

In-Vitro Cytotoxicity Assay of Curry Leaves Silver Nanoparticles Against Thp-1 Cell Line

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ABSTRACT

Medicinal plants are major crop in India and backbone of the Indian agriculture industry. It is very easily available in local market. We can produce very effective drug against various disorders like HIV, cancer, Skin disease, etc. To overcome these disorders, drugs can be prepared using combination of crude extract and nanotechnology. Nanoparticles can be synthesis by chemical, Physical, Biological method. The Biological method is most promising of all. In this study curry leaves was the biological material used for synthesis of NPs. Curry leaves and AgNO₃ were the important component for the synthesis. The synthesized silver nanoparticles were characterized by UV-Vis spectrometry which showed the presence of nanoparticles. SEM which showed that nanoparticles were spherical in shape, X-RD which showed that size of nanoparticles was range of 1-239.6nm, FTIR which showed the alkyl halides, Alkenes functional groups on nanoparticles. It has been seen that curry leaves plays an important role in the reduction and stabilization of silver to silver nanoparticles. Further, the anticancerous activity of these silver nanoparticles was studied on THP-1 cancerous cell lines using MTT assay. In cancer, some of the body cells begin to divide without stopping and spread into surrounding tissue. MTT dye stains mitochondrial succinate dehydrogenase enzyme in living cells. Using this process about 90% anticancerous activity was seen. This shows the wide application of curry leave silver nanoparticles in field of oncology.

Keywords : Curry leaves, AgNO₃, X-RD, SEM, FTIR, UV-Vis spectroscopy, THP-1 cell line, MTT Assay

I. INTRODUCTION

Curry leaves are a popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. "Let food be your medicine and let medicine be your food." Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies The important advantages claimed for

therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Curry leaf (*Murraya koenigii*) is an important leafy vegetable. Its leaves are widely used in Indian cookery for flavouring foodstuffs. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavour and other qualities even after drying. Curry leaf is also used in many of the Indian ayurvedic and unani prescriptions [11]. Curry leaves belong to kingdom plantae in division Magnoliopsida, Order Spindles and family Rutaceae. Scientific name

is *Murraya koenigii* [11]. *Murraya koenigii*, commonly known as curry leaf or karipatta in Indian dialects, belonging to Family Rutaceae which represents more than 150 genera and 1600 species. Carbazole carboxylic showed the presence of mukeic acid (1- methoxycarbazole- 3- carboxylic acid) and mukoeic acid. Koenoline was also isolated from root bark which exhibited cytotoxic activity. 9- formyl-3 methyl carbazole displayed weak cytotoxic activity against both mouse melanoma B 16 and Adriamycin resistant P 388 mouse leukemia cell lines [5]. The effects of extracts of *Murraya koenigii* in in-vitro (short term incubation method and in-vivo (Dalton's ascetic lymphoma (DAL) anticancer models have been evaluated in male Swiss albino mice. DAL cells were injected intraperitoneally (10⁶ cells) to the mice. The anticarcinogenic potential of curry leaf using benzo (a) pyrene-induced for stomach and 7, 12 dimethyl benzo (a) anthracene (DMBA) induced skin papillomas was studied [5]. *Murraya koenigii* has been found to induce apoptosis in human myeloid cancer cell (HL-60). Result shows that mahanine down-regulates cell survival factors by activation of caspase-3 through mitochondrion-dependent pathway, and disrupts cell cycle progression. Another study reported that mahanine, purified from the leaves of *Murraya koenigii*, has a dose- and time-dependent antiproliferative activity in acute lymphoid (MOLT-3) and chronic myeloid (K562) leukemic cell lines and in the primary cells of leukemic and myeloid patients, with minimal effect on normal immune cells including CD34 (+) cells [5]. Cancer is an abnormal type of tissue growth in which cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cells [12]. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are needed. These extra cells can divide without stopping and may form growths called tumours. Cancer cells are also often able to evade the immune system, a

network of organ, tissue, and specialized cells that protects the body from infection and other conditions [12].

In recent times, medicinal plants occupy an important position for being the paramount sources of drug discovery, irrespective of its categorized group herb, shrub or tree. Cancer is a dreadful disease characterized by the irregular proliferation of cells. As a cell progresses from normal to cancerous the biological imperative to survive and perpetuate drives fundamental changes in cell behavior [7].

THP-1 is a human monocytic cell line derived from an acute monocytic leukemia. It is used to test leukemia patient cell lines in immunocytochemical analysis of protein-protein interaction and immunohistochemistry [10].

Nanobiotechnology, an emerging field of nanoscience, utilizes nanobased-systems for various biomedical applications. This rapidly developing field of nanoscience has raised the possibility of using therapeutic nanoparticles in the diagnosis and treatment of human cancers [6]. The space required for nonmaterial's is so much less due to their small size. Human life is getting more suitability due to utilization of nonmaterial's in their daily life. Human got a remedy on Cancer in the roll of nanotechnology [1]. As there is very little or no research has been done on the synthesis of silver nanoparticles from *Murraya koenigii* (Curry Leaves).

II. METHODS AND MATERIAL

Materials

Fresh curry leaves, THP-1 Animal cell line. Silver nitrate 10 mM Silver nitrate (Sigma-Aldrich), Dulbecco's Modified Eagle Medium (DMEM) (Gibco), Trypsin (0.25%+EDTA 1Mm in PBSA), MT (3-

4,5dimethylthiazolyl- 3,5 diphenyltertraolium Bromide (Srichem), Fetal Bovine Serum (FBS 10%) (Gibco), Standard drug (Cyclozan).

Instruments

Spectrophotometer (Shimadzu), Auto clave (Bio technics India),Refrigerator (L.G), Hot air oven (Bio technics India), Weighing balance (Citizen), Laminar air flow (Micro Filt), CO2 Incubator (Thermo Scientific Meta lab), ELISA Reader (Bio-Rad), etc.

Glassware's: Beaker (100ml, 250ml), Conical flask (250 ml), Petri plates, Glass rod, Test tubes, Pipettes (1ml, 5ml), Measuring cylinder (100 ml), , Tissue culture Bottles, ELISA plate (Tarsons), Plastic wares and miscellaneous: Filter paper, Cork borer, Micro tip, Cell culture bottles (roller bottles), , Cotton, etc.

Methods

Preparation of Curry leaves extract. The curry leaves were removed from the stalk and 50 gm leaves were weighed. These leaves were washed twice with distilled water and kept in hot air oven for drying at 60°C for 30 minutes. The leaves were then crushed into fine powder. 5gm powdered leaves were then boiled in 100 ml distilled water and filtered to remove the debris. The filtered extract was preserved in refrigerator for future use. [6].

Preparation of silver nitrate solution A 10mM stock solution of AgNO₃ was prepared. Preparation of curry leaves silver nanoparticles. Curry leaf extract and silver nitrate solution were mixed in 1:1 ratio [13]. Drying of curry leave silver nanoparticles. The curry leaves silver nanoparticles solution was dried in hot air oven at for 4 hrs. to obtain dry powder of nanoparticles. This powder was collected in clean vials and stored [14].

Characterization methods of silver nanoparticles: UV Spectroscopy The reduction of pure Ag⁺ ions was monitored using UV-Vis spectrum of a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu) (Plate no 10). Spectrum of sample was taken with the frequency range of 400 nm to 700 nm at room temperature [2], Scanning electron microscope The topography of the nanoparticles was studied using Scanning Electron Microscope (SEM), Nova Nano SEM 450 [3]. Fourier transform infrared spectroscopy FT-IR (Fourier transform infrared spectroscopy) was performed in the range of 400 to 4500 cm⁻¹ using Jasco FT/IR-16100 to ascertain the presence of plant peptides that may have coated the particles during synthesis procedure. X ray diffraction this method was performed using 1gm of curry leave silver nanoparticles. Phase identification of crystalline material and unit cell dimension was obtained using the XRD machine [4].

Anticancer assay: This colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzyme in living cells to reduce the yellow water soluble substrate 3-(4,5- Dimethyl thiazol- 2- yl)- 2, 5diphenyl tetrazolium bromide(MTT) into an insoluble, colored formazan product which measured spectrophotometrically. Since reduction of MTT can only in metabolically active cells, the level of activity is a measure of the viability of the cells. 96 well plate was used for study of anticancerous activity. Blank was set using growth medium (DMEM) in column no. 1. Control was set using cells containing growth medium in column 11and 12. All the remaining column were filled with cells containing growth medium. The plate was incubated overnight at 37°C in a humidified incubator, (5% CO₂). 100µl of curry leaves AgNPs were added in column 2,3,4. 100µl of curry leaves extract were added in column 5, 6, 7. 100µl of

standard drug were added in column 8, 9, 10. The plate was incubated overnight at 37°C in a humidified incubator, (5% CO₂). 20 µl MTT reagent was added to all the 96 wells. Incubated at 37°C for 3-4 hours. 100 µl DMSO and 25 µl glycine buffer was added to wells. Supernatant were taken out of the wells into another microtiter plates. The absorbance was taken at 595 nm on plate reader. The result were tabulated and % viability (survival), % cell inhibition. The experiment was carried out on triplicates in two plates. Note: 100 µl DMEM medium was replaced after every 24 hrs.

Formula: -

$$\% \text{ of survival} = \frac{\text{O.D. of test sample}}{\text{O.D. of control}} \times 100$$

III. RESULTS AND DISCUSSION

Synthesis of silver nanoparticles was carried out using cold extract of curry leaves. The reduction of silver nitrate to silver nanoparticles resulted in colour change from dark green to pale brown. Characterizations of silver nanoparticles UV-Spectroscopy analysis of curry leaves silver nanoparticles. The colour change was attributed to coherent oscillation of electrons at the surface of nanoparticles resulting in the surface plasmon resonance (SPR). The silver nanoparticles were characterized by studying absorbance maxima at 700 nm. The broadening of peak was attributed to the polydispersed nature of the nanoparticles. Silver nanoparticles grow gradually due to bio reduction and that influences the SPR in extract.

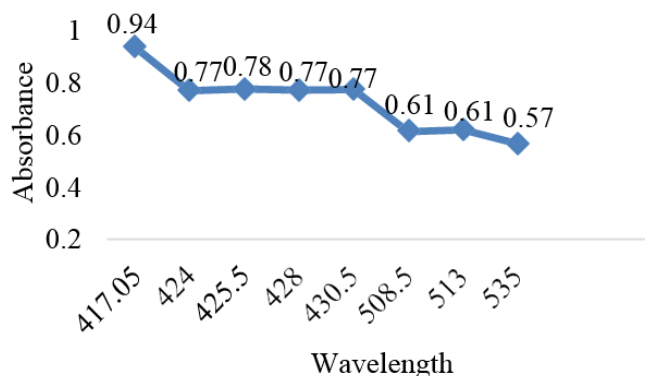


Figure 1. UV- Spectra analysis of curry leaves silver nanoparticles

Scanning Electron Microscopy analysis of curry leaves silver nanoparticles: SEM confers the shape particles were round and oval crystal in shape

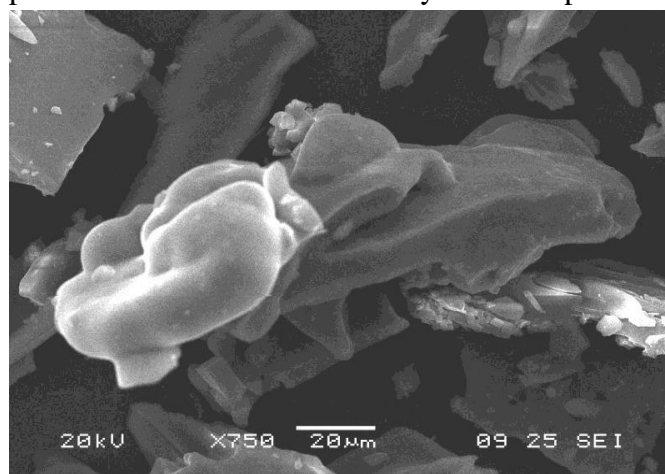


Figure 2. SEM analysis of curry leaves silver nanoparticles

Fourier Transform Infrared Microscopy analysis of curry leaves silver nanoparticles. FT-IR analyzed reveals a set of unique peak that proves the presence of amino group indicating involvement of plant peptides in biosynthesized of nanoparticles. The FTIR of bio- stabilized nanoparticles. FTIR analysis used to confirm the presence of the plant peptides visible due to the bending produced by amide bonds. The peaks obtained at 3350.071cm⁻¹, 1643.05cm⁻¹, 621.931cm⁻¹, indicates presence of amine, -C-N- stretching suggesting that the presence of plant protein and its role in synthesis and stabilization of silver nanoparticles.

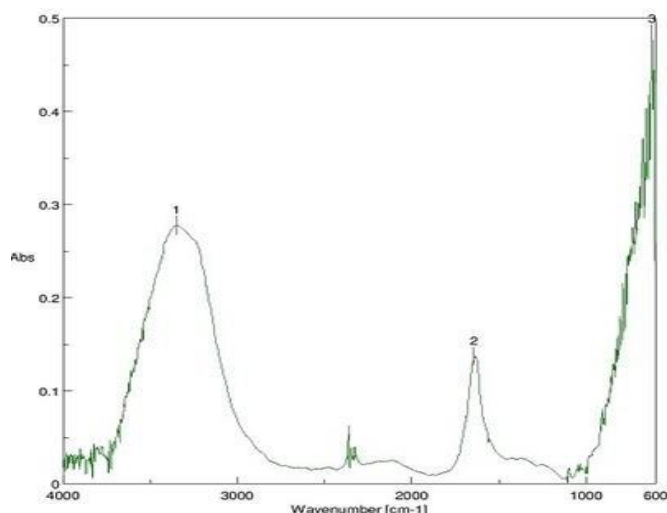


Figure 3. FTIR spectra analysis of curry leaves silver nanoparticles

Table 1 : FTIR Analysis of curry leaves silver nanoparticles

Sr.No	Positions	Intensity	Functional group	Bond
1	3350.71	0.277	Alkyl halides	C-Br
2	1643.05	0.137	Alkyl halides	C-Br
3	621.93	0.483	Alkenes	-C=C-

X-Ray diffraction analysis of curry leaves silver nanoparticles. The small amount of dry silver nanoparticles was used for the XRD analysis. The XRD curve confirmed that the nanoparticles nothing but the silver. The silver nanoparticles showed the peaks of silver at 2θ angle. XRD pattern spectra clearly shows the pure crystalline silver structures. The data was matched with database of joint committee on Powder Diffraction Standard (JCPDS NO. 04- 0783). A comparison of XRD spectrum with the standard and it was confirmed that the silver nanoparticles were in the form of nanocrystal. The average size of nanoparticle was 1- 239.29nm.

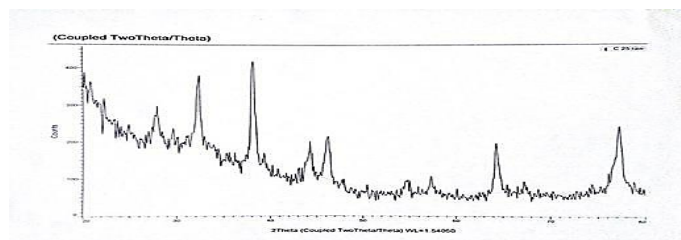


Figure 4. XRD spectra analysis of curry leaves silver nanoparticles

MTT Assays

THP-116 (Tamm Horsfall Protein).

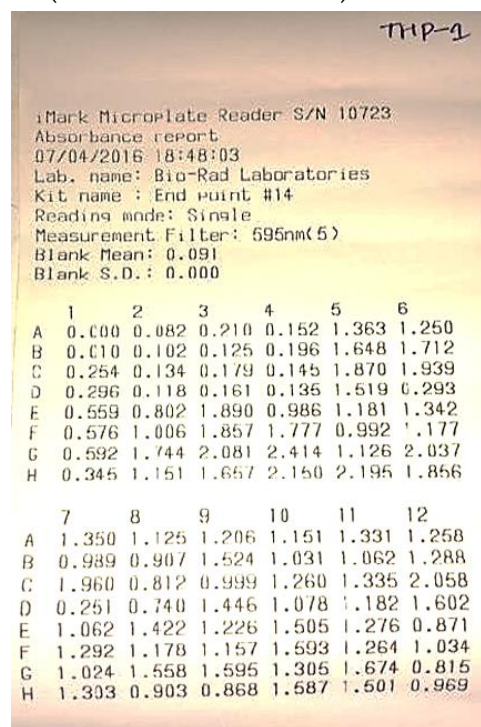


Figure 5. Readings of ELISA plate (Microtiter Plate) containing after incubation at 37°C for 3-4 hours

Table 2 : Effect of Curry leaves silver nanoparticles on THP-1 cell line

Sr. No	Concentrations $\mu\text{g/ml}$	Absorbance (595nm).			Avg	% inhibition
1	100	0.08	0.21	0.15	0.10	90.14
2	300	0.10	0.12	0.19	0.13	90.6
3	500	0.13	0.17	0.14	0.14	91.3

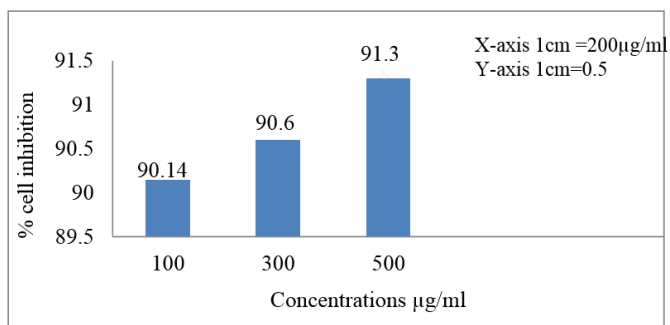


Figure 6. Effect of Curry leaves silver nanoparticles on THP-1 cell line.

It can be clearly seen that Curry leaves silver nanoparticles have considerable amount of anticancerous activity. It increases with increase in concentration.

Table 3 : Effect of Curry leaves Extract on THP-1 Cell line

Sr. No	Concentrations µg/ml	Absorbance(595nm).			Avg	% Cell inhibition
1	100	1.36	1.25	1.35	1.32	3.64
2	300	1.64	1.71	0.98	1.44	9.2
3	500	1.87	1.93	1.96	1.92	13.8

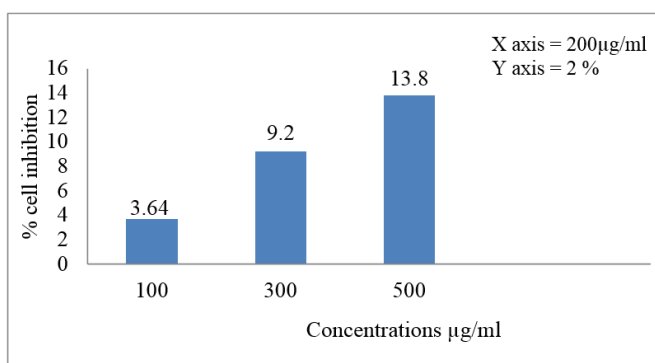


Figure 7. Effect of Curry leaves Extract on THP-1 Cell line

It can be clearly seen that Curry leaves extract show anticancerous activity but less than that of curry leaves silver nanoparticles. It can also be seen that cell inhibition increases with increase in concentration.

Table 4: Effect of standard Drug on THP-1 cell line

Sr. No.	concentrati on µg/ml	Absorbance (595nm).			Avg	% cell inhibition
1	100	1.12	1.20	1.15	1.15	22.63
2	300	0.90	1.52	1.03	1.23	23.07
3	500	0.81	1.99	1.26	1.35	27.47

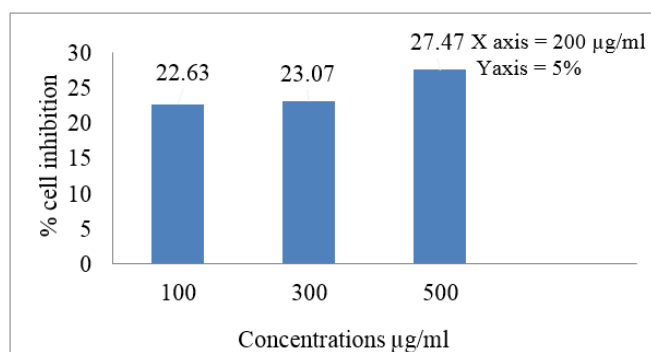


Figure 8. Effect of standard Drug on THP-1 cell line

It can be clearly seen that standard drug show anticancerous activity but less than that of curry leaves silver cell inhibition increases with increase in concentration.

Concentration µg/ml	Curry leave silver nanoparticles (% cell inhibition).	Curry leave Extract (% cell inhibition).	Drug (% cell inhibition)
100	90.14	3.64	22.63
300	90.6	3.92	23.07
500	91.3	13.8	27.47

Table 5: Comparative results of anticancerous activities

Concentrati on µg/ml	Curry leave silver nanoparticles (% cell inhibition).	Curry leave Extract (% cell inhibition).	Drug (% cell inhibition).
100	90.14	3.64	22.63
300	90.6	3.92	23.07
500	91.3	13.8	27.47

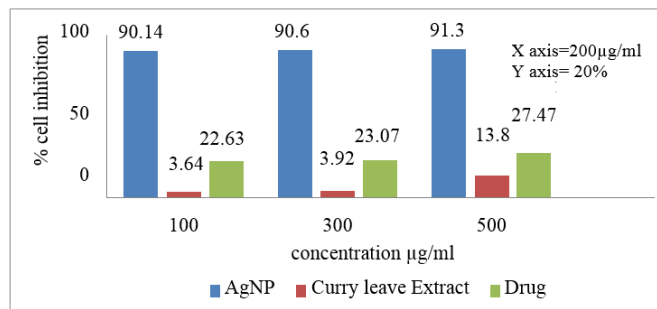


Figure 9. Comparative results of anticancerous activities.

Anticancerous activity of Curry leaves silver nanoparticles, curry leaves extract and standard drug was tested against THP-1 cancerous cell lines. It was seen that curry leaves silver nanoparticles showed maximum amount of anticancerous activity i.e. around 90%.

IV. CONCLUSION

In this present study the silver nanoparticles were synthesized by biological method using curry leaf extract (*Murraya koenigii*) which acts as a reducing agent to reduce silver metal to nanosize. The synthesized silver nanoparticles were analyzed by UV-Vis Spectroscopy, Scanning Electron Microscopy, Fourier Transform Infrared Microscopy and X-Ray Diffraction in order to characterize them. UV-Vis spectrophotometer confirmed the synthesis of silver nanoparticles from the leaf extract of *Murraya koenigii*. SEM used to confirm the nature of the silver nanoparticles. In that it was found that the particles are crystal in nature and size in the range of 1- 100nm. The dry silver nanoparticles used for the XRD analysis. The curve confirmed that the nanoparticles are nothing but of silver and it showed the peaks of silver at 2θ angle. FTIR analysis reveals a set of unique peak that proves the presence of amino peptides in biosynthesis of nanoparticles. However, to use SNPs in various fields, it is essential to prepare the SNPs with a cost effective method. Toxicity of silver

nanoparticles is concentration-size- shape dependent. In this studied anticancerous activity of curry leaves silver nanoparticles, curry leaves extract and standard drug was study. It was seen about 90% cancer cell were kill due to curry leaves silver nanoparticles from this it can be calculated that use of silver nanoparticles against cancerous cell is promising technique giving good results with less side effect. This opens the door to prepare a suitable pharmaceutical formulation using these nanoparticles. Taking into account the mobility of SNPs into cells and their fate in a bioprocess or even in the environment, the risk aspects for the application in larger scales and in the environment as well as studies on different biological activities in different fields should be strengthened in future studies.

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