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STUDY ON SILVER NANOPARTICLE ENCAPSULATED CURCUMIN FOR ANTICANCER ACTIVITY

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ABSTRACT

Curcumin is one of the poly phenols, which is known for its medicinal use since long time. Curcumin shows poor solubility and low absorption and therefore, its use as nanoparticles is beneficial due to their greater solubility and absorption. In this work curcumin was extracted from curcuma longa by Soxhlet method. The particle size of the curcumin was reduced by Ultrasonication method. Silver nanoparticle was synthesized by chemical reduction method. These two solutions were agitated about 30 minutes using magnetic stirrer to form silver encapsulated curcumin. Particle size of the curcumin (36.6 nm), silver nano particle (50.6 nm) and silver encapsulated curcumin (102.1 nm) was studied by using Particle size analyzer. The R_f value was calculated from TLC analysis. Purity and percentage of the curcumin content was found by HPLC analysis. Anticancer activity was assayed out for curcumin and silver nanoparticle encapsulated curcumin. The study summarizes the challenges to develop curcumin delivery platforms and up-to-date solutions for improving curcumin bioavailability and anticancer potential for therapy.

KEY WORDS: Anti cancer activity, Silver nano particles, Curcumin, HPLC, TLC.

1. INTRODUCTION

From ancient times silver and turmeric are used for medicinal purposes. Silver has good anti-oxidant properties. Turmeric has a compound called curcumin. Curcumin is responsible for the medicinal properties and colour of turmeric. The bio availability of curcumin is very less. It is hydrophobic and hence it is not absorbed by blood. Curcumin is all-in-one medicine for bacterial infections, cancer treatment, viral infections, inflammations, fungal infections, etc.

Nano medicines hold very good promise for target oriented reach of the drugs. The medicines which are generally available react in the same way to all the cells. They can not differentiate diseased cells and normal cells. So the available medicines kill the normal cells along with the diseased cells thereby damaging the body in the process of curing it. The approaches in nano medicine range from the medical use of nano materials, nano electronic biosensors and even possible future applications of molecular nanotechnology. Current problems for nano medicine involve understanding the issues related to toxicity and environmental impact of nano scale materials. Nano medicine seeks to deliver a valuable set of research tools and clinically helpful devices in the near future.

Topical drug delivery system for burn wound healing using SNPs prepared by the new method has been successfully developed.[1] Biosynthesized silver nanoparticles (b-Ag NPs) shows enhanced antibacterial activity compared to chemically synthesized silver nanoparticles. Silver nano particle is one of the plausible mechanisms for the anticancer and antibacterial activity.[2] Silver based nano particles are used in the field of infectious diseases treatment.[3] Turmeric is safe and non-toxic for most patients, it has been shown to have diverse biological effects in humans and animals. Turmeric/curcumin is a potent anti-inflammatory and antioxidant. The evidence suggests that it can suppress tumor genesis, tumor promotion, and metastasis and, therefore, has enormous potential as an anticancer agent. Further study is needed to determine whether it, like other antioxidants, should be avoided during chemotherapy.[4]

Pure curcumin and the crude ethanol extract have great potential in the prevention and cure of cancer.[5] Extraction of curcuminoids from turmeric powder with the help of methanol as solvent was carried out and curcuminoids were isolated in the form of crystals with 95% purity and 75% yield. The isolated crystals show very good anti-inflammatory activity against inflammation induced edema.[6] For curcumin extraction dichloromethane is the best solvent for extraction. Cost-effective, simple and rapid identification method for authentication of C. Longa rhizome have been developed.[7] The ethanol extract of curcuma rhizome exhibited significant antibacterial activity. Ethanol extract is effective than aqueous extract against bacteria and fungi.[8] Isolated turmeric extracts and pure curcumin showed very weak activity against the studied myco bacteria but showed very good antioxidant activity.[9] It is one of the best medicines to treat various life threatening diseases like Alzheimer, Huntington's disease, Various types of cancer and AIDS.[10]

1.1 Curcumin

Curcumin differentiates between the normal cells and the cancer cells. It will not kill the normal cells when it destroys the cancer cells. Such a medicine is hard to come by. But the problem is that the curcumin has very less bio availability. It is not soluble in water. So it is not present in the viable form in blood for long time after consumption to be effective for the treatment. The bio availability of nano curcumin is more than that of the conventional type. Attempts to improve the bioavailability of this novel compound curcumin are underway at various places as we speak. Adjuvants which absorb, retain curcumin and then release it in the blood stream after some time are being discovered. Nano particles of curcumin are being studied. Liposomes and phospholipid complexes are used to improve the delivery of curcumin.

1.2 Silver nanoparticle

Silver nanoparticles are nanoparticles of silver between 1 nm and 100 nm in size. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Their extremely large surface area permits the coordination of a vast number of ligands. The properties of silver nanoparticles applicable to human treatments are under investigation in laboratory and animal studies, assessing potential efficacy, toxicity and costs.

Introduction of silver into bacterial cells induces a high degree of structural and morphological changes, which can lead to cell death. As the silver nanoparticles come in contact with the bacteria, they adhere to the cell wall and cell membrane. Silver nanoparticles inhibit replication and are sufficient to cause the death of the cell. When silver dissolves in cytosol, it ionizes to produce nanoparticles that increases its bactericidal activity. When particle size decreases, reactivity increases because of increase in surface area to volume ratio.

Recently, silver nanoparticles have been extensively used in electronics, engineering, textiles, paints, food industry, cosmetics, bio-sensing, Chronic ulcers, and in many areas in medicine and biology. Therefore, design and development of simple, one-step, reliable, low-cost, non toxic and eco-friendly method for the fabrication of multifunctional silver nanoparticles is of greatest importance to expand their biomedical applications.

2. MATERIALS AND METHODS

2.1 Extraction of curcumin

Curcuma longa (turmeric) were collected from an organic farm which is located at Erode, Tamilnadu. Fresh rhizomes were cleaned using deionized water, then rhizomes were scrapped. It was dried in pollution free atmosphere at room temperature. After few days it was powdered using mixer grinder.20 g of the turmeric powder was taken into a thimble and placed in a Soxhlet apparatus, with ethanol as a solvent. Finally dark yellow extraction was obtained and filtered. The filtrate was kept in Ultrasonicator at 20 KHz frequency for about 20 minutes to reduce the particle size of the curcumin.

2.2 Synthesis of silver nanoparticle

A multitude of chemical reduction methods have been used to synthesize silver nanoparticles from silver salts. The reactions considered here were limited to those using silver nitrate as the starting material. They vary in the choice of reducing agent, the relative quantities and concentrations of reagents, temperature, duration of reaction, as well as the diameters of the nanoparticles produced. In nearly all of them the colloidal silver products are described as turbid and greenish-yellow or brown.[11–13]

A large excess of sodium borohydride is needed both to reduce the ionic silver and to stabilize the silver nanoparticles that form. A 10-mL volume of 1.0 mM silver nitrate was added drop wise (about 1 drop per second) to 30 mL of 2.0 M sodium borohydride solution that has been chilled in an ice bath. The reaction mixture was stirred vigorously on a magnetic stirrer plate. The solution turned light yellow after the addition of 2 mL of silver nitrate and a brighter yellow, when all of the silver nitrate has been added. The entire addition took about three minutes, after which the stirring was stopped and the stir bar removed.

Nanoparticles in colloidal sols can also be stabilized by adsorption to polyvinylpyrrolid (PVP).[14,15] Only a concentration of PVP is needed to prevent the aggregation. The procedure is repeated with diluted PVP until aggregation is observed upon addition of salt. The minimum concentration of PVP required to stabilize the sol synthesized according to the method described here was 0.01%.

2.3 Silver nanoparticle encapsulated curcumin

Silver nanoparticle (brown colour) and curcumin (dark yellow colour) was taken in the ratio of 2:3 and the sample was stirred using magnetic stirrer for about 30 minutes. By this process finally dark brownish yellow colour solution was obtained.

3. RESULTS AND DISCUSSION

3.1 Particle size

Particle size analyzer method was used for measuring the attenuation ultrasound at the set of frequencies in the MHz range. This attenuation spectrum is the raw data used for calculating the particle size distribution.

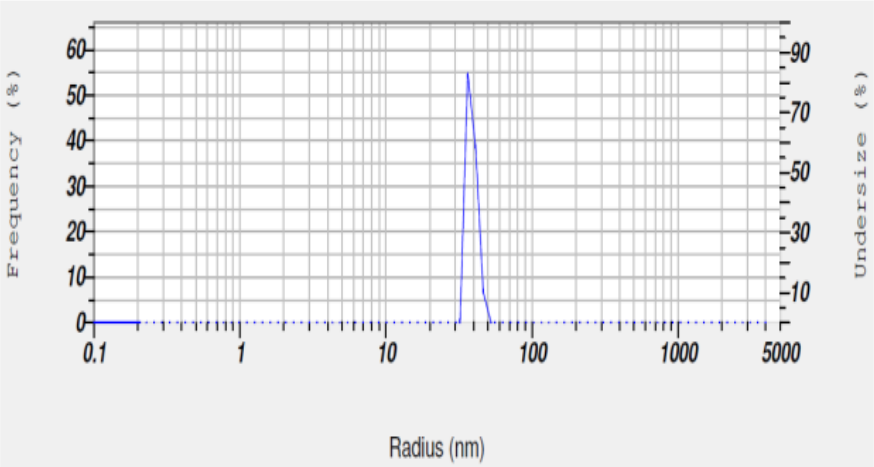

Fig.1 Particle size measurement for curcumin

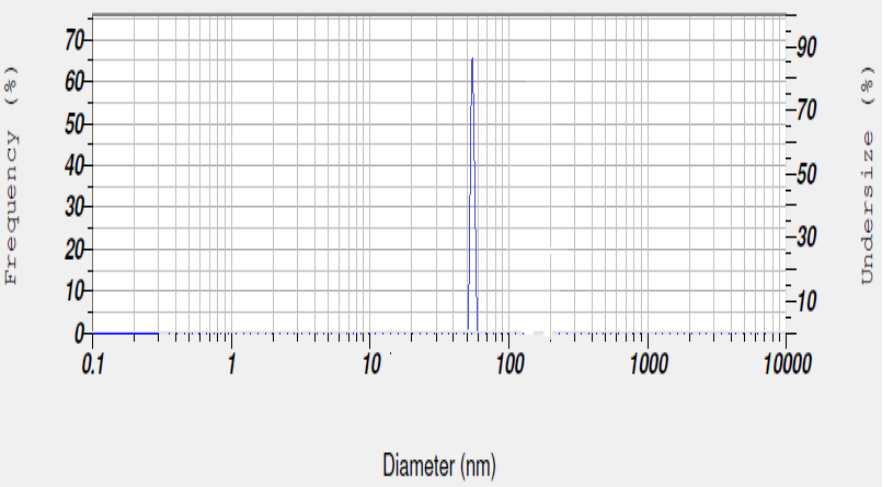

Fig.2 particle size measurement for silver nanoparticle

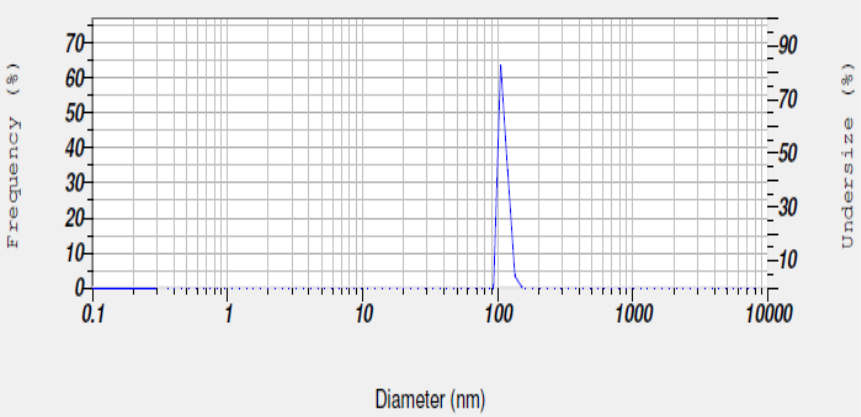

Fig.3 particle size measurement for silver nanoparticle encapsulated curcumin

In the present work, Nano Partica SZ-100 analyzer, Horiba Scientific make was employed to measure the particle size of the samples. The obtained results are shown in figures (1-3). From particle size analysis the size of curcumin, silver nanoparticle and the mixed solution are found to be 36.6 nm, 50.6 nm and 102.1 nm respectively. The increase in particle size is due to the encapsulation of silver nano particles with curcumin.

3.2 Thin layer chromatography

Thin layer chromatography of three solutions has been performed on TLC sheet using dichloromethane: methanol in the ratio 19:1 as a mobile phase. These conditions gave maximum resolution of spots. The Retention Factor was calculated using the formula, R_f = Distance travelled by the solute/ Distance travelled by the solvent. Three major bands were observed in extraction which corresponds to curcumin (C) (R_f = 0.885), demethoxycurcumin (DCM) (R_f = 0.704) and bisdemethoxycurcumin (BDMC) (R_f = 0.557). The chromatography of curcuminoids, silver nanoparticle and silver nanoparticle encapsulated curcumin are shown in fig. 4, 5 and 6 respectively.

Fig.4 curcuminoids

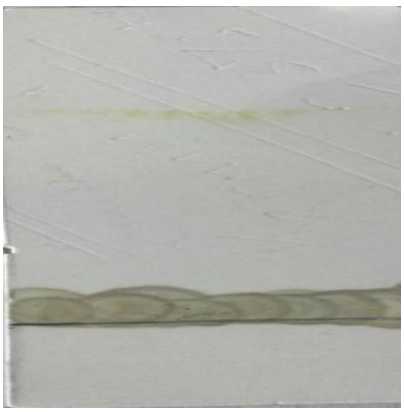

Fig.5 silver nanoparticle

Fig.6 silver nanoparticle Encapsulated curcumin

3.3 HPLC analysis

HPLC method was used for qualitative and quantitative estimation of curcuminoids. Samples containing different proportions of turmeric was accurately weighed and dissolved in 10 ml of distilled water. Samples were stirred and filtered through 0.45-mm filter membrane to get clear solution. Then the samples were injected into the column and analyzed under chromatographic condition.

Curcuminoids have immense biological properties in which curcumin (C) is reported for so many medicinal properties. Recently the analogs of curcumin were reported for biological activities. Demethoxycurcumin (DMC) were the best for inhibition of MCF -7 cells.[16] Bisdemethoxycurcumin (BDMC) is active for modulation of MDR-1 gene expression.[17] Compounds DMC and BDMC are not commercially available. Therefore to study biological properties of individual curcuminoids we need to isolate compounds at high purity.

Ethanol is the suitable solvent for extraction of curcuminoids. Ethanol extracted curcuminoids were quantified using HPLC. The chromatograms of 36.6 nm curcuminoids are shown in figure7. From the figure it is clear that C, DMC, BDMC of 50.6 nm curcuminoids in ethanol extract show single peaks at retention times of 10.3, 11.3, 12.3 min respectively. Hence ethanol is a good substance for the isolation of individual curcuminoids.

The identity of each peak was confirmed by determination of retention times and by spiking with standards. These purified compounds were further studied for biological activities and pharmaceutical properties.

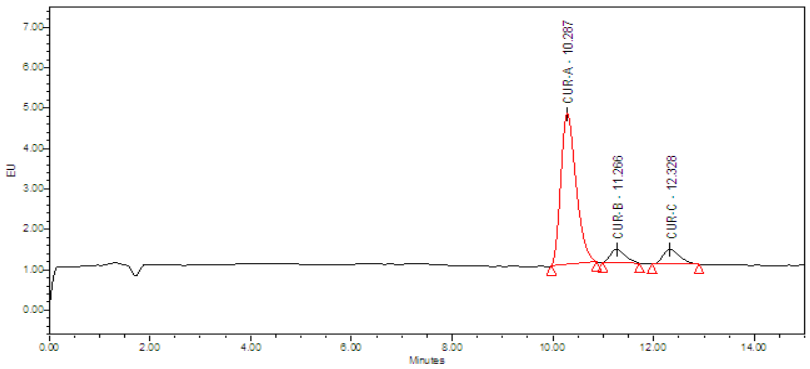

Fig.7 HPLC for curcuminoids

3.4 Anticancer activity

3.4.1 Methodology

Cell line

Human breast adenocarcinoma cell lines (MCF7) obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% Fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passed over weekly and the culture medium was changed twice a week.

3.4.2 Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24hr the cells were treated with serial concentrations of the test samples. They were initially dispersed in phosphate buffered saline by sonication and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing no samples served as control and triplicate was maintained for all concentrations.

3.4.3 Anticancer assay

Pure curcumin and silver nanoparticle encapsulated curcumin were chosen for anticancer assay. In brief, the cells were seeded into 4 wells of a 96-well micro titer plate at 2 × 104 cells per well with 100 μl growth medium and then incubated for 24 h at 37°C under 5% CO_2. Later, the medium was removed while fresh growth medium containing silver nanoparticle encapsulated curcumin (or pure curcumin) at 100, 50, 25, 2.5, 0.25μg/ml were added. After 3 days of incubation at 37°C under 5% CO_2, the medium was removed while 0.1 mg/ml MTT [3-(4, 5-dimethyl thiazole-2yl) - 2, 5-diphenyl tetrazolium bromide] reagent was then added. After incubation for 5 h at 37°C, the MTT [3-(4,5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazolium bromide] reagent was removed before adding 100 μl DMSO to each well and gently shaken. The absorbance was then determined by microplate reader at 570 nm. Control wells received only the media without the test samples. The conventional anticancer drug, cisplatin, was used as a positive control in this study. The inhibition of cell growth by silver nanoparticle encapsulated curcumin or pure curcumin were calculated as a percent anticancer activity using the following formula: percent anticancer activity (*Ac* – *As*/*Ac*) × 100% where *Ac* and As referred to the absorbance of control and the sample, respectively.

Curcumin

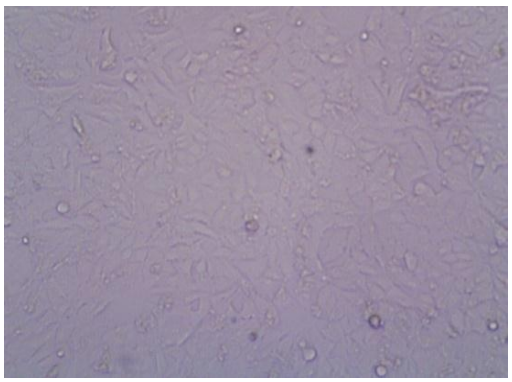

Fig.8a: 0.25 µg

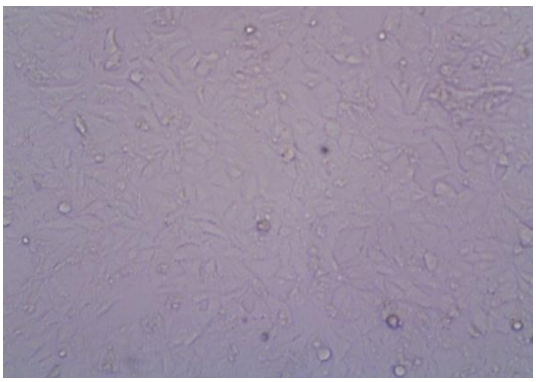

Fig.8b: 2.5 µg

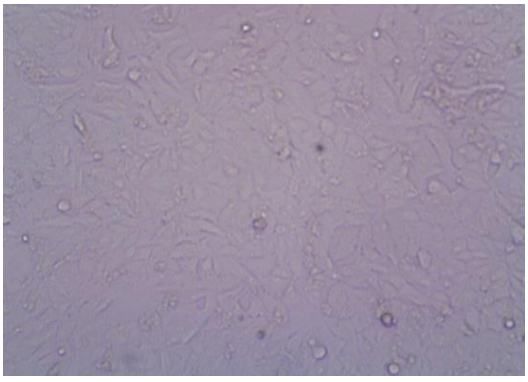

Fig.8c: 25µg

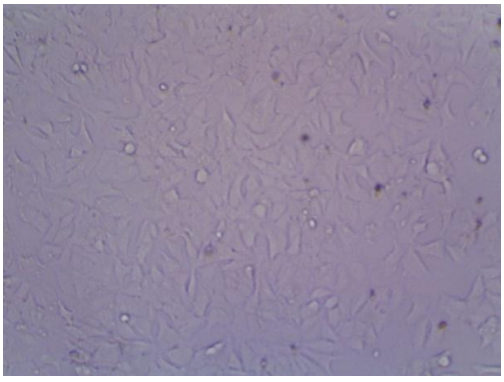

Fig.8d: 50µg

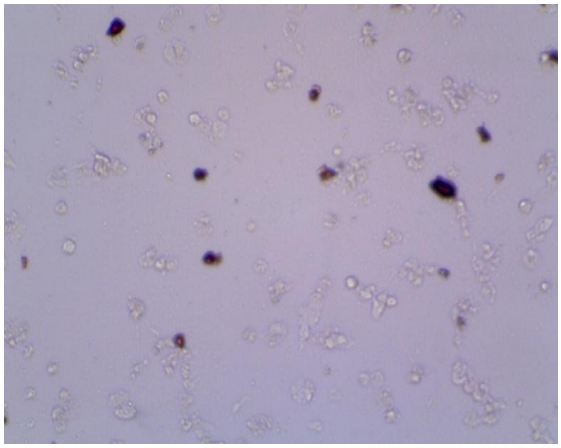

Fig.8e: 100µg

Silver nanoparticle encapsulated curcumin

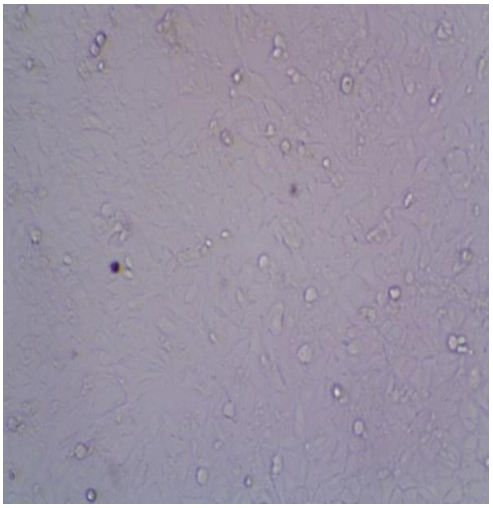

Fig.9a: 0.25 µg

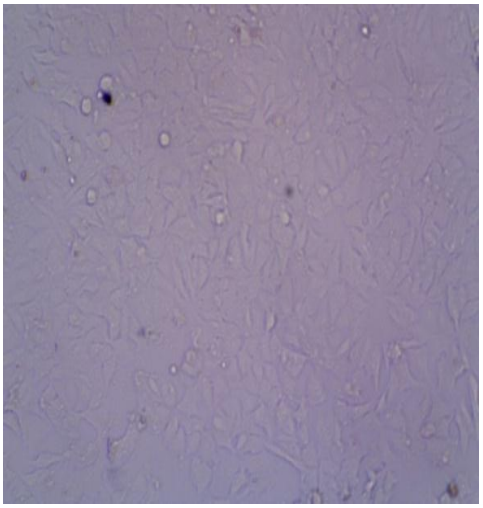

Fig.9b: 2.5 µg

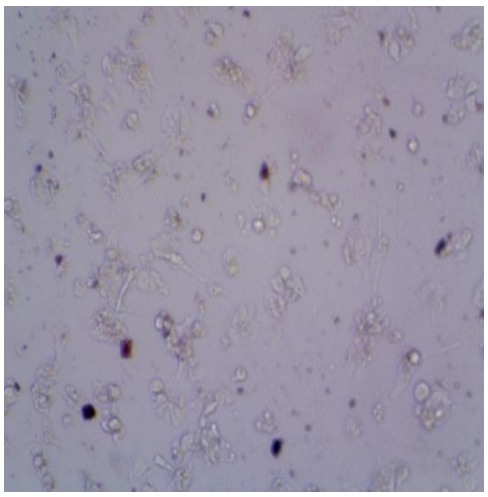

Fig.9c: 25 µg

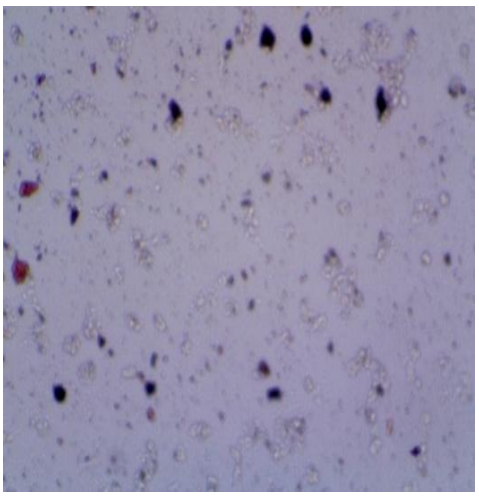

Fig.9d: 50 µg

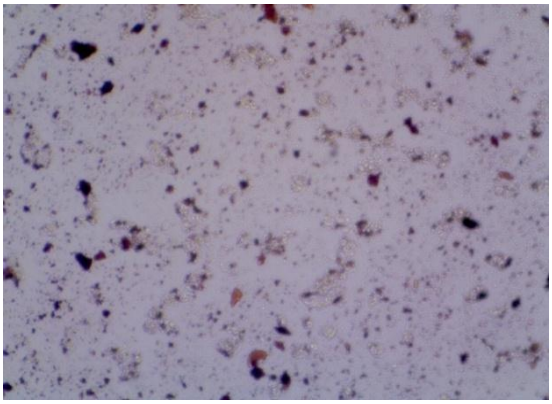

Fig.9e: 100 µg

The anticancer activity of the curcumin and silver nanoparticle encapsulated curcumin were analyzed by anticancer assay method. Figure (8a-8e) shows the percentage inhibition of curcumin in 0.25, 2.5, 25, 50 and 100µg/ml concentrations are increasing with increasing concentration. The concentration of curcumin at 50 and 100µg/ml show good inhibition value. Figure (9a-9e) shows the percentage inhibition of silver nanoparticle encapsulated curcumin. The silver nanoparticle encapsulated curcumin shows good inhibition value from 25µg/ml and have 100% inhibition at100µg/ml. It shows that silver nano particle encapsulated curcumin has high percentage inhibition than curcumin. Table 1 and 2 show the percentage of inhibition for curcumin and silver nano particle encapsulated curcumin at various concentrations.

Table 1: Percentage inhibition of curcumin

Concentration (µg/ml)	% cell inhibition	IC 50
0.25	0.770416	
2.5	2.619414	
25	8.782743	76.55 µg/ml
50	12.01849	
100	77.0416	

Table 2: Percentage inhibition of silver nanoparticle encapsulated curcumin

Concentration (µg/ml)	%cell inhibition	IC 50
0.25	1.694915	
2.5	9.707242	
25	79.12173	10.27 µg/ml
50	97.53467	
100	100	

Table 1 and 2 shows percentage inhibition of silver nano particle encapsulated curcumin (10.27µg/ml) has good toxicity compared with curcumin (76.55µg/ml).

3.4.4 MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed crystals were dissolved in 100µl of DMSO and then absorbance was measured at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software.

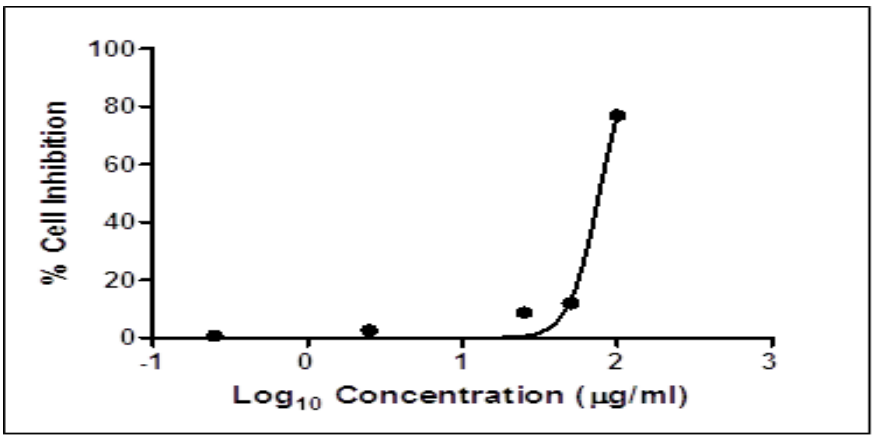

Fig.11 MTT assay of Curcumin (R^2= 0.9819)

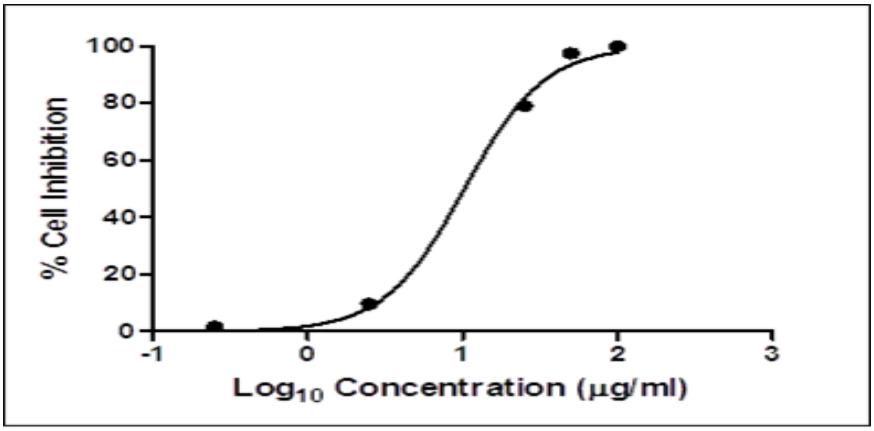

Fig. 12 MTT assay of Silver nano particle encapsulated curcumin (R^2= 0.9966)

Two releases of curcumin and silver were fitted to these kinetic models to determine the release kinetics and mechanisms from nanoparticles. The values of these kinetic rates, K and R^2, are presented in fig 11 and 12. In general, the release behavior for all nanoparticles did not obey zero order and first order kinetics based on the low R^2 values obtained.

Table 3 Absorbance of curcumin

Concentration(µg/ml)	Absorbance			
	1	2	3	Average
0.25	0.42	0.442	0.426	0.429333
2.5	0.424	0.422	0.418	0.421333
25	0.391	0.393	0.4	0.394667
50	0.382	0.377	0.383	0.380667
100	0.116	0.109	0.073	0.099333
Control	**0.421**	**0.438**	**0.439**	**0.432667**

Table 4 Absorbance of silver nanoparticle encapsulated curcumin

Concentration(µg/ml)	Absorbance			
	1	2	3	Average
0.25	0.437	0.424	0.415	**0.425333**
2.5	0.397	0.385	0.39	**0.390667**
25	0.081	0.074	0.116	**0.090333**
50	0.009	0.012	0.011	**0.010667**
100	0	0	0	**0**
Control	**0.421**	**0.438**	**0.439**	**0.432667**

In this study curcumin and Silver nanoparticle encapsulated curcumin were evaluated for preliminary estimation of the *in vitro* tumor inhibition activities against cell line of human breast carcinoma. The results revealed that curcumin and Silver nanoparticle encapsulated curcumin extract show some correlation between antitumor activity and the structures.

The concentrations that induce 50% inhibition of cell growth (IC50) in µg/ml are reported in Table 1 and 2. Compounds were classified by their activity as highly active (IC50 < 1 µg/ml), moderately active (1 µg/ml < IC50 < 10 µg/ml), or inactive (10 µg/ ml > IC50). The great majority of the curcumin and ethanol extract samples were strongly cytotoxic against all cell lines of human breast adenocarcinoma cell lines (MCF7) with IC50 below 1µg/ml, when compared to cisplatin, a fact that supports their anticancer activity.

4. CONCLUSION

Curcumin was extracted from curcuma longa by Soxhlet method. Silver nano particles were obtained by chemical reduction method. These two solutions were stirred to form silver nano particle encapsulated curcumin. The particle size of the solutions was found by using particle size analyzer. From the result it is concluded that the particle size is high for silver encapsulated curcumin. TLC analysis shows the presence of curcuminoids. From HPLC the purity and quantity of the curcumin content was found. Anticancer assay shows that silver nanoparticle encapsulated curcumin is effective in inhibiting cancer growth than curcumin. Pure curcumin and silver nanoparticle encapsulated curcumin have great potential in the prevention and cure of cancer. From the results obtained from this research work, it is now known that the Ag encapsulated curcumin has high potential to destroy cancer cells. The research in nanoparticle encapsulated curcumin should be strengthened to improve bioavailability and therapeutic efficacy in the treatment of various disorders.

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