CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.894496

Available online at: http://www.iajps.com Research Article

INVITRO STUDIES ON THE EFFECT OF *CURCUMA LONGA* METHANOL EXTRACTS IN COLORECTAL HCT – 116 CELL LINES

Madhuri B S[1], Dinesh Bhaskar[2], Balasubramanian Sathyamurthy*[3]

[1, 2]Department of Biochemistry, REVA Institute of Science and Management, Bangalore – 560064.

[3]*Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054

Abstract:

The leaves of Curcuma longa is commonly known as gummy gardenia/cambi gum tree. It is traditional medicinal plant grown in India, have many medicinal and therapeutic properties in diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis. This leaf extracts are found to have antioxidant, chemopreventive agents, anti-inflammatory and anti cancer activities. Curcumin is the active principle which is responsible for anticancer effects. This work was aimed to study the effect of Curcuma longa methanol extracts on HCT116 cell lines. It was found methanolic extracts of Curcuma longa leaves has phytochemical compounds having good inhibitory scavenging and anticancer activities.

Keywords: *Curcuma longa, HCT116 cell line, Curcumin.*

Corresponding author:

Dr. Balasubramanian Sathyamurthy,
Professor, Department of Biochemistry,
Ramaiah College of Arts, Science and Commerce,
Bangalore – 560054
Email: balasramaiah@gmail.com

QR code

Please cite this article in press as Balasubramanian Sathyamurthy et al, ***Invitro Studies on the Effect of Curcuma Longa Methanol Extracts in Colorectal HCT – 116 Cell Lines****, Indo Am. J. P. Sci, 2017; 4(09).*

INTRODUCTION:

Natural products have been considered as a valuable source of drug leads for many years. They have been selected to interact with biological targets. The high degree of their chemical diversity makes them attractive for discovery of new drugs. The testing of their extracts has been widely practiced in the pharmaceutical industry.

Epidemiological studies show that cancer is the second most common cause of death (after cardiovascular diseases) worldwide. According to estimates, the incidence of cancer will continue to increase, and cancer will become the first most common cause of death in the near future. For this reason, primary, secondary, and tertiary preventions for cancer are important. Primary prevention strategies entail blocking the formation of cancer and implementing precautions against risk factors for cancer. This is important from both the human health and economic (i.e., low cost) perspectives. Secondary prevention methods include early diagnosis and screening tests for the detection of the stages of cancer beginning at the cellular level, without clinical findings. In tertiary prevention, however, symptoms of cancer are present, and mortality can only be reduced with appropriate treatment. Particularly in Secondary and tertiary prevention strategies it requires significant financial funding. Recently, though the importance of primary prevention of cancer has been realized, considering the estimations of increase in the cancer incidence, developing new treatment approaches is essential. For instance, obtaining new chemotherapeutics from natural sources (i.e. plants, animals) may supply an advantage in terms of cost. Besides, the consumption of some herbal sources with diet may also contribute to primary prevention of cancer. Since several decades, many plants and plant derivatives have been used for the treatment of various diseases and as cosmetics. In recent years, herbal treatments have become very popular and are being increasingly used by alternative and complementary/integrative medical practitioners. Because plants can be easily exploited, their activities should be the focus of scientific investigations [1].

HCT116 CELL LINE

Organism	:	*Human sapiens*, Humans
Tissue	:	Colon
Disease	:	Colorectal carcinoma
Age	:	Adult
Gender	:	Male
Morphology	:	Epithelial
Growth Properties	:	Adherent

HCT116 cells are adherent epithelial cells that have been derived from the human colorectal carcinoma cell line. HCT116 cells are commonly used to study inflammatory responses in colon epithelial cells. [2]

Mutations of the APC, Ki-ras or p53 genes are considered to be necessary but individually insufficient for colorectal tumor formation. The disruption of activated Ki-ras in HCT116 and the subsequent correction of tumorigenicity in this cell lines suggested that this single genetic alteration could revert this cell lines to a more normal phenotype. Single chromosome transfer of chromosome 5 or 18 also is able to revert tumorigenic cells to a more normal phenotype, as is the reintroduction of a normal p53 gene. Introduction of APC by itself accomplishes a similar alteration in the phenotype of tumorigenic cells. HCT116 is characterized by a 13Asp in Ki-ras, no detectable mutation in p53 and a high degree of microsatellite instability. These results support the idea that more than one mutation is required for tumorigenicity and these mutations must accumulate in some complementary way to confer tumorigenicity. [3]

Many polyphenolic compounds may induce apoptosis through modification of the Mitogen –Activated Protein Kinases (MAPK) pathways. c-Jun N-terminal Kinase (JNK) is a member of the MAPK family which is activated by inflammatory cytokines and a variety of chemical stresses. Activated JNK phosphorylates, activates c-Jun and other transcription factors such as Activating Transcription Factor 2 (ATF-2) and Elk-1, but its role in apoptosis remains controversial. Activation of JNK may have pro-apoptotic or anti-apoptotic effects and may also promote cell proliferation. Similarly, c-Jun, the main downstream effectors of JNK, has also been shown to display both pro- and anti-apoptotic properties. Suppression of the transcription factor NFkB results in sustained activation of JNK after TNF treatment. Sustained JNK activity was found to be pro-apoptotic, whereas rapid, transient activation could be anti-apoptotic. Conflicting evidence shows that sustained JNK activity in the absence of NFkB can inhibit apoptosis. Some dietary chemicals with chemo preventive properties such as capsaicin and isothiocyanates have been shown to induce apoptosis through a JNK-dependent mechanism. Curcumin inhibits NFkB activation and has been shown previously to decrease JNK and AP-1 transcriptional activity initiated by cytokine stimulation. Data relating to effects of curcumin alone on JNK activation are lacking. Apoptosis which induced by curcumin may involve activation of JNK in addition to inhibition of constitutive NFkB activity and investigated these themes in HCT116 human colon

cancer cells. This cell line provides a suitable model of early APC/K-Ras-mediated neoplastic transformation relevant to colorectal cancer. For example, HCT116cells have intact APC but contain one wild-type allele and one mutant allele for an activating mutation of b-catenin (CTNNB1WT/D45). HCT116 cells also contain mutationally activated K-ras with intact JNK, Raf - Extracellular signal-Regulated Kinase (ERK) and p38responses. In addition, HCT116 cells retain wild-type inducible p53 and NFkB responsiveness. Here, we show that curcumin promotes activation of JNK and c-Jun, and stimulates AP-1 transcriptional activity. Blockage of these effects by a JNK-specific inhibitor impedes curcumin – induced apoptosis. These studies suggest a novel role for JNK effector pathways in curcumin-mediated apoptosis [4].

Curcuma longa

Turmeric has been used in India for more than 2,400 years and in China by 700 AD, East Africa by 800 AD and West Africa by 1200. It was introduced to Jamaica in the 18th century. Today, turmeric is widely cultivated throughout the tropics. Turmeric is the source of a bright yellow - orange culinary spice and dye and has important medicinal and cultural uses. *Curcuma longa* is a member of the ginger family (*Zingiberaceae*). Its rhizomes (underground stems) are the source of a bright yellow spice and dye.

The origin of *Curcuma longa* is not known, but it is thought to originate from South Asian country, most probably from Vietnam, China or India. It is only known as a domesticated plant and not found in the wild. India is the world's largest producer, consumer and exporter of turmeric. Turmeric is also cultivated extensively in Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia and the Philippines [5].

An upright, perennial herb to about 1 m tall. The rhizome (underground stem) is thick and ringed with the bases of old leaves. Turmeric only reproduces via its rhizomes. Leaves are large, oblong, upto 1 m long, dark green on upper surface, pale green beneath. Each leafy shoot (pseudostem) bearing 8–12 leaves. Flowers are Yellow-white, borne on a spike-like stalk 10–15 cm long. Flowers are sterile and do not produce viable seed. Seeds are small, ovoid and brown. Turmeric powder is yellow pigmented and has numerous curcuminoids that include curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%). Curcumin is a polyphenol (1, 7 – bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione). Turmeric extracts or "pure" active "curcumin" powder, the role of curcuminis most studied on chemopreventive agents, anti-inflammatory and cancer activity over the last half century has been well appreciated. Records from cell culture, animal research, and clinical trials indicate that curcumin may have potential therapeutic agent to treat diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis. The anti-cancer effect has been recorded in a few clinical trials, mainly as a chemoprevention agent in colon and pancreatic cancer, cervical neoplasia and Barrets metaplasia. The compound modulates several molecular targets and inhibits transcription factors (NF-kB, AP-1), enzymes (COX-1, COX-2, LOX), cytokines (TNF, IL-1, IL-6) and antiapoptotic genes (BCL2, BCL2L1). As a result, curcumin is able to induce apoptosis and has antiangiogenic activity [6].

Over 300 diarylheptanoids have been reported in the family *Zingiberaceae* and some non-closely related families. Curcuminoids belong to the group of diarylheptanoids diphenylheptanoids having an aryl-C7-aryl skeleton. These yellow pigments are usually used as food coloring agents and they are the main active compounds in turmeric. Usually, these polyphenols are present in 3-15% of turmeric rhizomes with curcumin as the principal compound. Curcumin ($C_{21}H_{20}O_5$), also known as diferuloyl methane or 1, 6-heptadiene-3, 5-dione-1, 7-bis (4-hydroxy-3-methoxyphenyl)-(1E, 6E), was isolated in 1815 and its chemical structure was determined in 1910. The compound is a yellow powder with a molecular weight of 368.37. It is water insoluble but can be dissolved well in ethanol, methanol, actone, and dimethysulfoxide. Commercial "cur-cumin" is usually a mixture of three curcuminoids. For example, the composition of a commercial "curcumin" is about 71.5% curcumin (curcumin I), 19.4% demethoxycurcumin (curcumin II), and 9.1% bisdemethoxycurcumin (curcumin III) [7].

There are a few reports on constituents of *C. longa* leaf essential oil from different origins. Sixty one compounds were identified from the leaf oil of *C. longa* constituting 99.8% of the oil and main constituents were α-phellandrene (53.4%), terpinolene (11.5%) and 1, 8-cineole (10.5%). Leaves essential of elite genotype of *C. longa* L. from South Eastern Ghats of Orissa showed α –phellandrene (57.8%) as a major constituent. The leaf oil of *C. longa* from Vietnam contained mainly α-phellandrene (24.5%), 1, 8-cineole (15.9%), p-cymene (13.2%) and β-pinene (8.9%), while that of a Nigerian chemotype contained mainly α-phellandrene (47.7%) and terpinolene (28.9%) [8].

Role of curcumin in Cancer cell lines

Cancer is proliferative disorder where a normal cell loses its cellular homeostasis and begins to

constitutively activate a plethora of genes that are involved in cell cycle, invasion, survival, metastasis, and angiogenesis. Curcumin is also a potent anti-inflammatory compound. Based on its distinct chemical properties, curcumin interacts with many extracellular and intracellular molecules that are actively involved in cancer initiation and progression, thereby inhibiting cancer progression. Increasing evidence suggests that deregulated inflammatory pathways play a pivotal role in a multitude of chronic diseases, including cancer. The mechanism by which chronic inflammation drives cancer initiation and progression is via increased production of pro-inflammatory mediators, such as cytokines, chemokines, reactive oxygen species , over expression of oncogenes, cyclooxygenase, Matrix Metallo Proteinase (MMPs), intracellular signaling pathway mediators, transcription factors such as nuclear factor κB (NF-κB), Signal Transducer and Activator of Transcription 3 (STAT3), protein kinase B (AKT), and activator protein 1 that drive tumor cell proliferation, transformation, invasion, metastasis, angiogenesis, chemo resistance, and radio resistance [9].

Curcumin induced apoptosis was known by inhibition of NFkB transcriptional activity and sustained activation of JNK and p38. Inhibition of JNK signaling pathways by use of the specific pharmacological inhibitor, attenuated curcumin induced apoptosis. Conversely, blockage of p38 signaling had no effect. Whilst JNK has been implicated in both pro- and anti-apoptotic pathways, it appears that the duration of its activation is a critical factor in determining cell survival or apoptosis [10].

Curcumin inhibition of a consitutively activated NFkB-dependent luciferase reporter construct, accord with previous observations. Sustained activation of JNK resulting from inhibition of NFkB has been attributed to disruption of the negative modulation of JNK activation exerted by NFkB responsive genes such as XIAP andgadd45b. Accordingly, curcumin has been shown to inhibit XIAP expression during apoptosis in melanoma cells. Dietary isothiocyanates are chemo preventive and induce JNK-dependent apoptotic signaling by suppressing expression of the JNK-specific phosphatase M3/6. Conceivably, curcumin could invoke similar pathways although further work is necessary to investigate the precise mechanisms responsible [11].

MATERIALS AND METHODS:

Sample Collection

Leaves wear collected from the *Curcumba longa* from Department of Horticulture, Ghandhi Krushi Vignana Kendra (GKVK), Bangalore.

Extraction procedure*:*

Plant leaves were washed thoroughly with distilled Water. The leaves were dried under shade at room temperature. The dried leaves of *Curcumba longa* were finely grinded using electrical grinder and stored in air tight containers for further use. A total of 250 g of the pulverized plant material was extracted for 4 d in Methanol. The extracts were then filtered through Whatman's No. 1 filter paper and then condensed to dryness using rotary evaporator. The thick extracted mass was then dried at room temperature. Dried extract was collect [12].

Phytochemical analysis

Phytochemical analysis of *Curcumba longa* seed extracts were done using the protocols described by ; Segelman AB, Fransworth NR, Quimbi MD, L Loide for the following[13].

Test for Sterols	-	Liebermann Burchard reaction
Tests for Alkaloids	-	Mayer's and Wagner's test
Tests for Tannins	-	Ferric chloride reagent test and
Tests for Saponins	-	Foam test
Tests for Phenols	-	Ferric chloride reagent test
Test for cardiac glycosides	-	Salkowiski test

Tests for Flavonoids

Test for Terpenoids

Hydroxyl Radical Scavenging Assay

Principle

Hydroxyl radicals, generated by the Fenton type reaction system (Fe^{+3} + EDTA / H_2O_2 / Ascorbic acid), are known to damage deoxyribose and form TBA reactive chromogen, which forms a pink color measured spectrophotometrically at 532 nm.

Procedure:

The deoxyribose assay is performed as described by Halliwell et al. with minor changes. The reaction volume of 1.0 ml contains 5.6mM deoxyribose, 2.8mM H_2O_2 , 40 µM $FeCl_3$, 100 µM EDTA, and varying concentrations of the sample in 2.5mM phosphate buffer, pH 7.4. Initiation of the reaction is by the addition of 0.1mM Ascorbic acid. The mixture is then incubated for 90 minutes at 37°C. After incubation of 1ml of TBA (0.7% in 0.05 N KOH) and 1ml of 2.5% TCA the mixture is heated at 100°C for 8 minutes, cooled and the pink color formed is measured spectrophotometrically at 532 nm. Controls are to be run, which are devoid of test samples. Quercitin is used as the reference standard [14].

HPLC analysis of Quercetin

Plant Extraction:

10gms plant powder was extracted with 50ml Methanol at 50°C for 4 hours. The Methanolic extracts were filtered through Whatmann No. 1 filter paper and filtrate was evaporated to dryness. Methanolic extract (10mg/ml) was used for HPLC analysis.

Quercetin Standard: 100ug/ml prepared in Methanol

HPLC Condition:

Instrument	**:**	Shimadzhu LC-Prominence 20AT
Column	**:**	C18 column 250 mm x 4.6 mm, 5u particle
Mobile Phase	**:**	Linear
A	**:**	HPLC grade Acetonitrile (60%)
B	**:**	HPLC grade Water (40%)
Flow Rate	**:**	1.0 ml/min
Injection volume:		10ul

Quantification of Quercetin in plant extracts

Concentration of Standard injected: 100µg/ml

Sample concentration: 10mg/ml

Formula used for quantification of quercetin in plant extract

Quercetin (Microgram/gram) = Sample area / Standard area X Standard concentration injected X Dilution factor.

4. Cytotoxicity studies using HCT116cell line by MTT assay

HCT116 cell line was obtained from American Type Culture Collection (ATCC) (Rockville, MD USA) (ATCC Number-CCL-247). The steps and procedure for cell culture, Thawing, Revival and Propagation of Cells were followed as described by Kangas, L. *et al*. [15].

Procedure

The collected cells should be about 70-80% confluency. Check the viability of the cells and centrifuge it. Take about 50,000 cells / well and seed it in 96 well plates and incubate for 24 hrs at 37^0C, 5% CO_2 incubator. Add plant samples which is to be tested from 0-320µg/ml (2 fold variation) concentration in RPMI without FBS & are incubated for 24 hr. Add 100µl/well of the MTT (5 mg/10ml of MTT in 1X PBS) to incubated plant samples to the respective wells and incubated for 3to 4 hours. Discard the MTT reagent by pipetting without disturbing cells and add 100 µl of DMSO to rapidly solubilize the formazan. Measure the Absorbance at 590 nm.

Calculating Inhibition

% Inhibition = 100 – (OD of sample/OD of Control) X 100.

RESULTS AND DISCUSSION:

Phytochemical analysis of *Curcumba longa*

Table.1: Phytochemical analysis of *Curcumba longa* leaves extracts

S.No	Tests	Observation	Inference
1	Froth formation test	Formation of stable froths was observed.	Presence of Saponins was confirmed.
2	Mayer's and Wagner's test	A brown color Precipitates was not observed.	Absence of Alkaloid was confirmed.
3	Ferric Chloride test	Dark green color was not developed.	Absence of Tannin was confirmed.
4	Liebermann-Burchard test	Formation of bluish green color was observed.	Presence of Steroid was confirmed.
5	Sodium hydroxide test	Change from yellow color to colorless was observed.	Presence of Flavonoid was confirmed.
6	Ferric chloride test	Violet color was not developed.	Absence of Phenol was confirmed.
7	Salkowski test	Reddish brown coloration was observed.	Presence of Terpenoid was confirmed.
8	Benedict's test	Formation of an orange red precipitate was not observed.	Absence of reducing sugar was confirmed.
9	Biuret test	Formation of pink color in the extract layer was found.	Presence of protein was confirmed.

From Table – 1, the *Curcumba longa* leaf extracts contains phytochemical compounds such as terpenoids, Saponins, flavonoids, proteins and steroids. The alkaloids, phenols, tannins and reducing sugars were absent.

Hydroxyl Radical Scavenging Assay:

Table.2: Hydroxyl Radical scavenging assay

Plants Name	Concentration (µg/ml)	Absorbance 546nm	% Inhibition	IC_{50}
Control	0.0	0.5934	0.00	30.35
Standard (Quercitin)	6	0.5592	5.76	
	12	0.5143	13.33	
	25	0.4265	28.13	
	50	0.2518	57.57	
	100	0.2158	63.63	
	200	0.1511	74.54	
Curcuma longa	3.12	0.5594	5.73	56.08
	6.25	0.5158	13.08	
	25	0.4271	28.02	
	100	0.3527	40.56	
	400	0.2765	53.40	
	600	0.1756	70.41	

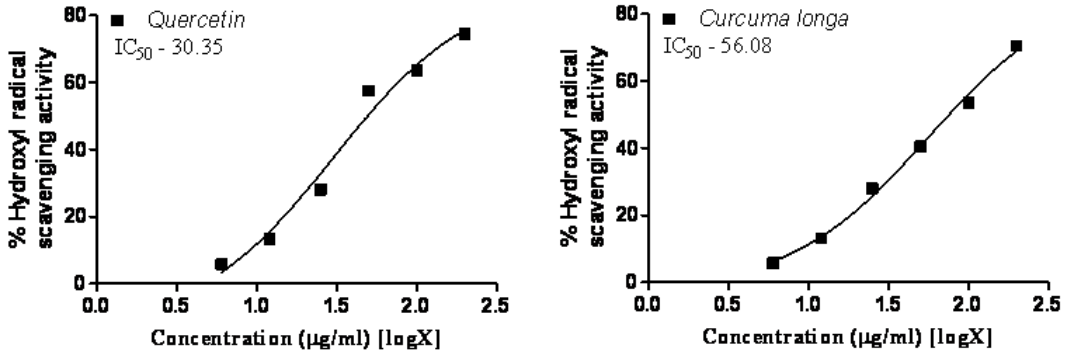

Fig1: Hydroxyl Radical scavenging assay ofQuercetin and *Curcumba longa*

From Table – 2 and Figure – 1, the studies on Hydroxyl radical scavenging assay using Quercetin as standard, it is understood that the *Curcumba longa* (IC_{50}:56.08µg/ml) have high inhibitory concentration when compare to Quercetin (IC_{50}: 30.35ug/ml). It may be due to the presence of phytochemicals which show Hydroxyl radical scavenging property.

HPLC analysis of Quercetin and *Curcumba longa*

Table 3: HPLC analysis of Standard Quercetin

S. No.	Retention. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.933	373.177	22.881	20.5	14.0	0.20
2	3.107	92.433	3.352	5.1	2.0	0.49
3	3.487	1296.195	133.916	71.3	81.6	0.14
4	4.207	55.054	3.869	3.0	2.4	0.22
	Total	1816.859	164.018	100.0	100.0	

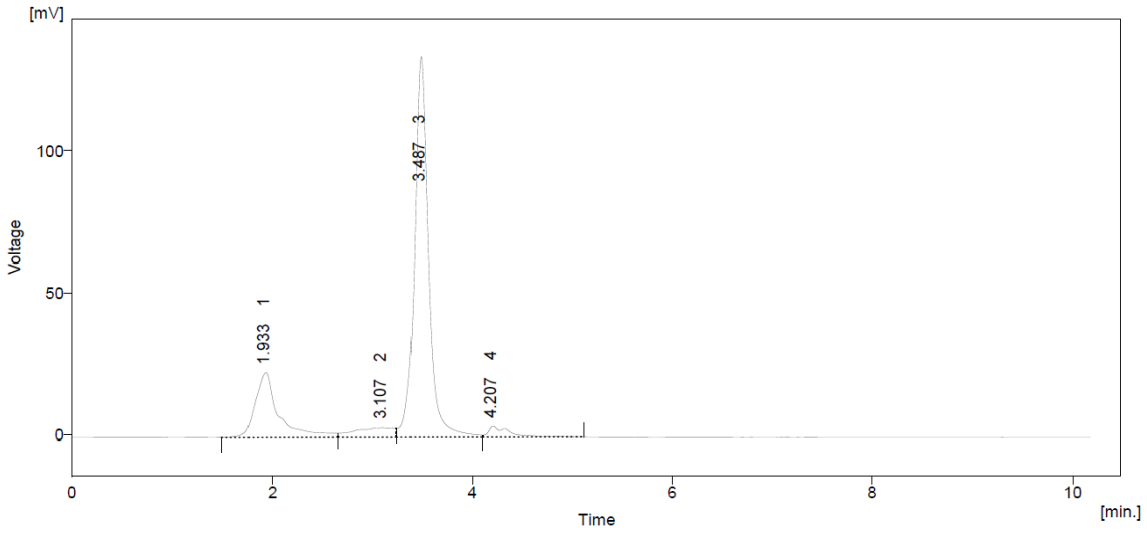

Fig 2: HPLC analysis of standard Quercetin

From Table – 3 and Figure – 4, the flavonoids were quantified at 254nm using peak area by comparison with a calibration curve derived from the quercetin.

Table 4: HPLC analysis of Quercetin content in *Curcumba longa*

S. No.	Retention. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.320	21.468	3.354	2.1	4.5	0.10
2	1.827	148.540	12.969	14.7	17.6	0.15
3	2.250	338.443	31.983	33.5	43.3	0.10
4	3.227	465.221	23.567	46.0	31.9	0.26
5	3.780	34.540	1.687	3.4	2.3	0.34
6	6.247	2.324	0.265	0.2	0.4	0.13
	Total	1010.533	73.826	100.0	100.0	

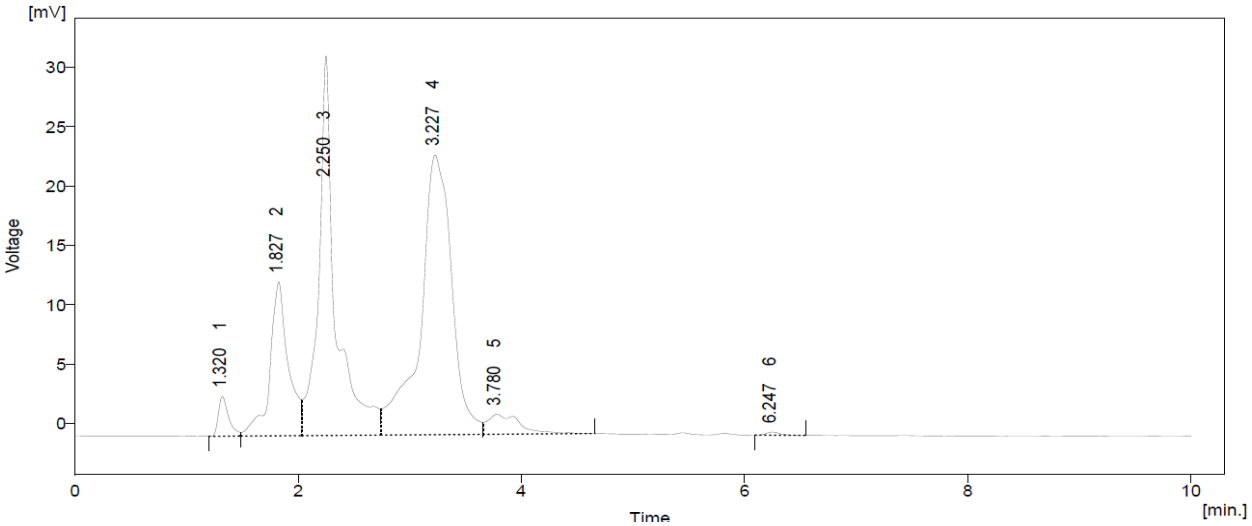

Fig.3: HPLC analysis of Quercetin content in *Curcumba longa*

From Table – 4 and Figure – 3, the HPLC chromatograms from leaves of *Curcumba longa* the main difference was in peak eluted at 3.4min. External flavonoids were already analyzed using HPLC method in various plant extracts.
The peaks in this study shown marked decreased in peak area in case of *Curcumba longa* leaves when compared with standard quercetin.

From the calibration curve results, the amount of Quercetin, in the sample injected was calculated. *Curcumba longa* leaves contain no quercetin. Other peaks in both the HPLC chromatogram *Curcumba longa* leaves extract indicated the presence of other chemical constituents. The present method was applicable for determining quercetin in any aerial part of plant material using HPLC technique.

Cytotoxicity studies using HCT116 cell lines by MTT assay

Table 5: cytotoxic study of *Curcumba longa*

Plants name	Conc. µg/ml	OD at 590 nm	% Inhibition	IC_{50}
	Control	0.5911	0.00	
Curcuma longa	10	0.5594	5.36	78.46
	20	0.5241	11.33	
	40	0.4172	29.42	
	80	0.3018	48.94	
	160	0.1738	70.60	
	320	0.1046	82.30	

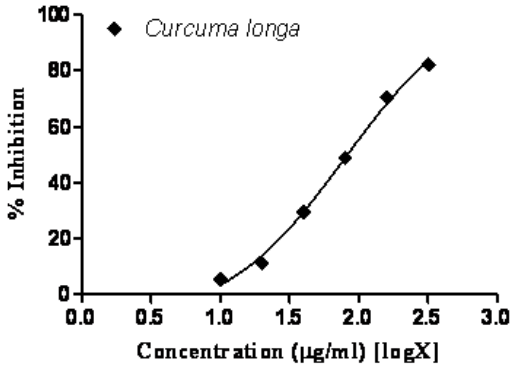

Fig 4: Cytotoxic study of *Curcumba longa*

In cytotoxic study of *Curcumba longa* (from table – 5 and figure – 4) on HCT116 cancer cell lines using MTT assay showed IC_{50} value was 78.46µg/ml.
These suggest that plant extracts showed significant inhibition of HCT116cell lines.

CONCLUSION:

The above observations of methanolic extracts of *Curcumba longa* possess maximum phytochemical components that are good in radical scavengers. The maximum Hydroxyl Radical IC50 valve of standard Quercetin and *Curcumba longa* was found to be 30.35 and 56.08 respectively. The MTT assay was found that there are cytotoxic effects with increasing concentration on HCT116cell line from 10µg to 320µg concentration when compared to the untreated HCT116cells.

ACKNOWLEDGEMENT

The authors are thankful to Skanda Life Sciences Private Limited, Bangalore for providing necessary facilities to complete this project successfully.

REFERENCES:

1.Hatice Gumushan Aktas, Seyhan Altun. In vitro antitumor effect of a new cultivar of trigonella foenum. Aktas and Altun., Afr J Tradit Complement Altern Med. 2015; 12(3):113-119
2.Awwad R A. Ishizuka J. Blagosklonny M V. Berry S E. Howell G M. HCT cell line Mesh review. Wiki gene publication.
3.Joanna Groden, Geoff Joslyn, Wade Samowitz, David Jones, Nitai Bhattacharyya, Lisa Spirio, Andrew Thilveris, Margaret Robertson, Sean Egan, Mark Meuth, and Ray. Response of Colon Cancer

Cell Lines to the Introduction of APC, a Colon-specific Tumour Suppressor Gene'. Cancer research 1995:55, 1531-1539

4.Gavin P.Collett and Frederick Charles Campbell. Curcumin induces c-jun N-terminal kinase-dependent apoptosis in HCT116 human colon cancer cells: Carcinogenesis 2004; 25(11) 2183--2189,

5.Aggarwal, B. B.; Bhatt, I. D.; Ichikawa, H.; Ahn, K. S.; Sethi, G.; Sandur, S. K.; Sundaram, C.; Seeram, N.; Shishodia, S. Curcumin Biological and medicinal properties. In Turmeric: The genus Curcuma, Ravindran, P. N.; Nirmal Babu, K.; Sivaraman, K., Eds. CRC Press: Boca Raton, London, New York, 2007; 297-368

6.Chavalittumrong, P.; Jirawattanapong, W. Variation of active constituents of Curcuma domestica rhizomes at different ages. Thai. J. Pharm. Sci., 1992; 16: 165-174.

7.Pfeiffer, E.; Hhle, S.; Solyom, A. S.; Metzler, M. Studies on the stability of turmeric constituents. J. Food Engineer., 2003; 56: 257-259.

8.Yasni, S.; Imaizumi, K.; Sin, K.; Sugano, M.; Nonaka, G. Identification of an active principle in essential oils and hexane-soluble fractions of Curcuma xanthorrhiza Roxb. showing triglyceride lowering action in rats. Food Chem. Toxicol., 1994; 32: 273-278.

9.Divya, C.S.; Pillai, M.R. Antitumor action of curcumin in human papillomavirus associated cells involves downregulation of viral oncogenes, prevention of NFkB and AP-1 translocation, and modulation of apoptosis. Mol. Carcinog. 2006; 45: 320–332.

10.Chen, Y.R.; Tan, T.H. Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. Oncogene 1998; 17: 173–178.

11.Shanmugam, M.K.; Kannaiyan, R.; Sethi, G. Targeting cell signaling and apoptotic pathways by dietary agents: Role in the prevention and treatment of cancer. Nutr. Cancer 2011; 63: 161–173.

12.Hemant RJ, Bhutani KK. Antioxidant properties of Indian Medicinal plants. Phytother. Res. 2002; 16(8):771- 773

13.Segelman AB, Fransworth NR, Quimbi MD, L Loide. Biological and phytochemical evaluation of plants. Pub met.1969; 1: 52-58.

14.Halliwell B, Gutteridge JMC and Aruoma OI. The deoxyribose method: a simple test tube assay-determination of rate constants for reaction of hydroxyl radicals. *Anal.Biochem.* 1987; 165: 215-219.

15.Kangas, L. *et al*. Bioluminescence of cellular ATP: A new method for evaluating cytotoxic agents in vitro. *Med. Biol*. 1984; 62: 338–43.