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Green synthesis of Ag NPs synthesized by using Cassia auriculata leaves (C.auriculata) and its applications of cancer activity

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Abstract: The use of herbal preparations in the treatment of diseases is very common in the rural communities of world. Cassia auriculata is frequently used for the treatment of infections, pathologies of the skin disease, fever, liver injury and others diseases involving inflammation and pain. By the help of Cassia auriculata leaves extract with silver nitrate source materials, silver NPs were prepared by using Reflux method. The Cassia auriculata leaves extract acts as a reducing agent and the reduced silver NPs are having the size of nearly 59 nm. XRD analysis explains the structural studies of the prepared NPs, FTIR studies interferes the vibrational modes of NPs. Quantitative phytochemical analysis of Cassia auriculata and anticancer activity results were tabulated in the Tables.

Keywords; Cassia auriculata leaves (C.auriculata**)**, Ag NPS, reflux method and phytochemical analysis

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I. INTRODUCTION

India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatments i.e. Ayurvedic and Unani systems since ancient times [1]. Ayurveda is considered not just as an ethnomedicine but also as a complete medical system that takes in to consideration physical, psychological, philosophical, ethical and spiritual well being of mankind. It lays great importance on living in harmony with the Universe and harmony of nature and science. This universal and holistic approach makes it a unique and distinct medical system. This system emphasizes the importance of maintenance of proper life style for maintaining positive health [2].

Plants are able to synthesize a broad range of different chemical compounds called secondary metabolites and these are easily degradable. Many of them provide new sources of natural compounds. Majority of world population in developing countries relay on herbal medicines. Currently 80% of the world population depends on plants derived medicine for the first line to primary health care because it has no side effects. In the present study, the effect of ethanolic extracts of *C.auriculata* leaves are selected in this study. On account of this, the present study has been aimed to investigate the pharmacological activities of *C. auriculata*.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [3].

In present situation, silver nanoparticles (AgNPs) are in great use in the medicinal, pharmaceutical, agricultural industry and in water purification. These nanoparticles can be synthesized either chemically or biologically. But the chemical process for synthesis of silver nanoparticles is more elaborate and leaves behind toxic effect that adversely affects the ecosystem. On the other hand, biological synthesis of silver nanoparticles is less time consuming, less costly, and more eco-friendly; therefore, in recent time, scientists are looking forward to the possible biological methods for the synthesis of silver nanoparticles [4]. AgNPs have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver

nanoparticles for their high electrical conductivity, stability and low sintering temperatures; in addition, AgNPs are applied in molecular diagnostics and photonic devices. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, keyboards, wound dressings and biomedical devices now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria. To study the anticancer activity of alcoholic extracts *C.auriculata* leaves extract and silver nanoparticle from various mammalian cell lines.

The preliminary phytochemical evaluation of leaves was carried on extract prepared by Soxhlet apparatus. The previously dried powdered leaves (50 gm) were extracted in a Soxhlet apparatus using ethanol and water. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc [5].

Green synthesis of silver nanoparticles by the help of green plants is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a large scale. *Cassia auriculata* showed great capability to synthesis AgNPs at optimum temperature conditions. The XRD patterns confirmed the purity, phase composition and nature of the synthesised nanoparticles FTIR studies confirmed the biofabrication of the AgNPs by the action of different phytochemicals with its different functional groups present in the extract solution [6].

II. EXPERIMENTAL

The *Cassia auriculata* (Figure. 1) leaf was collected, washed, cut into small pieces and dried at room temperature (28±1ºC) for two weeks and made into fine powder for further analysis. The shade-dried leaf powder of *Cassia auriculata* were subjected to extraction with 70% ethanol under reflux for 8 h and concentrated to a semisolid mass under reduced pressure (Rotavapor apparatus, Buchi Labortechnik AG, Switzerland). A dark semisolid (greenish-black) material was obtained and the yield was about 24% (w/w). It was stored at 4º C until used. When needed, the residual extract was suspended in distilled water and used for the study. The air dried, powdered plant material was extracted with petroleum ether, chloroform, alcohol, acetone-water and water separately in a conical flask at a room temperature. The total ash obtained from 2 gm of leaves powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. 2 gm of *Cassia auriculata* leaf powder was taken in a tarred glass bottle and initial weight was taken. The sample was heated at 105°C in an oven and weighed.

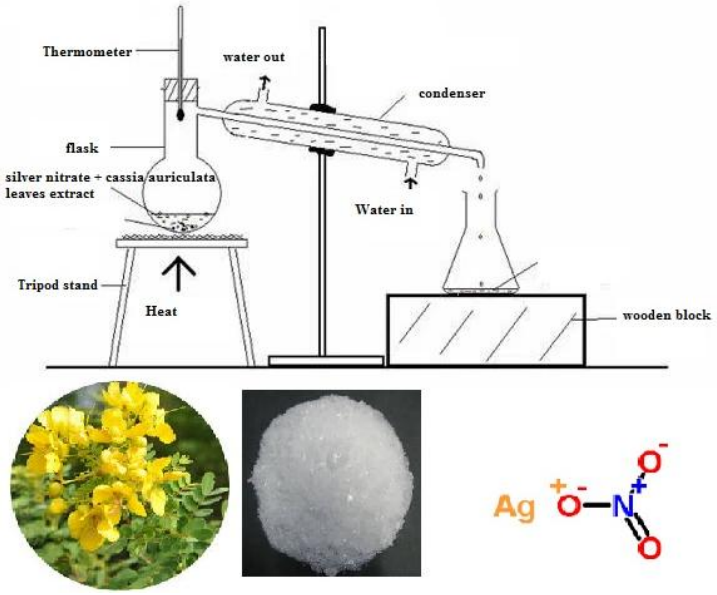

Figure 1 Synthesis of Ag NPs by using *Cassia auriculata leaves*

III. RESULTS AND DISCUSSION

3.1 XRD Analysis

The corresponding XRD trace patterns are shown in the Figure 2. In this case, the present peaks from the corresponding plane (111) clearly showed the cubic structure lattice pattern of Ag NPs. The calculated lattice constant a= 4.015 Å well matched with the results of JCPDS 65-2871. Form this observation, it is obvious that the formatted Ag has nanosize and it is important to note that the calculated lattice constant has well agreement with JCPDS results [7]. The parameters obtained in the results are shown in Table 1.

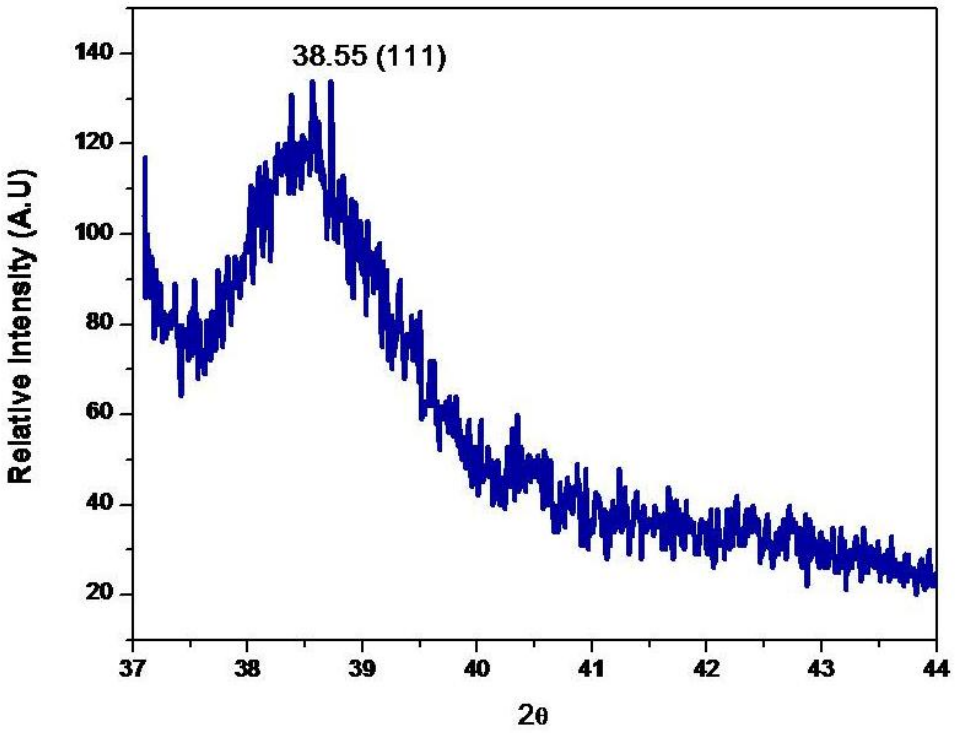

Figure 2 XRD analyses of Ag NPs at RT by using *Cassia auriculata leaves*

Table -1 Structural studies of Ag NPs at RT by using Cassia auriculata leaves

JCPDS 65-2871	2Θ	d-spacing	Crystal size (nm)	Lattice constant (Aº)
Standard from JCPDS	38.116	2.3591		4.086
Calculated	38.55	2.2960	59.54	4.015

3.2 FTIR Analysis

FTIR gives the information about functional groups present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the *C. auriculata* extracts. Figure 3 shows the FTIR spectrum of *C. auriculata* mediated synthesized silver nanoparticle, the silver nitrate salt and dried leaves petal extract, in $AgNO_3$ peaks were observed at $3697cm^{-1}$, $1761cm^{-1}$, $1390cm^{-1}$, $831cm^{-1}$ which are associated OH stretching, C=C stretching, CH stretching, NH stretching respectively. In this plant C. auriculata leaf extracts peak were observed which are associated OH stretching, CH stretching, C=N stretching, N-H stretching, CN stretching, C-Cl stretching. In the synthesized silver nanoparticle from C. auriculata peaks were observed which are associated with NH stretching, C=O stretching, N-O stretching, CH_2 and CH_3 deformation, C-O stretching and halogen group presence [8].

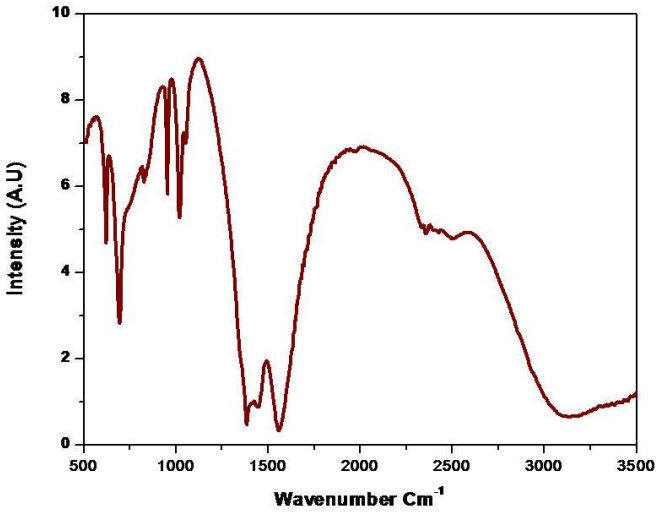

Figure 3. FTIR Analysis of Ag NPs synthesized by using ***Cassia auriculata leave***

3.3 Anticancer Activity

Quantitative phytochemical analysis of Cassia auriculata is shown in Table-2. Anticancer activity of *Cassiaauriculata* was studied in different mammalian cell line. Anticancer activity of ethanolic extract of *C. auriculata* as well as standard was determined through MTT cytotoxicity assay.In the preliminary study, the etahanolic extract showed the good yielding capacity of phytocompounds and antioxidant activity. In this regards, the present investigation the ethanolic extract of *C. auriculata* and silver nanoparticle were studied in HeLa cell lines and its result and also made with standard drug [9].

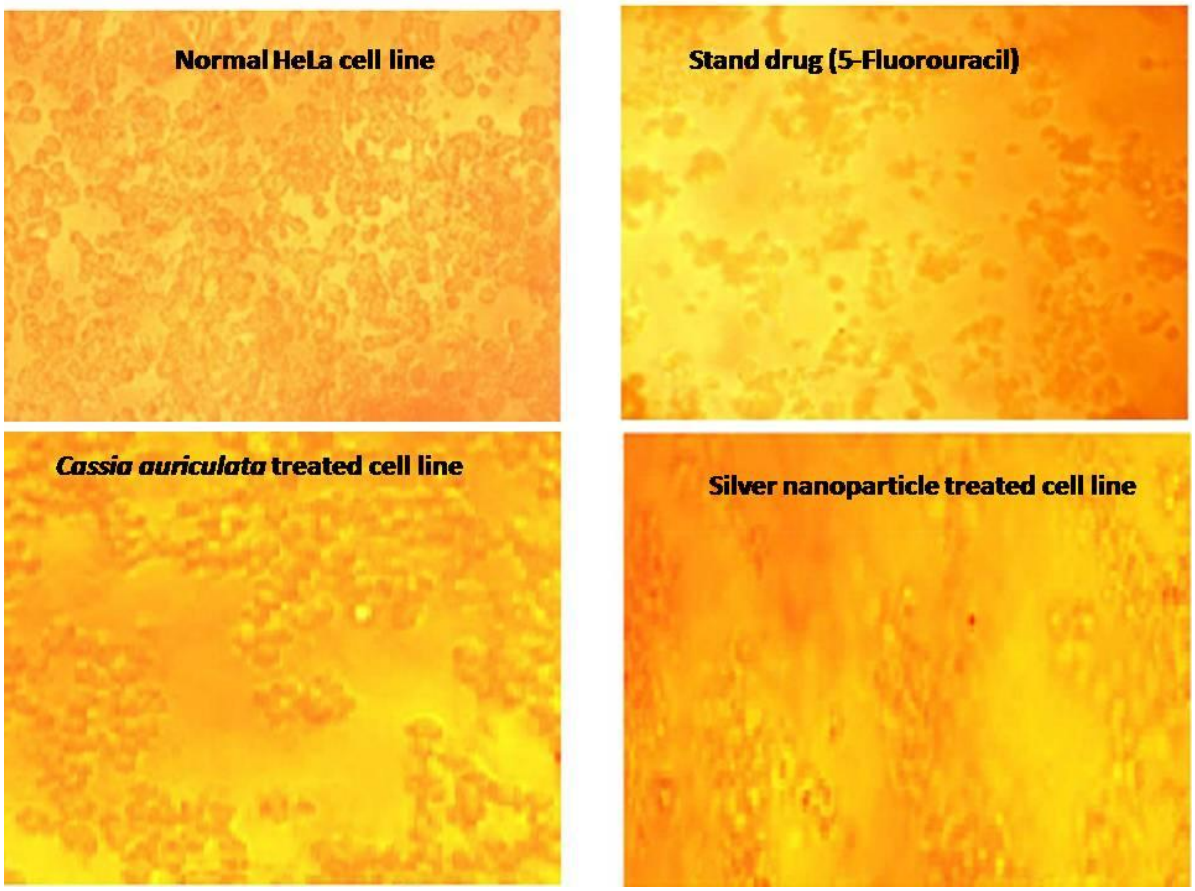

Figure 4 Plate showing the anticancer activity of HeLa cell line of silver nanoparticles Cassia auriculata leaf extracts compared with normal cell line

Table 2. Quantitative phytochemical analysis of *Cassia auriculata*

S. No	Parameters	Ethanol extract
1	Phenols (mg/g)	58.2 ± 0.22
2	Flavanoids (mg/g)	63.2 ± 0.26
3	Tannin (mg/g)	38.4 ± 0.12
5	Alkaloids (g/100g)	17.6 ± 1.35
8	Ascorbic acid (g/100g)	12.19 ± 0.24

Table 3 Anticancer activity of Ag NPs by using Cassia auriculata leaves

Concentrations (µg ml- ^{1})	**Cell viability (%)**	**Cell inhibition (%)**	**IC $_{50}$ (µg ml $^{-1}$)**
0.78	83.44	16.56	
1.56	75.21	24.79	
3.13	65.98	34.02	
6.25	62.23	37.77	**18.59**
12.5	53.40	46.60	
25	45.57	54.43	
50	34.44	65.56	
100	25.20	74.80	
Vehicle control (DMSO)	100	0	

The minimum cell viability (11.85%) and maximum cell inhibition (88.15%) were noted in 2000 µg concentration of *Cassia auriculata*. The IC_{50} value (98.41 µg/ml) was calculated for anticancer a ctivity of ethanolic extract of *C. auriculata*against HeLa cell line. The 5-Fluorouracil used as a standard for this study. In the standard, the minimum cell viability and maximum cell inhibition were observed in higher concentration of 1000 µg/ml. The percentage of cell inhibition was noted in the different concentrations of silver nanoparticle of *C. auriculata* ranges from 0.78 to 100 µg/ml [10]. The lowest cell inhibition (16.56%) was recorded in the lowest concentration and highest cell inhibition (74.80%) was noted in the higher concentration of silver nanoparticles. The IC_{50} value (18.59µg/ml) was calculated in the standard drug against HeLa cell line (Table 3).

The minimum cell viability (8.75%) and maximum cell inhibition (91.25%) were noted in 2000 µg/ml concentration of *Cassia auriculata* extract and followed by 1000 µg/ml concentration also recorded in the maximum cell inhibition (84.54%). The moderate levels of cell inhibition (56.67%, 62.45%, 71.18 and 79.79%) were observed in the different concentrations of ethanolic extract of *C. auriculata* in 62.5 µg/ml, 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. The IC_{50} value was noted in the concentration of 84.56 µg/m l against the MCF7 anticancer activity of ethanolic leaves extract of *C. auriculata*. In the standard, the minimum cell viability and maximum cell. Inhibition was observed in higher concentration (1000 µg/ml) [11].

IV. CONCLUSION

From the present analysis, it can be inferred that the selected plants *C. auriculata* have an anticancer activity against HeLa cell line. It is clear from the results it acts as excellent reducing agents to reduce silver nitrate to silver metal NPs, also *C. auriculata* leaves extract have significant anti-cancer activity in cell line. The extract is non-toxic even at relatively high concentrations. The *invitro* anticancer activity is *probably* due to the presence of phenolic compounds will helpful to the researcher to improve the activities of *C. auriculata leaves*.

REFERENCES

[1]. Surana S, Patra RK, Nedevschi S, Ramos M, Subramanian L, Ben-David Y, Brewer EA. Beyond Pilots: Keeping Rural Wireless Networks Alive. InNSDI, 2008 Apr 16 (Vol. 8, pp. 119-132).
[2]. Ravishankar B, Shukla VJ. Indian systems of medicine: a brief profile. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(3):319-37.
[3]. Ramamurthy V, Rajeswari DM, Gowri R, Vadivazhagi MK, Jayanthi G, Raveendran S. Study of the phytochemical analysis and antimicrobial activity of Dodonaea viscosa. International Journal of Pure and Applied Zoology. 2013;1(2).
[4]. Raja SK, Ganesh S. Avimanyu,"Evaluation of antibacterial activity of silver nanoparticles synthesized from Candida glabrata and Fusarium oxysporum. International Journal of Medicobiological Research. 2011;1(3):130-6.
[5]. Venkatpurwar V, Pokharkar V. Green synthesis of silver nanoparticles using marine polysaccharide: Study of in-vitro antibacterial activity. Materials Letters. 2011 Mar 31;65(6):999-1002.
[6]. Ahmad N, Sharma S, Alam MK, Singh VN, Shamsi SF, Mehta BR, Fatma A. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids and Surfaces B: Biointerfaces. 2010 Nov 1;81(1):81-6.
[7]. Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S, Saravanan M. Green synthesis of silver nanoparticles from leaf extract of Mimusops elengi, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. Colloids and Surfaces B: Biointerfaces. 2013 Aug 1;108:255-9.
[8]. Kaviyarasu K, Ayeshamariam A, Manikandan E, Kennedy J, Ladchumananandasivam R, Gomes UU, Jayachandran M, Maaza M. Solution processing of CuSe quantum dots: Photocatalytic activity under RhB for UV and visible-light solar irradiation. Materials Science and Engineering: B. 2016 Aug 1;210:1-9.
[9]. Ezhilarasi AA, Vijaya JJ, Kaviyarasu K, Maaza M, Ayeshamariam A, Kennedy LJ. Green synthesis of NiO nanoparticles using Moringa oleifera extract and their biomedical applications: Cytotoxicity effect of nanoparticles against HT-29 cancer cells. Journal of Photochemistry and Photobiology B: Biology. 2016 Nov 1;164:352-60.
[10]. Kannan RR, Arumugam R, Ramya D, Manivannan K, Anantharaman P. Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum. Applied Nanoscience. 2013 Jun 1;3(3):229-33.
[11]. Caprioli J, Noris M, Brioschi S, Pianetti G, Castelletti F, Bettinaglio P, Mele C, Bresin E, Cassis L, Gamba S, Porrati F. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood. 2006 Aug 15;108(4):1267-79.

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