content of curcumin in *C. longa* by HPTLC methods across Indian subcontinent. Linear concentration of curcumin was found to be 50–1000 ng/mL. The mobile phase consisting of toluene:chloroform:methanol (5:4:1, v/v/v) gave a sharp and well-defined peak at R$_F$ value of 0.31. The lowest amount of curcumin, which was limit of detected, was 50 ng/spot. The lowest amount of curcumin, which was limit of quantified, was found to be 200 ng/spot.[2] Recently, Ashraf *et al*. have carried out determination of curcuminoids in different samples of *C. longa* by UPLC/MS method.[37] Mudge *et al*. have carried out analysis of curcuminoids in turmeric roots and supplements. The method was optimized and validated and also reported that this validated method is suitable for quantitation of individual curcuminoids in turmeric raw materials and finished products.[38] In another study, Dhakal *et al*. have applied Fourier Transform-Raman (FT-Raman) and FT-infrared (FT-IR) spectroscopy as separate but complementary methods for detecting metanil yellow adulteration of turmeric powder in turmeric. Spectral analysis showed that the FT-IR method in this study could detect the metanil yellow at the 5% concentration, while the FT-Raman method appeared to be more sensitive and could detect the metanil yellow at the 1% concentration.[39] Recently, Sahne *et al*. have carried out comparative study of extraction of bioactive compound curcumin from turmeric (*C. longa*) through different routes.[40] In this experiment, extraction of curcumin was carried out through different extraction methods such as microwave-assisted, ultrasound-assisted, and enzyme-assisted extractions. These were used as modern extraction routes to extract curcumin from turmeric powder, and the yield was investigated. The presence of curcumin in the extracted samples was confirmed by UV-visible spectroscopy, and quantification of curcumin was carried out by HPLC analysis. Result showed that Soxhlet method gives the highest yield. In another recent study, Osorio-Tobón *et al*. carried out fast analysis of curcuminoids from turmeric (*C. longa* L.) by high-performance liquid chromatography using a fused-core column. They developed and validated the method as well. In this method, they used different extracts of turmeric rhizome and products in their formulation.[41] Monton *et al*. have carried out quantitation of curcuminoids contents, dissolution profile, and volatile oil content of

turmeric capsules produced at some secondary government hospitals.[42] In a very recent study, Jin *et al.*[43] have reported an integrated strategy for establishment of curcuminoids profile in turmeric using two LC–MS/MS platforms. Some of the phytochemical analysis work has been summarized in Table 1.

PHARMACOLOGICAL ACTIONS

Turmeric powder, curcumin demethoxycurcumin, and bisdemethoxycurcumin were found to be bioactive. Pharmacological action of *C. longa* is mainly due to its main active constituent curcumin present in *C. longa*. Different extract of turmeric such as ethanolic, aqueous, ether, and petroleum ether shows different types of activities.

Action on Gastrointestinal System

Turmeric powder increases mucin secretion in rabbits and may thus act as gastroprotectant against irritants.[57] It also shows both anti-ulcer[58] and ulcerogenic activities.[59,60] They also observed antiflatulent activity in both *in vivo* and *in vitro* experiments in rats. Curcumin was also found to increase intestinal lipase, sucrose, and maltase activity.[61,62] Lin *et al.*[28] have reported that curcumin suppresses intestinal fibrosis by inhibition of PPARΓ-mediated epithelial-mesenchymal transition. Zhang *et al.*[63] have carried out experiment on the protective effect of curcumin on TNBS-induced intestinal inflammation which is mediated through the JAK/STAT pathway and reported excellent results.

Dyspepsia and Gastric Ulcer

Curcumin has significant effect on dyspepsia and gastric ulcer. In a clinical trial phase II experiment, 45 subjects with endoscopically diagnosed peptic ulcers were given 600 mg curcumin 5 times daily for 12 weeks. It was seen that ulcers were absent in 12 patients (48%) after 4 weeks, 18 patients after 8 weeks, and 19 patients (76%) after 12 weeks. The remaining 20 patients, also given curcumin, had no noticeable ulcerations at the start of the study but were symptomatic-erosions, gastritis, and dyspepsia. Abdominal pain and other symptoms had decreased significantly within 1–2 weeks.[64] Kim *et al.*[65] discovered the defensive effect of turmeric ethanolic extract against gastric ulcers by blocking H2 histamine receptors (H2R) of male Sprague–Dawley (pylorus-ligated) rats. The effect of *C. longa* extract was compared to the properties of ranitidine. *Curcuma* was found to protect the gastric mucosal layer as effective as ranitidine. Orally administered ethanolic extract is believed to inhibit gastric acid, gastric juice secretion, and ulcer formation comparable to the properties of ranitidine. Rafatullah *et al.*[66] examined the antiulcer activity of an ethanolic extract of turmeric. Administration of turmeric extract led to a noteworthy decrease in ulcer index and acidity of stomach contents. Pre-treatment with the turmeric extract reduced the intensity of ulceration. Hypothermic-restraint stress reduction of gastric wall mucus was inhibited by turmeric extract treatment and reduced the severity of lesions induced by various necrotizing agents.

Action on Cardiovascular System

Turmeric acts as a protective action on the cardiovascular system. It causes lowering cholesterol and triglyceride levels, falling susceptibility of low-density lipoprotein (LDL) to lipid peroxidation, and preventing platelet aggregation.[67] Turmeric extract also causes to decrease predisposition of LDL to lipid peroxidation, furthermore to lower plasma cholesterol and triglyceride levels. Turmeric extract effect on cholesterol levels may be due to decreased cholesterol uptake in the intestines and increased conversion of cholesterol to bile acids in the liver. Inhibition of platelet aggregation by *C. longa* constituents is thought to be through potentiation of prostacyclin synthesis and inhibition of thromboxane synthesis.[68] Curcumin also causes protection against oxidative damage produced during atherosclerosis development by mobilizing α-tocopherol from adipose tissue. It also increases VLDL cholesterol transport in plasma, which results in increasing levels of α-tocopherol. Curcumin mobilizes α-tocopherol from adipose tissue, thus protecting their body against oxidative damage produced during the development of atherosclerosis. The study showed that increasing levels of α- tocopherol in plasma are due to more LDL cholesterol transportation. As a whole, the fatty acids in the animals were less susceptible to oxidation in the vessel wall.[69] In another experiment, it was observed that oral intake of 500 mg/d curcumin for 7 days resulted in a significant decrease in the level of serum lipid peroxides (33%), increase in high-density lipoprotein cholesterol (29%), and a decrease in level of total serum cholesterol (12%).[70] LDL in plasma and total cholesterol level in liver were found to decrease by action of curcumin. It also increases *a*-tocopherol level in rat plasma, suggesting *in vivo* interaction between curcumin and *a*-tocopherol.[71] The fatty acid content was found to increase after ethanol-induced liver damage is significantly decreased by curcumin treatment.[72] Curcumin causes the severity of pathological changes, and thus, it protects the damage of the heart from myocardial infarction.[73] Calcium transport was improved by curcumin helps in pharmacological interventions to correct the defective Ca^{2+}homeostasis in the cardiac muscle.[74] Curcumin has significant hypocholesteremic effect in hypercholesteremic rats.[75]

Antidepressant Properties and Effect on Nervous System

The antidepressant effect of curcumin was explored in chronic mild stress (CMS) model. In assessment with normal rats, rats suffering from the CMS procedure have a considerable

Table 1: Previous phytochemicals analysis studies on *Curcuma longa*				
Bioactive constituents of *C. longa*	**Analytical methods**	**Mobile phase**	**Wavelength**	**Reference**
Curcumin	A HPTLC method for chemotaxonomic evaluation of some *Curcuma* species and their commercial samples	Chloroform:ethanol: acetic acid (95:4:5 v/v/v)	260 nm	[44]
Curcuminoids	Determination of curcuminoids pigments in turmeric by reverse phase HPLC	Acetonitrile: water (55:45% v/v)	425 nm	[45]
Curcumin, dimethoxycurcumin, and bisdemethoxycurcumin	Extraction and purification of curcuminoids from *C. longa* using HPTLC	Chloroform: methanol (95:5 v/v)	420 nm	[34]
Curcumin	Validated method for estimation of curcumin from different varieties of *C. longa* by HPTLC	Chloroform: methanol (9.5:0.5 v/v)	421 nm	[46]
Curcumin, demethoxycurcumin, and bisdemethoxy curcumin	HPTLC method for quantitative determination of curcuminoids in *C. longa* germplasm	Chloroform: methanol (48:2, v/v)	425 nm	[47]
Curcumin, demethoxycurcumin, and bisdemethoxycurcumin	Occurrence of curcuminoids in *C. longa* quality standardization by HPTLC	Chloroform: methanol (48:2, v/v)	425 nm	[48]
Curcumin	Standardization of an Ayurveda formulation - kalyanavleha and estimation of curcumin using HPTLC	Toluene:ethyl acetate (8:2) and toluene:ethyl acetate: methanol (9:1:1) as mobile phase for hexane soluble and chloroform-soluble fractions, respectively	366 nm	[49]
Curcumin	Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations	Chloroform: methanol (9.25:0.75 v/v)	430 nm	[32]
Curcumin	Validated HPTLC method for estimation of curcumin content in dietary supplement formulation	n-Hexane:ethyl acetate: methanol:formic acid (8:2:1:2–3 drops v/v)	421 nm	[50]
Curcumin, demethoxycurcumin, and bisdemethoxycurcumin	Development and standardization of turmeric cream by HPTLC	Chloroform:ethanol: acetic acid (48:2:0.1 v/v/v	300 nm	[51]
Curcumin	Stability-indicating RP-HPLC determination of curcumin *in vicco* turmeric cream and Haridrakhandchurna	ACN: THF: 2% acetic acid: water (35:30:20:15 v/v/v/v)	429 nm	[52]
Curcumin, desmethoxy curcumin, and bisdesmethoxy curcumin	A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts	Acetonitrile and 2% v/v acetic acid (40:60, v/v)	425 nm	[53]
Curcumin	A sensitive reversed phase HPLC method for the determination of curcumin	Acetonitrile tetrahydrofuran: 2% acetic acid (50:30:20 (2%), v/v/v)	425 nm	[54]
Curcumin	Validated method for estimation of curcumin in turmeric powder	Dichloromethane and methanol (99:1, v/v)	427 nm	[55]
Curcumin, demethoxycurcumin, and bisdemethoxycurcumin	Development of HPTLC method and its validation for the estimation of curcuminoids from polyherbal mouth ulcer gel formulation	Chloroform:methanol: glacial acetic acid (7.5:2.0:0.5 v/v/v)	430 nm	[56]

HPLC: High-performance liquid chromatography, HPTLC: High-performance thin-layer chromatography, *C. longa*: *Curcuma longa*

lower consumption of sucrose, increased interleukin (IL-6), tumor necrosis factor alpha (TNF-α) levels, CRF, and cortisol levels. Ethanolic extract of turmeric causes to increase the sucrose intake to normal control levels reduced the CMS-induced increase in serum IL-6 and TNF-α level and reduced the CRF levels in serum and medulla oblongata to lower than normal. It also lowered the cortisol levels in serum to normal levels. Antidepressants properties of turmeric are mediated through inhibition of monoamine oxidize A.[76] Ethanolic extract of *C. longa* causes to reverse the decrease in serotonin, noradrenaline, and dopamine concentrations as well as the increase in serotonin turnover, cortisol levels, and serum corticotrophin-releasing factor.[77] Xu *et al*.[78] examined the consequence of orally directed curcumin on behavior in a long-lasting stress model of depression in rats. The antidepressant imipramine was used as a control. Administration of curcumin exhibited similar properties as imipramine. These discoveries propose that the properties of chronic administration of curcumin on the conduct of chronic stressed rats may be connected to the controlling properties of the dysfunction of the hypothalamic-pituitary-adrenal axis, through a discerning increase in brain-derived neurotrophic factor in the frontal cortex and the hippocampus of the rats. A direct effect of curcumin in decreasing the amyloid pathology of Alzheimer's disease (AD) has been shown by an experimental model of AD.[79] Several studies showed that curcumin possessed multiple actions in the brain. It can be a future drug of therapy for the treatment of various neurological disorders such as major depression, tardive dyskinesia, and diabetic neuropathy.[80] Curcumin and manganese complex of curcumin affectionate defensive acts next to vascular dementia by showing antioxidant activity.[81,82]

Anti-inflammatory Activity

C. longa plays very vital role in reducing inflammatory swelling. Oral administration of curcumin was found to be as effective as cortisone or phenylbutazone in acute inflammation. Anti-inflammatory properties of *C. longa* may be endorsed to its skill to restrain both biosynthesis of inflammatory prostaglandins from arachidonic acid and neutrophil function during inflammatory states. Curcuminoids also prevent LOX, COX, phospholipases, leukotriene's, prostaglandins, thromboxane, nitric oxide elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1, interferon-inducible protein, TNF, and IL-12. It also decreases prostaglandin formation and inhibits leukotriene biosynthesis through the lipoxygenase pathway.[83] Carrageenan-induced edema in rats and mice experiment was done to check the anti-inflammatory effect of curcumin and found to be effective.[84-87] FHM and BHM (natural analogs of curcumin) also possess anti-inflammatory activity. Another study showed that petroleum ether, alcohol water, and more importantly volatile oil extract of *C. longa* have anti-inflammatory effect.[88,89] The anti-inflammatory

effect of curcumin has acted through inhibition of NF*k*B activation.[90] Curcumin also enhances wound healing in diabetic rats and mice.[91]

Antioxidant Effect

Several works have been done in the past for the antioxidant property of curcumin. Sharma[92] supported antioxidant property of curcumin. It acts as a scavenger of oxygen free radicals.[93,94] It can protect hemoglobin from oxidation.[95,96] *In vitro*, curcumin can significantly reduce speed of the production of reactive oxygen species such as superoxide anions, hydrogen peroxide, and nitrite radical production. This is done by activated macrophages, which take part in a vital job in inflammation.[97] Different extracts of turmeric such as water and fat soluble and its curcumin component exhibit strong antioxidant activity. Pre-treatment of curcumin causes and decreases ischemia-induced changes in the heart.[98] In another study *in vitro* measuring, the effect of curcumin on endothelial heme oxygenase-1, an inducible stress protein, was carried out by utilizing bovine aortic endothelial cells.[19] Curcumin was found to reduce the testicular damage caused by exposure to di-n-butyl phthalate, by an increase in glutathion (GSH), testosterone levels, and glucose-6-phosphate dehydrogenase activity and decrease in malondialdehyde (MDA) levels. These properties may be due to intrinsic antioxidative abilities of curcumin.[99] Curcumin is only antimutagenic against mutagens which require metabolic activation. Curcumin was found to block cyclosporine A-resistant phorbol myristate acetate + anti-CD28 pathway of T-cell proliferation.[100] Clinical research on curcumin's therapeutic advantage for pancreatitis is inadequate and has mainly focused on its antioxidant properties. However, research indicates that the inflammatory response plays a critical role in the development of pancreatitis and subsequent tissue damage. Consequently, it appears likely an anti-inflammatory agent like curcumin, effective against a variety of inflammatory molecular targets, and shown to decrease inflammatory markers in an animal model of pancreatitis. One pilot study examined the effect of curcumin for tropical pancreatitis in patients.[101] Action consequence on pain patterns as well as erythrocyte MDA (MDA; an indicator of lipid peroxidation) and GSH was evaluated at baseline and after 6 weeks. In the curcumin group, there was a significant drop in MDA levels. Further research is needed to determine the role of lipid peroxidation in pain and other symptomology associated with pancreatitis.[102]

Anticarcinogenic Effect - Induction of Apoptosis

Curcumin plays an important role as anti-carcinogenic agent. Apoptosis by induction plays a significant task in its anti-carcinogenic effect. Actually, apoptosis was caused by this and block cell-cycle series, both of which are involved in reducing cancerous cell enlargement in rat aortic smooth muscle cells.[103] Although curcumin may act differently

on different cell lines. However, leukemia, breast, colon, hepatocellular, and ovarian carcinoma cells go away through apoptosis in the occurrence of curcumin, lung, prostate, kidney, cervix, and central nervous system malignancies and melanoma cells illustrate fight to cytotoxic effect of curcumin.[104] Curcumin also decreases the production of rat thymocytes. These powerfully involve that cell growth and cell death contribute to a common lane.[104,105]

In vitro and *in vivo* studies clearly indicated that turmeric and curcumin are proficient of suppressing the activity of several common mutagens and carcinogens in a variety of cell types.[106] The direct antioxidant and free-radical scavenging properties of turmeric and curcumin caused anticarcinogenic effect as well as their ability to indirectly increase glutathione levels, thereby aiding in hepatic detoxification of mutagens and carcinogens, and inhibiting nitrosamine formation and curcumin also induces apoptosis of cancer cells and it inhibits angiogenesis.[107] Supplementation with turmeric and curcumin may not be without harm. Laboratory findings show that dietary turmeric may actually inhibit the antitumor activity of chemotherapeutic agents such as cyclophosphamide and doxorubicin.[108] The use of both curcumin and turmeric extract during carcinogenesis and promotion resulted in less papilloma production, compared to controls. This specifies that both curcumin and turmeric extracts produce their best properties during tumor promotion. The effect of *C. longa* on myocardial apoptosis in experimentally induced myocardial ischemia-reperfusion injury was also investigated.[109] *C. longa* demonstrated significant antiapoptotic property, which might contribute to the observed preservation in cardioprotective properties and cardiac function. Some evidence showed that curcumin inhibits the activity of certain chemotherapy drugs. Research discloses curcumin decreased camptothecin-induced death of cultured breast cancer cells and prevented cyclophosphamide-induced breast tumor regression in mice.[110] Curcumin might also interfere with the absorption and efficacy of the chemotherapy drug irinotecan, which is used to treat colon cancer. On the other hand, curcumin may enhance the effects of some chemotherapy drugs. In a mouse xenograft model of human breast cancer, curcumin in conjunction with paclitaxel (Taxol) significantly inhibited breast cancer metastasis to the lung to a greater degree than either curcumin or paclitaxel alone.[111]

Antimicrobial Properties

Composition and antimicrobial activity of the essential oil from leaves of *C. longa* L. kasur variety was investigated. The antimicrobial properties of leaves of *C. longa* were tested by disc diffusion method against various human pathogens, including eight fungal and five bacterial strains. Essential oil showed maximum resistance against *Fusarium miniformes* MAY 3629 followed by *Bacillus subtilis* ATCC 6633, whereas it exhibited least resistance against *Fusarium oxysporum* ATCC 48122.[112] Another study, in which guinea pigs were infected with dermatophytes, pathogenic molds,

or yeast, found that topically applied turmeric oil inhibited dermatophytes and pathogenic fungi. Lesions developments were detected in the dermatophyte- and fungi-infected guinea pigs, and it disappeared at 7 days post-turmeric application. Curcumin has also been found to have moderate activity against *Plasmodium falciparum* and *Leishmania major* organisms.[113] Khattak *et al*.[114] studied the antifungal, antibacterial, phytotoxic, cytotoxic, and insecticidal activity of an ethanolic extract of turmeric. The extract exhibited antifungal activity toward *Trichophyton longifusus* and *Microsporum canis* and meager antibacterial activity against *Staphylococcus aureus*. Toxic activity was detected against *Lemna minor*. The *C. longa*-treated rabbit group showed a notable higher mean value for the decline of the wound related to controls. Furthermore, the wounds showed less inflammation and an increasing trend in the formation of collagen.[115]

Antimutagenic Activity

Curcumin shows together pro- and anti-mutagenic effects. Curcumin is shown to decrease the numeral of abnormal cells in cyclophosphamide- induced chromosomal aberration in Wistar rats at 100 and 200 mg/kg body wt doses.[116] Turmeric also prevents mutation in urethane (a powerful mutagen) models.[117]

Anticoagulant Activity

Curcumin has found to possess anticoagulant activity. Its mechanism of action is to inhibit collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta.[118] Grag[19] reported antifertility activity about 100% in rats when fed orally in petroleum ether and aqueous extracts of turmeric rhizomes. Again Garg *et al*.[119] also reported that implantation is totally repressed by these extracts. Curcumin is also found to inhibit 5*a*-reductase, which changes testosterone to 5*a*-dihydrotestosterone, in that way inhibiting the enlargement of flank organs in hamster. Curcumin also inhibits human sperm motility, and it is a sign of possible for the progress of a novel intra-vaginal contraceptive.[120]

Antidiabetic Effect

A number of experiments carried out in the past which showed the antidiabetic property of curcumin. A hexane extract (containing ar-turmerone), ethanolic extract (containing ar-turmerone, curcumin, demethoxycurcumin, and bisdemethoxycurcumin), and ethanolic extract from the residue of the hexane extraction (containing curcumin, demethoxycurcumin, and bisdemethoxycurcumin) were found to dose-dependently stimulate adipocyte differentiation. In this experiment, it was found that ethanolic extract of turmeric containing both curcuminoids and sesquiterpenoids is more powerfully hypoglycemic than either curcuminoids or

sesquiterpenoids.[121] Wickenberg *et al.*[122] studied the effects of turmeric on postprandial plasma glucose and insulin in healthy subjects; they found that the ingestion of 6 g *C. longa* had no considerable effect on the glucose response. The alteration in insulin reported was appreciably higher 30 min and 60 min after the OGTT including *C. longa*. The insulin AUCs were also significantly higher after the ingestion of *C. longa* after the OGTT. Turmeric was found to reduce blood sugar level in alloxan-induced diabetes in rat.[123] In another experiment, it showed that, at very low dose, curcumin prevents galactose-induced cataract formation.[124] Turmeric (curcumin) and its oil portion suppress growth of a number of bacteria such as *Streptococcus*, *Staphylococcus*, and *Lactobacillus*.[125] The aqueous extract of turmeric rhizomes is reported to act as antibacterial effects.[126]

Antifungal Effect

Due to wide traditional use of turmeric in food products, several studies have been done to study the turmeric and curcumin with the aspect of regulatory fungal related spoilage and fungal pathogens. Concentration of curcumin plays very important role in prevention of growth of fungus. Addition of turmeric powder in plant tissue culture at the 0.8 and 1.0g/L had considerable inhibitory activity against fungal infections.[127] The methanol extract of turmeric showed antifungal activity against *Cryptococcus neoformans* and *Candida albicans* with minimum inhibitory concentration (MIC) values of 128 and 256 µg/mL, respectively.[128] Hexane extract of *C. longa* at 1000 mg/L confirmed antifungal effect against *Rhizoctonia solani, Phytophthora infestans*, and *Erysiphe graminis*. The study also revealed that 1000 mg/L of ethyl acetate extract of *C. longa* shown inhibitory effect against *R. solani, P. infestans*, *Puccinia recondita*, and *Botrytis cinerea*. At the concentration of 500 mg/L, curcumin displayed antifungal activity against *R. solani, P. recondita*, and *P. infestans*.[129] Curcumin and turmeric oil showed antifungal effect against two phytophagous fungi, namely, *Fusarium solani* and *Helmintho sporium*. Oil of turmeric showed the most effective antifungal activity against *F. solani* and *H. oryzae* with IC_{50} of 19.73 and 12.7 µg/mL, respectively.[64] The methanol extract of *C. longa* has inhibitory effect against some clinical isolates of dermatophytes. It was established that 18-month-old and freshly distilled oil isolated from rhizome of *C. longa* exhibited the most potent antifungal effect against 29 clinical isolates of dermatophytes with MIC values of 7.2 and 7.8 mg/mL, respectively.[130] *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum gypseum* were suppressed by 1:40–1:320 dilutions of turmeric oil. In another *in vivo* study conducted on infected guinea pigs with *T. rubrum*, dermal application of turmeric oil (dilution 1:80) induced an improvement in healing of the lesions after 2–5 days which were and it caused the lesions after 6–7 days of consumption to vanish. Turmeric oil also showed activity against pathogenic molds such as *Sporothrix schenckii*, *Exophiala jeanselmei*, *Fonsecaea pedrosoi*, and *Scedosporium apiospermum* with MIC values of 114.9, 459.6, 459.6, and 114.9 µg/mL, respectively.[131] However, curcumin showed more potent significant effect against *Paracoccidioides brasiliensis* than fluconazole, though it did not affect the growth of *Aspergillus* species.[132]

Antiviral Effect

Curcumin also plays important role as an antiviral agent. It acts as an inhibition of Epstein–Barr virus key activator BamH fragment z left frame 1 protein transcription in RajiDR-LUC cells. It also restrains UV light-induced HIV gene expression.[133] Plants as a rich source of phytochemicals with different biological activities including antiviral activities are in interest of scientists. Due to the absence of successful therapeutics for the most of viral diseases, rise of antiviral drug resistance and high cost of some antiviral therapies require finding new effective antiviral compounds.[134,135] Moreover, the offered antiviral therapies are not always well-tolerated or to a certain extent effective and satisfactory.[136] Henceforth, in recent days, the increasing requirement for antiviral substances is more emphasized. It has been well-known that curcumin as a plant derivative has a widespread range of antiviral activity against different viruses. An enzyme called inosine monophosphate dehydrogenase (IMPDH) is suggested as a therapeutic target for antiviral and anticancer compounds. Curcumin in the course of inhibitory activity against IMPDH effect in either noncompetitive or competitive way is flexible as a potent antiviral compound through this process among the 15 different polyphenols.[137] One study showed curcumin to be an effective compound to inhibit the HIV-1 LTR-directed gene expression without any major effects on cell viability.[138] It was also found that curcumin and its derivatives, specifically, reduced curcumin, allyl-curcumin, and tocopheryl-curcumin, discovered 70–85% inhibition in Tat protein transactivation of HIV-1 LTR measured by β-galactosidase activities of HeLa cells which in HIV-1 LTR was fused to the indictor of lac Z gene.[139] Furthermore, curcumin reserved the acetylation of Tat protein of HIV significantly by p300 related with invasion of HIV-1 multiplication. Curcumin by targeting the acetyltransferase proteins of p300/CREB binding protein can be a potent compound for combinatorial HIV therapeutics.[140]

MOLECULAR MARKER ANALYSIS IN *C. LONGA*

C. longa is well-known and valued medicinal plant. Several molecular studies have been reported for the assessment of different accessions. Authentication and genuinely of plant is essential part in marker analysis of plant. As dissimilar parts of the same species can be used as different drugs and as the same drug comes from several species that lead to confusion on the taxonomy of these *Curcuma* species. Besides this, by

distributing this species in a large area, phenotypic variation of morphological characters of rhizomes and leaves is very common in this species that lead to phenotypic plasticity of the species and wrong taxonomic treatment of individuals. However, the correct identity is important to confirm the sources of origin of herbal drugs within the genus.[141,142] It is necessary to adopt various methods to identify the sources of origin of different *Curcuma* species and evaluate their genetic relationship.[143] Among all markers, random amplification polymorphism DNA (RAPD)-based molecular marker is the best approach for identification and genetic diversity analysis in turmeric plant. The declining number of natural populations and rapid fragmentation of natural habitat may have severe impact on the genetic potential of this species.[144] Besides this, taxonomic identity of the species is also confusing. Their potential use, genetic diversity at species, and population level are also unfamiliar. Taxonomic identity of the species is important to search and confirm the origins of different potential. Extending to that knowledge of genetic diversity, it will greatly help to utilize and conserve *C. longa* genetic resource of the country. Even though germplasm collections represent the main source of variability for turmeric genetic improvement studies which is mostly restricted to phenotypic makers. However, the use of phenotypic traits in germplasm characterization may be limited due to the small number of descriptors available and the influence of environment and genotype x environment interactions. Molecular marker techniques may overcome many restrictions of the morphological and biochemical markers for the inequity of turmeric cultivars by providing genetic background for the observed phenotypic variability since they are not affected by the environment or developmental stage and can detect variation at the DNA level.[145] Hussain *et al*.[146] studied RAPD analysis for 30 accessions of 5 *Curcuma* species including *Curcuma latifolia*, *Curcuma malabarica*, *Curcuma manga*, *Curcuma Raktakanta*, and 13 *C. longa* conserved morphotypes. Their RAPD data corroborated the morphological classification of the morphotypes. The efficiency of individual RAPD primers was also compared showing that RAPD markers were highly informative in discriminating the germplasm of *Curcuma*. Islam *et al*.[147] had reported that existence of a high level of genetic diversity within populations of *Curcuma zedoaria* may be present due variations in ecological conditions. However, RAPD markers are known to yield low levels of polymorphism when compared to other molecular markers, such as microsatellites (simple sequence repeats, SSR).[148,149] In recent study, ISSRs, SSRs, and chloroplast or nuclear markers (matK, rbcL, rpl16, and trnK) were used for characterization of inter- and intra-specific diversity of turmeric cultivars.[150] The genetic fingerprints of 15 *Curcuma* species using RAPD and ISSR markers were done by Syamkumar and Sasikumar[151] A RAPD-based assay was carried out and reported that the genetic diversity of C. *zedoaria* was maintained to a greater extent in hilly populations than in plain and plateau populations.[152] RAPD analysis of genetic divergence in 20 accessions of *C. longa* showed a small divergence between accessions.[153] A simple and rapid molecular identification method for six medicinal *Curcuma* species from China using the *trnk* nucleotide sequences was reported by Cao and Komats.[154] Khan *et al*.[155] reported that the ecology of the species varied so much that their habitat ranges from sea level (sandy coastal habitat) to high altitude such as above 2000 m in the Himalayas. Isozyme polymorphism in a germplasm collection of *C. longa* was done by using acid phosphatase, superoxide dismutase, esterase, polyphenol oxidase, peroxidase, and catalase, and it showed good polymorphism in the 15 accessions.[156] An isozyme polymorphism was used to identify some early seven flowering *Curcuma* species.[157] RAPD markers are markers of choice, because of its simplicity and low-cost nature, rapid, inexpensive, and effective system for studying plant genetic relationships.[153,158] The RAPD markers could also be used in the study of genetic variability of species or natural populations.[147,159] This could also be used in the study of genotype identification.[160-162] In another study, Sasaki and Nagumo reported rapid identification of *C. longa* and *C. aromatica* by LAMP method.[163] A study conducted by Sigrist *et al*. showed genetic diversity of turmeric germplasm (*C. longa*; Zingiberaceae) using microsatellite markers to discover genetics and molecular research.[164] Sasikumar *et al*. carried out polymerase chain reaction (PCR)-based detection of adulteration in the market samples of turmeric powder.[165] In another experiment, Hayakawa *et al*. carried out molecular identification of turmeric (*C. longa*, Zingiberaceae) with high curcumin content.[166] Das *et al*.[167] have reported genetic relationship of *Curcuma* species from northeast India using PCR-based markers. They elucidated intra- and inter-specific genetic diversity important for utilization, management, and conservation. In another experiment, identification of different *Curcuma* species using RAPD markers were carried out by Donipati and Sreeramulu[168] They established relationships among six medicinal species of *Curcuma* assessed by RAPD markers. Recently, ISSR fingerprinting of genetic relationship of *Curcuma* sp. of Tripura was carried out by Saha *et al*.[169] Molecular fingerprints of four different species of *Curcuma, namely, Curcuma amada, Curcuma caesia, Curcuma Longa*, and *Curcuma zedoaria* found in Tripura were developed using inter simple sequence repeats. Twenty ISSR primers generated 116 loci amplified in the range of 200–5000 bp with an average of 5.8 alleles and 1.6 effective alleles per locus. The percentage of polymorphic band was found to be 86.29 with an average of 5.15 per primer.

CONCLUSION

The present review attempts to summarize and document the phytochemical, pharmacological, and molecular work done on turmeric. Turmeric and many of its chemical constituents were shown to have useful pharmacological actions to treat various types of disease. Curcumin is one such phytoconstituent and nutraceutical substance with

numerous pharmacological activities verified experimentally and clinically. It has been recognized advantageous in treating anti-inflammatory, anti-allergic, antioxidant, antihyperglycemic, and anticancer properties. Molecular study of turmeric including finger printing by RAPD, SCAR, ISSR, and AFLP plays an important role in the development of molecular marker for authenticity and diversity of plants. Different marker analysis shows that there is a high level of polymorphism among different accessions and pattern varied with respect to environmental factors and genetic parameters.

FUTURE PERSPECTIVE OF *C. LONGA*

Comprehensive traditional knowledge on turmeric can be validated by modern pharmacological studies emphasizing the chemical nature of turmeric, its effects on various parameters and detailed studies of the mechanisms of the observed biological actions and molecular study. Although this information alone is not adequate to provide proof for safety and efficacy of a natural product and requires further exploration. Studies on molecular, phytochemical, and pharmacological effects allow greater perceptive of the factors sustaining the safe use of the medicine, including interactions with other drugs or nutritional factors. If the whole of these results endorses and explains the traditional uses, a clinical study in volunteers or patients may be vindicated for the specific outcome being projected.

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REFERENCES

1. Purseglove JW, Brown EG, Green CL, Robbins SR. Spices. Vol. 1. London, New York: Longman; 1981.
2. Ashraf K, Mujeeb M, Altaf A, Ahmad S, Amir M, Mallick MN, *et al*. Validated HPTLC analysis method for quantification of variability in content of curcumin in *Curcuma longa* L (turmeric) collected from different geographical region of India. Asian Pac J Trop Biomed 2012;5:584-8.
3. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm 2007;330:155-63.
4. Priyadarsini KI. The chemistry of curcumin: From extraction to therapeutic agent. Molecules 2014;19:20091-112.
5. Rana M, Maurya P, Reddy SS, Singh V, Ahmad H, Dwivedi AK, *et al*. A standardized chemically modified *Curcuma longa* extract modulates IRAK-MAPK signaling in inflammation and potentiates cytotoxicity. Front Pharmacol 2016;7:223.
6. Vavilova NI. Turmeric a new spice and medicinal plant. Skogo Inst Rastenievodstva Im 1990;4:77-9.
7. Afaq F, Adhami VM, Ahmad N, Mukhtar H. Botanical antioxidants for chemoprevention of photocarcinogenesis. Front Biosci 2002;7:d784-92.
8. Ammon HP, Wahl MA. Pharmacology of *Curcuma* longa. Planta Med 1991;57:1-7.
9. Polasa K, Sesikaran B, Krishna TP, Krishnaswamy K. Turmeric (*Curcuma* longa)-induced reduction in urinary mutagens. Food Chem Toxicol 1991;29:699-706.
10. Govindarajan VS. Turmeric-chemistry, technology, and quality. Crit Rev Food Sci Nutr 1980;12:199-301.
11. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Pauli GF, *et al*. The essential medicinal chemistry of curcumin. J Med Chem 2017;60:1620-37.
12. Verma MK, Najar IA, Tikoo MK, Singh G, Gupta DK, Anand R, *et al*. Development of a validated UPLC-qTOF-MS method for the determination of curcuminoids and their pharmacokinetic study in mice. Daru 2013;21:11.
13. Kapoor LD. Handbook of Ayurvedic Medicinal Plants. Boca Raton, Florida: CRC Press; 1990. p. 185.
14. Thakur R, Puri HS, Husain A. Major Medicinal Plants of India. Lucknow: Central Institute of Medicinal and Aromatic Plants; 1989.
15. MacGregor H. Out of the spice box, into the lab: Turmeric, an Indian staple, has long had medicinal uses. Now the West is Taking Notice. Los Angeles, CA: Los Angeles Times; 2006.
16. Pandeya N. Old wives tales: Modern miracles turmeric as traditional medicine in India. Trees Life J 2005;1:3.
17. Tilak JC, Banerjee M, Mohan H, Devasagayam TP. Antioxidant availability of turmeric in relation to its medicinal and culinary uses. Phytother Res 2004;18:798-804.
18. Unnikrishnan MK, Rao MN. Inhibition of nitrite induced oxidation of hemoglobin by curcuminoids. Pharmazie 1995;50:490-2.
19. Garg SK. Effect of *Curcuma longa* (rhizomes) on fertility in experimental animals. Planta Med 1974;26:225-7.
20. Shishodia S, Sethi G, Aggarwal BB. Curcumin: Getting back to the roots. Ann N Y Acad Sci 2005;1056:206-17.
21. US Food and Drug Administration. Detention without Physical Examination of Turmeric Due to Lead Contamination. US Food and Drug Administration. Available from: http://www.FDA.gov. [Last accessed on 2014 Dec 03; Last retrieved on 2015 Dec 09].
22. Food Standards Agency. Producing and Distributing Food-Guidance: Chemicals in Food: Safety Controls; Sudan Dyes and Industrial Dyes Not Permitted in Food, Food Standards Agency, UK Government. Available from: http://www.gov.uk. [Last accessed on 2012 Oct 08; Last retrieved on 2015 Dec 12].
23. Tomren MA, Másson M, Loftsson T, Tønnesen HH. Studies on curcumin and curcuminoids XXXI.

Symmetric and asymmetric curcuminoids: Stability, activity and complexation with cyclodextrin. Int J Pharm 2007;338:27-34.

24. Tiyaboonchai W, Tungpradit W, Plianbangchang P. Formulation and characterization of curcuminoids loaded solid lipid nanoparticles. Int J Pharm 2007;337:299-306.

25. Jayaprakasha GK, Rao LJ, Sakariah KK. Antioxidant activities of curcumin, desmethoxycurcumin and bisdemethoxycurcumin. Food Chem 2006;98:720-4.

26. Ravindran PN, Nirmal BK, editors. Ginger: The Genus *Zingiber*. New York: CRC Press; 2005. p. 15-85.

27. Gupta AP, Gupta MM, Kuma SJ. Simultaneous determination of curcuminoids in *Curcuma* samples using high-performance thin-layer chromatography. J Liq Chromatogr Realat Technol 1999;22:1561-9.

28. Lin X, Xue L, Zhang H, Zhu C. Determination of curcumins in turmeric by micellarelectro kinetic capillary chromatography. Can J Anal Sci Spectrosc 2006;51:35-42.

29. Bos R, Windono T, Woerdenbag HJ, Boersma YL, Koulman A, Kayser O. HPLC-photodiode array detection analysis of curcuminoids in *Curcuma* species indigenous to Indonesia. Phytochem Anal 2007;18:118-22.

30. Payton F, Sandusky P, Alworth WL. NMR study of the solution structure of curcumin. J Nat Prod 2007;70:143-6.

31. Paramasivam M, Aktar MW, Poi R, Banerjee H, Bandyopadhyay A. Occurrence of curcuminoids in *Curcuma* longa: A quality standardization by HPTLC. Bangladesh J Pharmacol 2008;3:55-8.

32. Zhang J, Jinnai S, Ikeda R, Wada M, Hayashida S, Nakashima K. A simple HPLC-fluorescence method for quantitation of curcuminoids and its application to turmeric products. Anal Sci 2009;25:385-8.

33. Taylor S, McDowell I. Determination of curcuminoid in turmeric (*C. domestica* Val.) by reverse phase high performance liquid chromatography. Chromatographia 1992;34:73-7.

34. Paramapojn S, Gritsanapan W. Free radical scavenging activity determination and quantitative analysis of curcuminoids in *Curcuma zedoaria* rhizome extract by HPLC method. Curr Sci 2009;97:1069-73.

35. Herebian D, Choi JH, Abd El-Aty AM, Shim JH, Spiteller M. Metabolite analysis in *Curcuma domestica* using various GC-MS and LC-MS separation and detection techniques. Biomed Chromatogr 2009;23:951-65.

36. Li R, Xiang C, Ye M, Li HF, Zhang X, Guo DA. Qualitative and quantitative analysis of curcuminoids in herbal medicines derived from *Curcuma sp*ecies. Food Chem 2011;126:1890-5.

37. Ashraf K, Mujeeb M, Ahmad A, Ahmad N, Amir M. Determination of curcuminoids in *Curcuma longa* Linn. by UPLC/Q-TOF-MS: An application in turmeric cultivation. J Chromatogr Sci 2015;53:1346-52.

38. Mudge E, Chan M, Venkataraman S, Brown PN. Curcuminoids in turmeric roots and supplements: Method optimization and validation. Food Anal Methods 2016;9:1428-35.

39. Dhakal S, Chao K, Schmidt W, Qin J, Kim M, Chan D. Evaluation of turmeric powder adulterated with metanil yellow using FT-Raman and FT-IR spectroscopy. Foods 2016;5. pii: E36.

40. Sahne F, Mohammadi M, Najafpour GD, Moghadamnia AA. Extraction of bioactive compound curcumin from turmeric (*Curcuma longa* L.) Via different routes: A comparative study. Pak J Biotechnol 2016;13:173-80.

41. Osorio-Tobón JF, Carvalho PI, Barbero GF, Nogueira GC, Rostagno MA, Meireles MA. Fast analysis of curcuminoids from turmeric (*Curcuma longa* L.) by high-performance liquid chromatography using a fused-core column. Food Chem 2016;200:167-74.

42. Monton C, Charoenchai L, Suksaeree J, Sueree L. Quantitation of curcuminoid contents, dissolution profile, and volatile oil content of turmeric capsules produced at some secondary government hospitals. J Food Drug Anal 2016;24:493-9.

43. Jin S, Song C, Jia S, Li S, Zhang Y, Chen C, *et al*. An integrated strategy for establishment of curcuminoid profile in turmeric using two LC-MS/MS platforms. J Pharm Biomed Anal 2017;132:93-102.

44. Srivasta S. A HPTLC method for chemotaxonomic evaluation of some *curcuma* species and their commercial samples. J Sci Ind Res 2009;68:876-80.

45. Kulkarni SJ, Maske KN, Budre MP, Mahajan RP. Extraction and purification of curcuminoids from turmeric. Int J Pharmcol Pharm Technol 2012;1:81-4.

46. Kandasamy J. Validated method for estimation of curcumin from different varieties of *curcuma* longa. Int J Pharm Biosci 2013;4:1004-10.

47. 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;113:640-4.

48. Khatoon S. Standardization of an ayurvedic formulation-kalyanavleha and estimation of curcumin using HPTLC. Indian J Tradit Knowl 2014;13:535-42.

49. Ansari MJ, Ahmad S, Kohli K, Ali J, Khar RK. Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. J Pharm Biomed Anal 2005;39:132-8.

50. Kekre VA, Walode SG. Validated HPTLC method for estimation of curcumin content in dietary supplement formulation. Int J Pharm Sci Res 2012;3:3796-800.

51. Khan S, Makhija IK, Khamar D, Rani S. Development and standardization of turmeric cream by HPTLC. Int J Biomed Adv Res 2010;1:109-16.

52. Jayaprakasha JK, Lingamullujagan MR, Kunnumpurath KS. Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. J Agric Food Chem 2012;50:3668-72.

53. Wichitnithad W, Jongaroonngamsang N, Pummangura S, Rojsitthisak P. A simple isocratic HPLC method for

the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochem Anal 2009;20:314-9.

54. Ramshankar YV, Suresh S. A sensitive reversed phase HPLC method for the determination of curcumin. Pharmacogn Mag 2009;5:71-4.

55. Gantait A, Barman T, Mukherjee PK. Validated method for estimation of curcumin in turmeric powder. Indian J Tradit Knowl 2011;10:247-50.

56. Sheikh S, Asghar S, Ahmad S. Development of HPTLC method and its validation for the estimation of curcuminoids from polyherbal mouth ulcer gel formulation. J Pharm 2013;3:29-34.

57. Lee CJ, Lee JH, Seok JH, Hur GM, Park YC, Seol IC, *et al*. Effects of baicalein, berberine, curcumin and hespiridin on mucin release from airway goblet cells. Planta Med 2003;69:523-6.

58. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, *et al*. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;13:784-9.

59. Prasad DN, Gupta B, Srivastava RK, Satyavati GV. Studies on ulcerogenic activity of curcumin. Indian J Physiol Pharmacol 1979;20:92.

60. Bhavani Shankar TN, Sreenivasa Murthy V. Effect of turmeric (*Curcuma* longa) fractions on the growth of some intestinal and pathogenic bacteria *in vitro*. Indian J Exp Biol 1979;17:1363-6.

61. Platel K, Srinivasan K. Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. Int J Food Sci Nutr 1996;47:55-9.

62. Su X, Jiang B, Wang H, Shen C, Chen H, Zeng Li. Curcumin suppresses intestinal fibrosis by inhibition of PPARγ-mediated epithelial-mesenchymal transition. Evid Based Complement Altern Med 2017;92:57-66.

63. Zhang X, Wu J, Ye B, Wang Q, Xie X, Shen H. Protective effect of curcumin on TNBS-induced intestinal inflammation is mediated through the JAK/STAT pathway. BMC Complement Altern Med 2016;16:299.

64. Prucksunand C, Indrasukhsri B, Leethochawalit M, Hungspreugs K. Phase II clinical trial on effect of the long turmeric (*Curcuma longa* Linn) on healing of peptic ulcer. Southeast Asian J Trop Med Public Health 2001;32:208-15.

65. Kim DC, Kim SH, Choi BH, Baek NI, Kim D, Kim MJ, *et al*. *Curcuma longa* extract protects against gastric ulcers by blocking H2 histamine receptors. Biol Pharm Bull 2005;28:2220-4.

66. Rafatullah S, Tariq M, Al-Yahya MA, Mossa JS, Ageel AM. Evaluation of turmeric (*Curcuma longa*) for gastric and duodenal antiulcer activity in rats. J Ethnopharmacol 1990;29:25-34.

67. Ramírez-Tortosa MC, Mesa MD, Aguilera MC, Quiles JL, Baró L, Ramirez-Tortosa CL, *et al*. Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. Atherosclerosis 1999;147:371-8.

68. Srivastava R. Inhibition of neutrophil response by curcumin. Agents Actions 1989;28:298-303.

69. Lee HS. Antiplatelet property of *Curcuma longa* L. Rhizome-derived ar-turmerone. Bioresour Technol 2006;97:1372-6.

70. Soni KB, Rajan A, Kuttan R. Reversal of aflatoxin induced liver damage by turmeric and curcumin. Cancer Lett 1992;66:115-21.

71. Kamal-Eldin A, Frank J, Razdan A, Tengblad S, Basu S, Vessby B. Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. Lipids 2000;35:427-35.

72. Akrishnan VR, Menon VP. Potential role of antioxidants during ethanol-induced changes in the fatty acid composition and arachidonic acid metabolites in male Wistar rats. Cell Biol Toxicol 2001;17:11-22.

73. Nirmala C, Puvanakrishnan R. Protective role of curcumin against isoproterenol induced myocardial infarction in rats. Mol Cell Biochem 1996;159:85-93.

74. Sumbilla C, Lewis D, Hammerschmidt T, Inesi G. The slippage of the Ca2+ pump and its control by anions and curcumin in skeletal and cardiac sarcoplasmic reticulum. J Biol Chem 2002;277:13900-6.

75. Patil TN, Srinivasan M. Hypocholesteremic effect of curcumin in induced hypercholesteremic rats. Indian J Exp Biol 1971;9:167-9.

76. Yu ZF, Kong LD, Chen Y. Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. J Ethnopharmacol 2002;83:161-5.

77. Xia X, Cheng G, Pan Y, Xia ZH, Kong LD. Behavioral, neurochemical and neuroendocrine effects of the ethanolic extract from *Curcuma longa* L. in the mouse forced swimming test. J Ethnopharmacol 2007;110:356-63.

78. Xu Y, Ku B, Tie L, Yao H, Jiang W, Ma X, *et al*. Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res 2006;1122:56-64.

79. Ringman JM, Frautschy SA, Cole GM, Masterman DL, Cummings JL. A potential role of the curry spice curcumin in Alzheimer's disease. Curr Alzheimer Res 2005;2:131-6.

80. Kulkarni SK, Dhir A. An overview of curcumin in neurological disorders. Indian J Pharm Sci 2010;72:149-54.

81. Vajragupta O, Boonchoong P, Watanabe H, Tohda M, Kummasud N, Sumanont Y. *Manga*nese complexes of curcumin and its derivatives: Evaluation for the radical scavenging ability and neuroprotective activity. Free Radic Biol Med 2003;35:1632-44.

82. Thiyagarajan M, Sharma SS. Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Life Sci 2004;74:969-85.

83. Bundy R, Walker AF, Middleton RW, Booth J. Turmeric extract may improve irritable bowel syndrome symptomology in otherwise healthy adults: A pilot study. J Altern Complement Med 2004;10:1015-8.

84. Ghatak N, Basu N. Sodium curcuminate as an effective anti-inflammatory agent. Indian J Exp Biol 1972;10:235-6.

85. Srivastava R, Srimal RC. Modification of certain inflammation-induced biochemical changes by curcumin. Indian J Med Res 1985;81:215-23.

86. Brouet I, Ohshima H. Curcumin, an antitumor promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. Biochem Biophys Res Commun 1995;206:533-40.

87. Srimal RC, Dhawan BN. In: Arora BB, editor. Development of Unani drugs from Herbal Sources and the Role of Elements in their Mechanism of Action. New Delhi: Hamdard National Foundation Monograph; 1985.

88. Yegnanarayan R, Saraf AP, Balwani JH. Comparison of anti-inflammatory activity of various extracts of *Curcuma longa* (Linn). Indian J Med Res 1976;64:601-8.

89. Chandra D, Gupta SS. Anti-inflammatory and anti-arthritic activity of volatile oil of *Curcuma longa* (Haldi). Indian J Med Res 1972;60:138-42.

90. Singh S, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem 1995;270:24995-5000.

91. Sidhu GS, Mani H, Gaddipati JP, Singh AK, Seth P, Banaudha KK, *et al*. Curcumin enhances wound healing in streptozotocin induced diabetic rats and genetically diabetic mice. Wound Repair Regen 1999;7:362-74.

92. Sharma OP. Antioxidant activity of curcumin and related compounds. Biochem Pharmacol 1976;25:1811-2.

93. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett 1995;94:79-83.

94. Subramanian M, Sreejayan, Rao MN, Devasagayam TP, Singh BB. Diminution of singlet oxygen-induced DNA damage by curcumin and related antioxidants. Mutat Res 1994;311:249-55.

95. Rao TS, Basu N, Siddiqui HH. Anti-inflammatory activity of curcumin analogues. Indian J Med Res 1982;75:574-8.

96. Joe B, Lokesh BR. Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. Biochim Biophys Acta 1994;1224:255-63.

97. Dikshit M, Rastogi L, Shukla R, Srimal RC. Prevention of ischaemia-induced biochemical changes by curcumin and quinidine in the cat heart. Indian J Med Res 1995;101:31-5.

98. Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic Biol Med 2000;28:1303-12.

99. Ranjan D, Johnston TD, Wu G, Elliott L, Bondada S, Nagabhushan M. Curcumin blocks cyclosporine A-resistant CD28 costimulatory pathway of human T-cell proliferation. J Surg Res 1998;77:174-8.

100. Okamoto T, Yamagishi S, Inagaki Y, Amano S, Koga K, Abe R, *et al*. Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. FASEB J 2002;16:1928-30.

101. Gukovsky I, Reyes CN, Vaquero EC, Gukovskaya AS, Pandol SJ. Curcumin ameliorates ethanol and nonethanol experimental pancreatitis. Am J Physiol Gastrointest Liver Physiol 2003;284:85-95.

102. Durgaprasad S, Pai CG, Vasanthkumar, Alvres JF, Namitha S. A pilot study of the antioxidant effect of curcumin in tropical pancreatitis. Indian J Med Res 2005;122:315-8.

103. Chen HW, Huang HC. Effect of curcumin on cell cycle progression and apoptosis in vascular smooth muscle cells. Br J Pharmacol 1998;124:1029-40.

104. Khar A, Ali AM, Pardhasaradhi BV, Varalakshmi CH, Anjum R, Kumari AL. Induction of stress response renders human tumor cell lines resistant to curcumin mediated apoptosis: Role of reactive oxygen intermediates. Cell Stress Chaperones 2001;6:368-76.

105. Piwocka K, Jaruga E, Skierski J, Gradzka I, Sikora E. Effect of glutathione depletion on caspase-3 independent apoptosis pathway induced by curcumin in Jurkat cells. Free Radic Biol Med 2001;31:670-8.

106. Boone CW, Steele VE, Kelloff GJ. Screening for chemopreventive (anti-carcinogenic) compounds in rodents. Mutat Res 1992;267:251-5.

107. Thaloor D, Singh AK, Sidhu GS, Prasad PV, Kleinman HK, Maheshwari RK. Inhibition of angiogenic differentiation of human umbilical vein endothelial cells by curcumin. Cell Growth Differ 1998;9:305-12.

108. Somasundaram S, Edmund NA, Moore DT, Small GW, Shi YY, Orlowski RZ. Dietary curcumin inhibits chemotherapy-induced apoptosis in models of human breast cancer. Cancer Res 2002;62:3868-75.

109. Mohanty I, Arya DS, Gupta SK. Effect of *Curcuma longa* and *Ocimum sanctum* on myocardial apoptosis in experimentally induced myocardial ischemic-reperfusion injury. BMC Complement Altern Med 2006;6:3.

110. Sakano K, Kawanishi S. Metal-mediated DNA damage induced by curcumin in the presence of human cytochrome P450 isozymes. Arch Biochem Biophys 2002;405:223-30.

111. Frank N, Knauft J, Amelung F, Nair J, Wesch H, Bartsch H. No prevention of liver and kidney tumors in long-evans cinnamon rats by dietary curcumin, but inhibition at other sites and of metastases. Mutat Res 2003;523-524:127-35.

112. Parveen Z, Nawaz S, Siddique S, Shahzad K. Composition and antimicrobial activity of the essential oil from leaves of *Curcuma longa* L. Kasur variety. Indian J Pharm Sci 2013;75:117-22.

113. Rasmussen HB, Christensen SB, Kvist LP, Karazmi A. A simple and efficient separation of the curcumins, the antiprotozoal constituents of *Curcuma* longa. Planta Med 2000;66:396-8.

114. Khattak S, Saeed-ur-Rehman, Ullah Shah H, Ahmad W,

Ahmad M. Biological effects of indigenous medicinal plants *Curcuma longa* and *Alpinia galanga.* Fitoterapia 2005;76:254-7.

115. Kundu S, Biswas TK, Das P, Kumar S, De DK. Turmeric (*Curcuma longa*) rhizome paste and honey show similar wound healing potential: A preclinical study in rabbits. Int J Low Extrem Wounds 2005;4:205-13.

116. Shukla Y, Arora A, Taneja P. Antimutagenic potential of curcumin on chromosomal aberrations in Wistar rats. Mutat Res 2002;515:197-202.

117. Hamss R, Analla M, Campos-Sanchez J, Alonso-Moraga A, Muñoz-Serrano A, Idaomar M. A dose dependent anti-genotoxic effect of turmeric. Mutat Res 1999;446:135-9.

118. Srivastava R, Dikshit M, Srimal RC, Dhawan BN. Anti-thrombotic effect of curcumin. Thromb Res 1985;40:413-7.

119. Garg SK, Mathur VS, Chaudhury RR. Screening of Indian plants for antifertility activity. Indian J Exp Biol 1978;16:1077-9.

120. Rithaporn T, Monga M, Rajasekaran M. Curcumin: A potential vaginal contraceptive. Contraception 2003;68:219-23.

121. Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, *et al*. Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in Type 2 diabetic KK-Ay mice. J Agric Food Chem 2005;53:959-63.

122. Wickenberg J, Ingemansson SL, Hlebowicz J. Effects of *Curcuma longa* (turmeric) on postprandial plasma glucose and insulin in healthy subjects. Nutr J 2010;9:43.

123. Arun N, Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr 2002;57:41-52.

124. Suryanarayana P, Krishnaswamy K, Reddy GB. Effect of curcumin on galactose-induced cataractogenesis in rats. Mol Vis 2003;9:223-30.

125. Kumar S, Narain U, Tripathi S, Misra K. Syntheses of curcumin bioconjugates and study of their antibacterial activities against beta-lactamase-producing microorganisms. Bioconjug Chem 2001;12:464-9.

126. Upendra RS, Khandelwal P, Reddy AH. Turmeric powder (*Curcuma longa* Linn.) as an antifungal agent in plant tissue culture studies. Int J Eng Sci 2011;3:7899-904.

127. Ungphaiboon S, Supavita T, Singchangchai P, Sungkarak S, Rattanasuwan P, Itharat A. Study on antioxidant and antimicrobial activities of turmeric clear liquid soap for wound treatment of HIV patients. Songklan J Scitech 2005;27:269-578.

128. Kim MK, Choi GJ, Lee HS. Fungicidal property of *Curcuma longa* L. Rhizome-derived curcumin against phytopathogenic fungi in a greenhouse. J Agric Food Chem 2003;51:1578-81.

129. Chowdhury H, Banerjee T, Walia S. *In vitro* screening of *Curcuma longa* L and its derivatives as antifungal agents against *Helmintho sporrumoryzae* and *Fusarium solani*. Pestic Res J 2008;20:6-9.

130. Wuthi-udomlert M, Grisanapan W, Luanratana O, Caichompoo W. Antifungal activity of *Curcuma longa* grown in Thailand. Southeast Asian J Trop Med Public Health 2000;31 Suppl 1:178-82.

131. Apisariyakul A, Vanittanakom N, Buddhasukh D. Antifungal activity of turmeric oil extracted from *Curcuma longa* (*Zingiberaceae*). J Ethnopharmacol 1995;49:163-9.

132. Martins CV, da Silva DL, Neres AT, Magalhães TF, Watanabe GA, Modolo LV, *et al*. Curcumin as a promising antifungal of clinical interest. J Antimicrob Chemother 2009;63:337-9.

133. Taher MM, Lammering G, Hershey C, Valerie K. Curcumin inhibits ultraviolet light induced human immunodeficiency virus gene expression. Mol Cell Biochem 2003;254:289-97.

134. Tomei L, Altamura S, Paonessa G, De Francesco R, Migliaccio G. HCV antiviral resistance: The impact of *in vitro* studies on the development of antiviral agents targeting the viral NS5B polymerase. Antivir Chem Chemother 2005;16:225-45.

135. Lemoine M, Nayagam S, Thursz M. Viral hepatitis in resource-limited countries and access to antiviral therapies: Current and future challenges. Future Virol 2013;8:371-80.

136. De Clercq E. Strategies in the design of antiviral drugs. Nat Rev Drug Discov 2002;1:13-25.

137. Dairaku I, Han YN, Yanaka N, Kato N. Inhibitory effect of curcumin on IMP dehydrogenase, the target for anticancer and antiviral chemotherapy agents. Bios Biotech Biochem 2010;74:185-7.

138. Li CJ, Zhang LJ, Dezube BJ, Crumpacker CS, Pardee AB. Three inhibitors of Type 1 human immunodeficiency virus long terminal repeat-directed gene expression and virus replication. Proc Natl Acad Sci U S A 1993;90:1839-42.

139. Barthelemy S, Vergnes L, Moynier M, Guyot D, Labidalle S, Bahraoui E. Curcumin and curcumin derivatives inhibit Tat-mediated transactivation of Type 1 human immunodeficiency virus long terminal repeat. Res Virol 2008;149:43-52.

140. Balasubramanyam K, Varier RA, Altaf M, Swaminathan V, Siddappa NB, Ranga U, *et al*. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. J Biol Chem 2004;279:51163-71.

141. Cao H, Sasaki Y, Fushimi H, Komatsu K. Molecular analysis of medicinally-used Chinese and Japanese *Curcuma* based on 18S rRNA gene and trnK gene sequences. Biol Pharm Bull 2001;24:1389-94.

142. Saiki R, Scharf S, Faloona F, Mullis K, Horn G, Erlich H, *et al*. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1995;230:1350-4.

143. Xuemei P, Yan V, Ziyi Y, Weiming Z, Qitang L, Changmei L. DNA sequence and RAPD information

re-affirms the taxonomic relationships between *Apocynum venetum* L. *Poacynum pictum* (Schrenk) Baill. Pak J Bot 2012;44:1261-6.

144. Paisooksantivatana Y, Kako S, Seko H. Genetic diversity of *Curcuma alismatifolia* Gagnep (*Zingiberaceae*) in Thailand as revealed by allozyme polymorphism. Genet Resour Crop Evol 2001;48:459-65.

145. Tingey SV, del Tufo JP. Genetic analysis with random amplified polymorphic DNA markers. Plant Physiol 1993;101:349-52.

146. Hussain Z, Tyagi K, Sharma R, Agrawal A. Genetic diversity in *in vitro*-conserved germplasm of *Curcuma* L. as revealed by RAPD markers. Biol Plant 2008;52:627.

147. Islam MA, Meister A, Schubert V, Kloppstech K, Esch E. Genetic diversity and cytogenetic analysis in *Curcuma zedoaria* (Christm) roscoe from Bangladesh. Genet Resour Crop Evol 2005;54:149-56.

148. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey SM, *et al*. Thecomparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers form germplasm analysis. Mol Breed 1996;2:225-38.

149. Akbar F, Rabbani MA, Masood MS, Shinwari ZK. Genetic diversity of sesame (*Sesamum indicum* L.) germplasm from Pakistan using RAPD markers. Pak J Bot 2011;43:2153-60.

150. Pervaiz ZK, Rabbani MA, Shinwari ZK, Masood MS, Malik SA. Assessment of genetic variability in rice (*Oryza sativa* L.) germplasm from Pakistan using RAPD markers. Pak J Bot 2010;42:3369-76.

151. Syamkumar S, Sasikumar B. Molecular marker based genetic diversity analysis of *Curcuma sp*ecies from India. Sci Hortic 2007;112:235-41.

152. Pinheiro JB, Zucchi MI, Teles FL, Ázara NA. Molecular genetic diversity and RAPD. Minutes 2003;25:1.

153. Aslam D. Source of income from fruits and vegetables. In: Proceedings of National Workshop on Horticultural Crops and their Importance in Pakistan; 1996. p. 3.

154. Cao H, Komatsu K. Molecular identification of six medicinal Curcuma plants produced in Sichuan: Evidence from plastid trnK gene sequences. Yao Xue Xue Bao 2003;38:871-5.

155. Khan S, Shama N, Reema, Naeem R. Genetic fingerprinting of local turmeric genotypes using rapds. Pak J Bot 2013;45:339-46.

156. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990;18:6531-5.

157. Ashraf K, Ahmad A, Chaudhary A, Mujeeb M, Ahmad S, Amir M, *et al*. Genetic diversity analysis of *Zingiber officinale* Roscoe by RAPD collected from subcontinent of India. Saudi J Biol Sci 2014;21:159-65.

158. Lashermes PH, Cros J, Marmey PH, Charrier A. Use of random amplified polymorphic DNA to analyse genetic variability and relationship of *Coffea* species. Crop Evol Genet Resour 1993;40:91-9.

159. Wilkie SE, Isaac PG, Slater RJ. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium.* Theor Appl Genet 1993;86:497-504.

160. Wilde J, Waugh R, Powell W. Genetic finger printing of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 1992;83:871-7.

161. Koller B, Lehmann A, McDermott JM, Gessler C. Identification of apple cultivars using RAPD markers. Theor Appl Genet 1993;85:901-4.

162. Wolff K, Peters-van Rijn J. Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora* Tzvelev) using random primers. Heredity (Edinb) 1993;71:335-41.

163. Sasaki Y, Nagumo S. Rapid identification of *Curcuma longa* and *C. aromatica* by LAMP. Biol Pharm Bull 2007;30:2229-30.

164. Sigrist MS, Pinheiro JB, Filho JA. Genetic diversity of turmeric germplasm (*Curcuma longa*; *Zingiberaceae*) identified by microsatellite markers genetics and molecular research. Genet Mol Res 2011;10:419-28.

165. Sasikumar B, Syamkumar S, Remya R, Zachariah JT. PCR based detection of adulteration in the market samples of turmeric powder. Food Biotechnol 2004;18:3.

166. Hayakawa H, Kobayashi T, Minamiya Y, Ito K, Miyazaki A, Fukuda T, *et al*. Molecular identification of turmeric (*Curcuma* longa, *Zingiberaceae*) with high curcumin content. J Jpn Bot 2010;85:263-9.

167. Das A, Kesari V, Satyanarayana VM, Parida A, Rangan L. Genetic relationship of *Curcuma sp*ecies from Northeast India using PCR-based markers. Mol Biotechnol 2011;49:65-76.

168. Donipati P, Sreeramulu SH. Relationships among six medicinal species of *curcuma* assessed by rapd markers. Int J Recent Sci Res 2015;6:5909-12.

169. Saha K, Sinha RK, Basak S, Sinha S. ISSR fingerprinting to ascertain the genetic relationship of *Curcuma* sp. of Tripura. Am J Plant Sci 2016;7:259-66.