

ANATOMICAL STUDIES ON CURCUMA LONGA L. (TURMERIC) FOR PRESENCE OF CURCUMIN AND CURCUMINOIDS

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Abstract-

Curcuma longa L. (Turmeric) is an herbaceous plant and its rhizome yield a commonly used spice- turmeric powder. Turmeric rhizome has observable pharmacokinetics and its medicinal uses are proven in numerous clinical researches. It has more than 35 important bioactive compounds of which Curcumin (2methoxy feruloyl methane) is most widely studied and other phytochemical collectively termed as curcuminoids are also of great significance. This study was planned for phytochemical estimation of curcumin and curcuminoids in various anatomical zones of turmeric rhizomes. The processed turmeric rhizomes were sectioned from cover, cortex and pith regions to prepare samples for HPTLC analysis! It was observed that cortex of IR dried samples of rhizome has more curcumin and curcuminoids compared to microwave dried samples. We studied four varieties of turmeric as IR dried and MW dried samples namely Roma, Roma Pahari, CL-70, NDH-18 and phytochemical estimation of their anatomical zones was performed. It was found that cortex region of IR dried Roma pahari and Roma has maximum amount of curcumin and curcuminoids respectively. The cortex region of IR dried Roma pahari has curcumin-1.68% and Roma variety IRdried samples has curcuminoids 4.47%. So from this study we could say that cortex region of turmeric rhizome are rich in phytochemical content as compared to cover and pith.

Key words- phytochemicals, pharmacokinetics, curcumin, curcuminoids,

INTRODUCTION-

Turmeric plant (*Curcuma longa* L.) is a perennial herbaceous plant of zingiberaceae family. It blossoms with red, violet, pink and white flowers arranged as racemose spike floescence in winters. In Asian countries like India, China, Japan and Indonesia turmeric is used for spiritual and religious purposes. Underground rhizomes are processed before use, Rhizomes are dug and cleaned dried or boiled and then dried. The processed dried rhizomes are powdered for use as culinary spice¹. The phytochemicals present in *Curcuma longa* are Curcumin, bisdesmethoxy curcumin, curcumenone, curdione, curleone, ar-curcumenone, curdione, linalool, p- coumaric acid, -methoxy cinnamic acid, Terpinene, D-a phyllandrene, d-camphene, zingiberene, Ascorbic acid, water, carbohydrates, proteins, oils and fats^{2,3}.

curcumin is an active chemical compound of turmeric with molecular formulae C₁₇H₂₁O₆. It has defined clinical and therapeutic medical value for human biology.

REVIEW OF LITERATURE-

Bioactive molecules like curcumin and curcuminoids effective in medical treatments like skin infections, ulcers, wounds and inflammation². Curcumin also called di feruloyl methane is a natural polyphenol with antioxidant activities and marks the active absorption of distress molecules like superoxide mutase and helps in reducing free radicals⁴. Calcium hydrogenase and serum oxides also increase in blood with active bioabsorption of curcumin in human blood serum thus marking an antioxidant activity^{4,5}. Anti-inflammatory and antirheumatic activity of curcumin is observed by formation of TNF alpha factors of recovery that help in reducing inflammation⁶. Curcumin checks metastasis and helps formation of factors like TNF-alpha, cyclooxygenase-2, NF-kB particles that works for wound healing or anticarcinogenic effect on human body⁷. Curcumin ethanol extract and oil fraction show antimicrobial activity. It suppresses the growth of bacteria like staphylococcus, streptococcus, helicobacter and lactobacillus⁸.

Pharmacological effects of curcumin and other effects of curcuma longa-

Effect on gastrointestinal tract- turmeric powder has curative effect on stomach.it increases mucin secretion in rabbit irritants. However antiulcer activities have made turmeric a potent medicine. Curcumin has shown to prevent stomach from ulcerogenic effects of phenylbutazone in guinea pigs at 50mg/kg dosage^{9,10}. Recent studies in laboratories indicate that curcumin can block

indomethacin, ethanol, and stress induced gastric ulcer and can also block pylorus ligation induced acid secretion in rats. The antiulcer effect is mediated by scavenging of reactive oxygen species by curcumin.

Effect on intestine- curcumin has antispasmodic activity of sodium curcumin as observed in related guinea pig ileum. Hikino H observed antitflatulent activity is cited in vivo and invitro experiments in rats. It is also observed that curcumin also enhances intestinal lipase, sucrase and maltase activity¹¹.

Effects on liver- curcumin and its analogue has protective activity in culture hepatocyte in carbontetrachloride, DGalactosamine, peroxide, ionophore induced toxicity^{12,13}. Curcumin also protects against diethylnitrosamine and 2,acetyl amino flavine induced altered hepatic foci development¹⁴. Increased bile production was reported in dogs by curcumin and essential oils of curcuma longa¹⁵.

Effect on Pancreas- 1-phenyl-1-hydroxy n pentane, asynthetic derivative of p-tolyl methyl carbinol an ingredient of curcuma longa has increased activity of pancreatic amylase, lipase, trypsin and chymotrypsin¹⁵.

Effect on cardiovascular system- curcumin proves Ca^{2+} transport and its slippage from the cardiac muscle sarcoplasmic reticulum thereof raising the pharmacological intervention that correct the effective homeostasis in the cardiac muscle.

Effect on nervous system- Curcumin and manganese complex acts protective against vascular dementia by being antioxidant¹⁶.

Effect on lipid metabolism- curcumin reduces low density lipoprotein (ldl) and very high density in plasma and cholesterol level in liver alongwith increasing tocopherol level in plasma. Suggesting invivo interaction between curcumin and bioavailability of vitamin E reduces cholesterol level (Kamal Eldwin A 2000). Increase in fatty acid content after ethanol induced liver damage is significantly decreased by curcumin treatment and archidionic level is increased (krishanan A and menon VR 2001).

Antiinflammatory Activity- curcumin is effective in carragenin induced odema in rats. The natural analogues of curcumin viz- feruloyl(4- hydroxy cinnamoyl methane and bis 4- hydroxy feruloyl methane (BHM) are also potent anti-inflammatory. The volatile oil and also the peteroleum ether, alcohol and water extract are also potent anti-inflammatory. The volatile oil and also the peteroleum ether, alcohol and water extract curcuma longa show anti-inflammatory effect Oral administration of curcumin has proven effective in rheumatism (Sharma Op 1976).

Antioxidant effect- the antioxidant activity of curcumin was reported as early as 1975. It act as oxygen free radical scavenger. It can protect haemoglobin from oxidation (Frankel EN 1998). In vitro curcumin can effectively inhibit generation of reactive oxygen species (ROS) like superoxide ions, H_2O_3 and nitrite radical generation by activated macrophages which play important role in inflammation¹⁷.

Anticarcinogenic activity- induction of apoptosis and anti-tumor effect apoptosis lead to anticarcinogenic effect and reduces tumor formation. It induces cell cycle inhibition to prevent cell maturation and cell proliferation¹⁸. Recently S480 cells were transfected or with hsp sense but showed positive result on sense hsp and caused apoptosis¹⁹.

- a) Curcumin inhibit tumorigenesis – curcumin checks proliferation of various cell adhesion molecules. Curcumin also induces upregulation of caspase 3 and down regulation of expression of NF-KB²⁰. Due to this function curcumin is able to significantly inhibit growth of ovarian cancer. Curcumin is effective in checking metasis because it prevents ectodermal skin carcinoma and breast cancer. Deeb et al 1994 explored that curcumin and TNF related apoptosis inducing ligand (TRAIL) cooperatively act to promote death of LN cap cells. Curcumin also encounter many cell proliferation genes which are resistant to chemotherapy and reports also suggest curcumin is effective in multiple myeloma^{20,23}.
- b) Curcumin down regulates cyclin D1 expression. Cyclin D1 a component unit of cyclin dependent kinase cdk4 and cdk6 a rate limiting factor in expression of progression of cells. Anti proliferative expression of curcumin maybe related to inhibition of cd expression²².
- c) Curcumin inhibit proliferation of VSMCs. For cardiac ailments curcumin inhibit proliferation of peripheral blood mononuclear cell²².

Inhibition of platelet aggregation- Srivastava et al 1995 founded that curcumin increases the synthesis of PGI₂ thus could be favourable for patients prone to thrombosis and requiring anti arthritic activity. Curcumin inhibit collagen and adrenaline induced platelet aggregation invitro as well as invivo in rats thoracic aorta²⁴.

Suppression of diabetes- Babu et al (1995) founded that curcumin feeding improve metabolic breakdown of sugars despite no effect on glycemic status and body weight. It is due to hypercholesterolemic influence and its antioxidant properties²⁵.

Antibacterial effect- curcumin and its oil extract suppress growth of several bacteria like streptococcus, lactobacillus etc. Recent studies states that curcumin prevents growth of bacteria like staphylococcus, helicobacter, pseudomonas aeruginosa, E coli etc

Antifungal activity- Ethanol extract of curcuma longa shows antifungal activity against aspergillus flavus, fusarium moniforme etc²⁶

Antivenom effect- Ar-turmerone isolated from curcuma longa neutralizes hemorrhagic activity of Bothrops venom and 70% lethal effect of crotalus venom in mice. It acts as an enzymatic inhibitor of venom enzymes with proteolytic activity.

Antifertility effect- Curcumin has strong antifertility effect. It inhibits 5-alpha reductase which converts testosterone to 5-dihydroxytestosterone and inhibit growth of flank organs. (Garg sk 1994, 1978, Shukla Y 2002, Shukla Y arora 2003).

Checks gall stone formation- Curcumin reduces the biliary cholesterol concentration and potent antinucleating activity²³.

Protection against Alzheimer's disease- A study published in Italian Journal of biochemistry (December 2003) discussed curcumin role in induction of antioxidant bilirubin, which protects the brain from oxidative free radical injury. Such oxidation is responsible for aging and neurodegenerative disease like alzheimers. Curcumin strongly induces expression of gene called hemeoxygenase-1 (HO-1) in astrocytes. Curcumin also inhibit amyloid A aggregation in brain cells, thus preventing Alzhiemer's disease. Lim et al (2001) found that curcumin reduced oxidative cange and amyloid pathology in alzhiemer's disease. With low dosage insoluble beta amyloid and plaque burden is significantly reduced²⁷.

MATERIAL AND METHOD-

Morphologically turmeric rhizomes are formed of central thick part called bulb from which finger like projections arise. Anatomical distinction of bulb and fingers is shown in three zones-

1. Cover- formed of epidermis including part of hypodermis
2. Cortex- middle part
3. Pith- inner part

Turmeric is processed by cleaning, polishing and then boiling and drying before it is available for market sale. So here in this study we will observe the amount of curcumin and curcuminoids in different anatomical zones of processed rhizomes of four turmeric varieties viz. Roma, Roma Pahari, CL-40, NDH-18. The transverse section (TS) of bulb and fingers of turmeric rhizome show the anatomical regions epidermis, hypodermis, cortex and pith distinctly. So the boiled microwave dried rhizomes and boiled infrared dried rhizomes are sectioned to reveal its epidermis, cortex and pith. Further the sections of epidermis or cover, cortex and pith are cut to get samples for methanol extract of turmeric rhizomes under study.

Preparation of sample-

1. Boiled microwave dried sample of cover, cortex and pith- 50mg of turmeric sample were taken and extracted with methanol (15x3) on a water bath. Extracts were filtered and filtrate were made to 50ml with methanol. Sample prepared is ready for HPTLC analysis.
2. Boiled infrared dried sample of cover, cortex and pith- 50mg of turmeric sample were taken and extracted with methanol (15x3) on a water bath. Extracts were filtered and filtrate were made to 50ml with methanol. Sample prepared is ready for HPTLC analysis.

Equipment used-

A Camag's HPTLC system, consisting of TLC scanner3, Linomat and a twin trough development chamber with cats 4.05 evaluation software was used,

Calculation-

To calculate amount of curcumin and total curcuminoids linear regression equation is plotted between amount of standard and area of sample and amount of sample and area of standard was plotted using following formulae-

% of curcumin = conc of std(microgram) x area of test/conc of sample x area of std * 100

% of curcuminoids =conc of total cucuminoids in std x area of test/conc of test sample x area of std *100

OBSERVATION AND RESULT-

As this study is done to know the quantitative distribution of curcumin and curcuminoids in various anatomical zones (cover, cortex, pith) of four varieties of turmeric namely Roma, Roma Pahari, CL-70, NDH-18. It is found that **curcumin(CM)** and **curcuminoids(CMD)** are more in cortex region of microwave (MW) dried and IR dried turmeric rhizomes.

The result of HPTLC analysis of Roma, Roma pahari, CL-70, NDH-18 are tabulated in table no. 1-

The MW dried Roma finger pith has maximum amount of curcumin (1.95%) and curcuminoids (5.06%). This study revealed IR dried cortex sample of Roma Pahari (curcumin-1.68% and curcuminoids- 4.28%), Roma (curcumin-1.59% and curcuminoids-4.47%) and CL-70(curcumin-0.80% and curcuminoids-2.06%) have more curcumin and curcuminoids content than MW dried samples. So it is evident that cortex of turmeric rhizome has more CM and CMD as compared to other anatomical zones.

Usually cortex of IR dried samples has more CM and CMD as compared to MW dried cortex region samples.

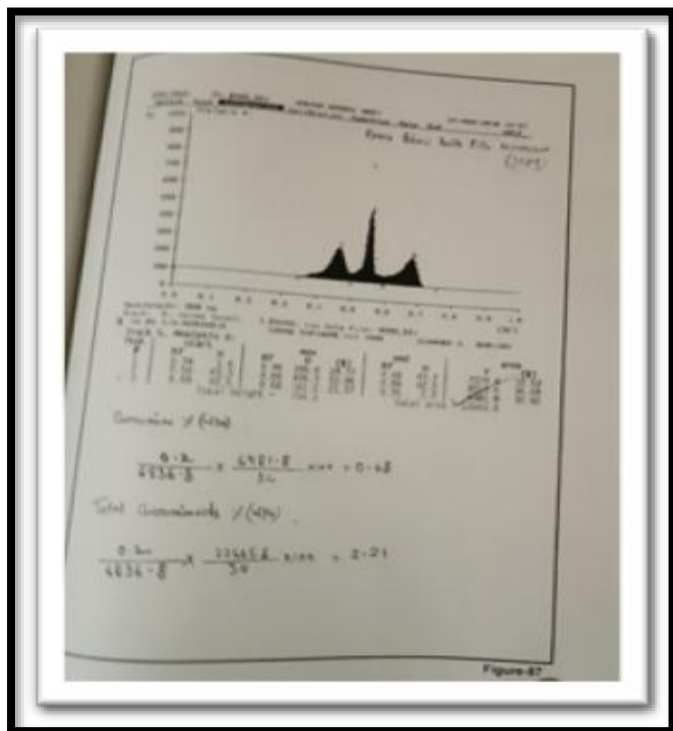
CONCLUSION AND DISCUSSION-

Biological and pharmacological studies on an effective medicinal plant require qualitative and quantitative estimation of phytochemicals or bioactive molecules. Curcuma longa L. is an important medicinal plant and its rhizome contain distinct amount of curcumin and curcuminoids. Our result showed that cortex is rich in CM and CMD amount compared to cover and pith. We also observed dark yellow or deep orange coloured spots in the cortex region of turmeric rhizomes representing high concentration of phytochemicals in that zone. The phytochemical content of cover and pith is also comparatively less than that of cortex region of turmeric rhizomes. HPTLC analysis results of bulbs and fingers of Roma, Roma Pahari, CL-70 and NDH-18 support IR dried samples of Roma and Roma pahari finger cortex is high and therefore CL-70 may be considered a good variety of turmeric. Roma Pahari IR dried finger cortex has curcumin- 1.68% and Roma IR dried finger cortex has curcuminoids-4.47% which maximum among the studied MW dried and IR dried samples of turmeric rhizome.

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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) SCANS FOR ESTIMATION OF CURCUMIN AND CURCUMINOIDS IN BULB PITH AND ROMA PAHARI FINGER COVER

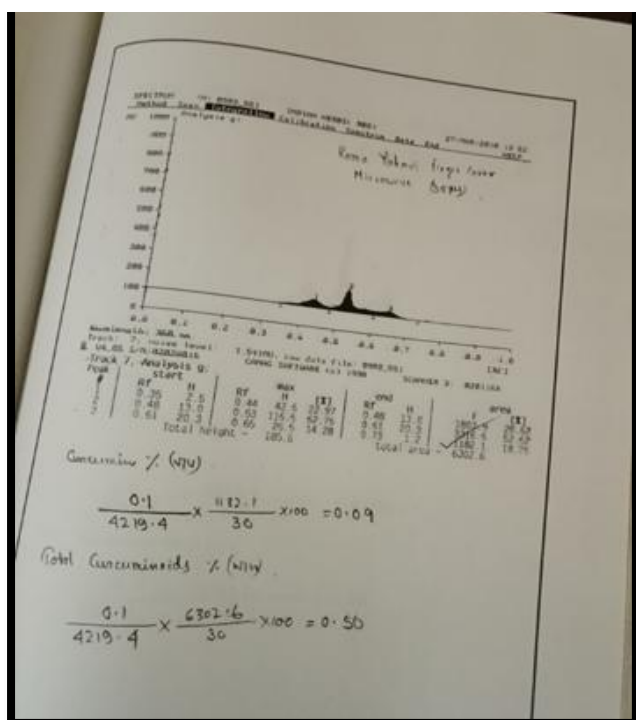


TABLE-1

CONTENT OF CURCUMIN AND CURCUMINOIDS IN DIFFERENT ANATOMICAL SECTIONS OF VARIOUS CURCUMA LONGA VARIETIES

s.no.	Variety	<u>BULB(MW)</u>						<u>FINGER(MW)</u>						<u>BULB(IR)</u>						<u>FINGER(IR)</u>					
		<u>cortex</u>		<u>pith</u>		<u>cover</u>		<u>cortex</u>		<u>pith</u>		<u>cover</u>		<u>cortex</u>		<u>pith</u>		<u>cover</u>		<u>cortex</u>		<u>pith</u>		<u>cover</u>	
		CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD	CM	CMD
1	Roma	1.30	3.89	0.91	2.90	1.37	4.02	1.95	2.87	1.95	5.06	1.75	4.84	1.52	4.25	1.11	3.38	1.23	3.51	1.59	4.47	1.32	3.23	1.33	3.29
	Roma Pahari	0.42	1.36	0.68	2.21	0.70	0.50	0.40	1.06	0.66	1.68	0.09	0.50	1.55	4.01	0.78	2.52	1.46	3.81	1.68	4.28	1.93	4.83	1.56	4.21
3	CL-70	1.56	4.70	0.83	2.41	0.63	2.00	1.09	2.67	1.24	2.90	0.90	2.40	1.29	3.55	0.77	2.27	1.00	2.87	0.80	2.06	1.79	4.39	1.33	3.41
4	NDH-18	0.32	0.86	0.43	1.21	0.22	0.69	0.66	1.37	0.66	1.43	0.32	0.90	0.39	1.20	0.46	1.60	0.31	1.20	0.56	1.50	0.49	1.58	0.41	1.21

CM= CURCUMIN : CMD= CURCUMINOIDS

CURCUMIN AND CURCUMINOID VALUES ARE MEASURED IN % W/W

MW= MICROWAVE

IR= INFRA RED

MW AND IR DRYING METHODS ARE APPLIED TO ANATOMICAL SECTIONS NAMELY CORTEX, COVER AND PITH OF BULB AND FINGER OF TURMERIC RHIZOMES. ESTIMATED CONTENT OF CURCUMIN AND CURCUMINOIDS ARE TABULATED.