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Research Article

Characterization, Antimicrobial and Anticancer Activity of Biosynthesized Silver Nanoparticles using *Garcinia gummi-gutta* (L.) Robs. Leaf Extracts

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ABSTRACT

Garcinia gummi-gutta is commercially an important plant as its leaves and fruits contain valuable chemical components and are used for various ailments and treatments. Even though, there are minimum reports that have been seen in their exploration of this plant in the field of nanotechnology. So the present study deals with the synthesis of silver nanoparticles from the aqueous and ethanol leaf extracts of *G. gummi-gutta* and screening its antimicrobial and anticancer properties. Green synthesized silver nanoparticles was characterized by UV-visible (UV-vis) spectroscopy, transmission electron microscopy (TEM), fourier transforms infrared spectroscopy (FTIR) and X-ray diffraction (XRD) studies. In UV-vis spectroscopy, the surface plasmon resonance (SPR) spectrum of silver nanoparticles produced prominent peaks at 365 and 363 nm in aqueous and ethanol leaf extracts of *G. gummi-gutta*, respectively. Scanning electron microscopy (SEM) and XRD studies showed that the biosynthesized silver nanoparticles from aqueous and ethanol extracts of *G. gummi-gutta* leaves are spherical and crystalline in nature with an average size of 25 and 23 nm. FTIR spectroscopy reveals that the functional groups are responsible for the synthesis and stabilization of silver nanoparticles. The biosynthesized silver nanoparticles from both plant extracts have remarkable antibacterial and anticancer properties. Through these studies, it was found that silver nanoparticles from the ethanol leaf extract of *G. gummi-gutta* is more potential than silver nanoparticles from the aqueous leaf extract of *G. gummi-gutta*. So the present study is a novel attempt to synthesize the silver nanoparticles from this plant and elucidate its antimicrobial and anticancer potential.

INTRODUCTION

Science and technology help each other in their advance in many different ways. One such breakthrough in the world of science is the use of nanotechnology. Nanotechnology is a quickly growing field with its application for producing new materials at the nanoscale level such as 1 to 100 nm. Because of its tremendous application in various fields of industry, technology as well as medicine, its demand has increased worldwide.^[1,2] Metal nanoparticles such as silver, gold and copper get more attention for their unique electronic, catalytic, optic magnetic and antibacterial properties.^[3] Among these, silver nanoparticles play a major role in solar energy absorption, biolabeling, optical receptors in electrical batteries, electrical conductors,

catalysts in chemical reactions and act as antibacterial materials, etc.^[4] Silver nanoparticles are even more powerful because of their larger surface area-to-mass ratio. It is very useful in the field of medicine as an alternative antibiotic for antibiotic-resistant microorganisms^[5] and also possesses antifungal, antiviral, anticancer, antidiabetic as well and antiplatelet activities.^[6] Methods like photochemical, electrochemical, thermal, radiation, microwave-assisted and chemical reductions are generally used for the synthesis of silver nanoparticles, which are often associated with toxic and hazardous chemicals that cause potential environmental and health issues.^[7,8] So the researchers have great interest in finding a novel method for the synthesis of silver nanoparticles. Recently several

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green synthesis techniques have been reported for the synthesis of silver nanoparticles, which is a safe and eco-friendly method to reduce the use of toxic chemicals and related environmental issues.^[9] Plants are found to be rich in bioactive compounds. The biomolecules present in plant extracts have the capacity to reduce metal ions into nanoparticles with great intrinsic value.^[10] Silver nanoparticles produced from plants or natural extracts such as *Azadirachta indica*,^[11] *Morinda citrifolia*,^[12] *Aloe vera*,^[13] *Pedaliium murex*,^[14] *Citrus limon*^[15] and *Cycas circinalis*^[16] have different shapes and sizes, which possess significant biological activities.

The plant *Garcinia gummi-gutta* (former name, *Garcinia cambogia*) is commonly known as Malabar tamarind or kudam puli (pot tamarind) and belongs to the family Clusiaceae. It is a medium-sized, deciduous tree with velvety leaves and round fruits. The rinds of the fruit are dried and stored for a long time for food. Recent studies have revealed that the fruits of this plant contain hydroxy citric acid, guttiferone, xanthochymol, flavonoids, alkaloids, phenols, tannins and saponins which have different pharmacological significance such as anti-inflammatory, antioxidant, anti-cancer and antimicrobial activities. Fruits of this plant are widely used in Ayurveda and traditional medicine for various diseases.^[17-19] Due to the presence of hydroxy citric acid, It also increases the levels of hemoglobin, blood cells and cellular immunological indicators and decreases the level of glucose, cholesterol and low-density lipoprotein (LDL) in catfish.^[20-22] Previous studies had also shown that leaf extracts of this plant had strong antimicrobial and antioxidant properties.^[23] The leaf of *G. gummi-gutta* also contains hydroxy citric acid, even though the leaves are not much explored as its fruits are done for its biological activities.^[24,25] Cancer and microbial infections are the leading causes of death worldwide. Chemical radiation and synthetic drugs are used for cancer and long-term use of antibiotics can cause severe health issues and side effects.^[26] The main aim of this study to find out an alternative to cancer and infectious diseases. The present study deals with the synthesis of silver nanoparticles from aqueous and ethanol leaf extracts of *G. gummi-gutta*. The antimicrobial effect of the green synthesized silver nanoparticles was studied against different strains of human pathogenic bacteria. The trypan blue exclusion test of the synthesized silver nanoparticles was evaluated using Dalton lymphoma ascites (DLA) tumor cells and the highest value showed the sample was subjected to the trypan blue exclusion test on rat spleen cells. The cytotoxic effect of these silver nanoparticles was checked by MTT assay on MCF-7 cell lines.

MATERIALS AND METHODS

Collection and Identification of the Plant Material

Plant: *Garcinia gummi-gutta*

Family: Clusiaceae

Genus: *Garcinia*

Species: *gummi-gutta*

Common name: Malabartamarind/Kudampuli (Malayalam)

The plant *Garcinia gummi-gutta* (L.) Robs. is a small or medium-sized dioecious tree with horizontal or drooping branches. Leaves are opposite, petiolate and elliptic to obovate in shape. Flowers are usually in clusters and red or yellow. Fruits are green berries, that become yellow or red while ripe. Seeds are large, 6 to 8 in number covered by a succulent aril. Plant materials collected from Panangad, a suburban village in Kochi, Ernakulam district were taxonomically identified^[27] and the voucher specimen No: 9753 was deposited at the herbarium of Botany department of St. Teresa's College (Autonomous), Ernakulum for further studies.

Preparation of Plant Extract and Silver Nitrate Solution

The leaves *G. gummi-gutta* were collected and washed thoroughly in distilled water to remove dust particles. The leaves were cut into small pieces and dried under shade for a few days. The shade-dried powdered leaves were used for the solvent extraction. In 50 g of powdered sample was subjected to soxhlet extraction using distilled water and ethanol as solvents. The extracts thus obtained were cooled, filtered through a Whatman no. 1 filter paper and concentrated by removing the complete content of the solvent. It was stored in amber-colored glass bottles at 4°C for further experiments. The silver nitrate (A. R. grade) was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Silver nitrate solution (0.1N) was prepared by dissolving 1.692 g of silver nitrate crystals in 100 mL distilled water. The solution was transferred into a dark bottle and stored for further use.^[28]

Biosynthesis of Silver Nanoparticles

The aqueous and ethanolic leaf extracts of the plant samples were added separately to the freshly prepared silver nitrate solutions for the synthesis of AgNPs. In 10 mL of the plant extract was slowly added to 90 mL of 0.1 N AgNO₃ solution in an Erlenmeyer flask under agitation for two to three minutes. The mixture was transferred to the water bath at 60°C for a few hours. The solution was left undisturbed in the dark for 48 hours. On complete reduction, the solutions turned into a yellowish-brown color, indicating the synthesis of silver nanoparticles.^[29] The solutions were wrapped with aluminum foil and stored in the refrigerator.

Physicochemical Characterization of Silver Nanoparticles

UV-visible spectrophotometric analysis

The formation of silver nanoparticles was initially identified by the color change of the solution from

green color to yellowish brown. The absorbance of the samples was measured at a wavelength range of 200 to 600 nm using a Thermo Scientific spectrophotometer. The absorption spectrum of the reduced nanoparticles of both aqueous and ethanol leaf extracts of *G. gummi-gutta* was recorded against the reagent blanks.^[30]

Scanning electron microscopy

The surface topography and elemental composition of silver nanoparticles were analyzed by scanning electron microscopy (SEM) micrograph (JEOL Model JSM-6390LV).^[31] The SEM uses an electron beam for surface imaging. The solution was centrifuged at 12,000 rpm for 10 minutes and the pellet was dispersed in sterile distilled water. The centrifugation and dispersion were repeated three times to ensure the complete separation of silver nanoparticles. The dried pellet of silver nanoparticles dissolved in distilled water is used for SEM scanning. SEM Characterization of nanoparticles was done in order to understand the size and shape of the nanoparticles.

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectroscopic analysis was carried out to identify the potential functional groups that capped the nanoparticles formed in the solution. The study was done using a Thermo Nicolet, Avatar 370 spectrophotometer at a spectral range of 4000 to 400 cm^{-1} and a resolution of 4 cm^{-1} . Dried pellets were washed and used for scanning. The FTIR spectra of the nanoparticles, which contain some adsorbates, possess additional peaks in comparison with the FTIR pattern of a bare nanoparticle, which can be easily detected.^[32]

X-ray diffraction studies

X-ray diffraction (XRD) analysis was done using Bruker AXSD8 ADVANCE. The biosynthesized silver nanoparticles were added dropwise on a piece of slide and dried using a hot plate which was then subjected to XRD. The images obtained were compared with the data available at the Joint Committee on Powder Diffraction Standards (JCPDS) library to account for the crystalline structure. The crystalline nature and average size of the silver nanoparticles were measured using the Deby-Scherrer equation.

$$D = k\lambda / \beta \cos\theta$$

Where D = Thickness of the silver nanoparticles, K = Scherrer constant, 0.94, λ = Wavelength of the X-ray, 1.5406 $\times 10^{-10}$, β = Full width of half maxima of reflection pf Bragg's angle, it is calculated by fitting a Gaussian curve, θ = Bragg diffraction angle.^[33]

Assay of antimicrobial efficacy

Antibacterial activity of the green synthesized silver nanoparticles from *G. gummi-gutta* plant extracts was tested against gram-positive bacteria such as *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC

2413) and gram-negative bacteria namely *Salmonella typhi* (MTCC 3231) and *Pseudomonas aeruginosa* (MTCC 741) by agar well diffusion method. The microbial strains for the antimicrobial screening were obtained as the microbial type culture collection (MTCC) from the Institute of Microbial Technology, Chandigarh (India). Sterilized agar plates were prepared using Muller Hinton agar. Molted agar media was poured into the plates to a uniform depth of 5 mm and allowed to solidify. The microbial suspensions were streaked over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. The wells with a diameter of 10 mm were punched aseptically with a sterile corkborer and 100 $\mu\text{g/mL}$ concentration of silver nanoparticles of aqueous and ethanolic leaf extracts of *G. gummi-gutta* dissolved in DMSO were poured onto them. Standard antibiotic gentamycin (160 $\mu\text{g/mL}$) was used as positive control. The plates were incubated at 37°C for 24 hours and the growth inhibition zones, including the well diameters, were measured.^[34]

Cytotoxicity Study

Trypan blue dye exclusion test

For the cytotoxicity assay, 1×10^6 DLA cells, rat spleen cells in PBS and the test material at different concentrations were made up to 1-mL using PBS and incubated for 3 hours at 37°C in an incubator. Cell suspension without the test material was used as the control. After incubation 100 μL of 0.1%, trypan blue was added to the tubes containing different concentrations of samples and kept undisturbed for 2 to 3 minutes, after which it was loaded in a hemocytometer. Dead cells took up the blue color of trypan blue while live cells did not become colored. Live and dead cell numbers were counted using a compound microscope. The percentage of cell death was calculated using the formula

$$\text{Percentage of cell death} = \frac{\text{Number of dead cells}}{\text{Total number of cells counted}} \times 100$$

The trypan blue dye exclusion method was conducted twice, first on both the test samples and once the results were obtained, the sample showing the highest cytotoxic potential was tested against the normal spleen cells extracted from the rat, in order to analyze the cytotoxicity towards the normal cells.^[35]

MTT Assay

A human breast cancer cell line, MCF-7, was procured from National Centre for Cell Sciences, Pune, India and maintained in DMEM media, supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin and kept at 37°C in an incubator with 5% CO_2 . Cytotoxicity of the test material was estimated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay.^[36] Approximately 1×10^5 cells/mL were seeded in a 48-well plate, with complete growth medium (DMEM) and allowed to



attach and grow. Fresh medium containing different concentrations of test material (25–200 µg/mL) were used. The cells were further incubated for 48 hours. Then 20 µL of 0.5% MTT was added to the test and control wells and incubated for 4 hours. The formazan crystals formed were dissolved in dimethyl sulfoxide and the absorbance was measured at 540 nm wavelength in UV-vis spectrophotometer (Systronics, India) and the percentage of viability was calculated according to the formula.

$$\text{Percentage of Cell Viability} = \frac{\text{Mean OD of Samples}}{\text{Mean OD of Control}} \times 100$$

Statistical Analysis

Antimicrobial and cytotoxicity assays were carried out in a completely randomized block design and each treatment was replicated three times. The statistical analysis of the data was done using Origin software (version 7.0383, origin Lab Corporation, Northampton, MA 01060, USA) and the results were represented as mean ± standard deviation.

RESULTS

UV-vis Spectrophotometric Analysis

The plant extracts added to 0.1N silver nitrate solution in a 1:1 ratio led to the formation of silver nanoparticles. The presence of silver nanoparticles was confirmed by the formation of a yellowish-brown color (Fig. 1). The sample solutions were labeled separately for further studies. The reduced silver nanoparticles formed from aqueous leaf extract was labeled as AL and that from ethanol leaf extract was indicated as EL. The UV-vis spectrophotometric analysis of the two samples was done between a range of 200 to 600 nm wavelengths. In this study, the maximum absorption peak was observed at 365 and 363 nm wavelengths due to the characteristic surface plasmon resonance (SPR) of the samples AL and EL, respectively (Fig. 2). SPR peaks indicated the presence of silver nanoparticles. It is correlated to the findings of Caroling^[37] who have reported that the noble metal silver displays characteristic absorbance at around 360 to 460 nm wavelengths.

SEM, FTIR and XRD Studies

The FTIR images of the samples AL and EL are shown in Fig. 3. The FTIR spectrum of the samples showed

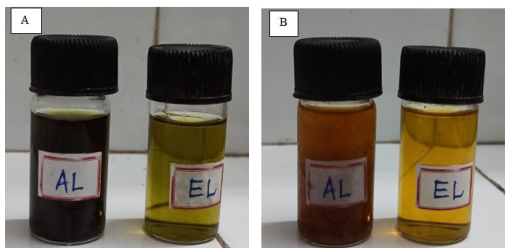


Fig. 1: A&B are aqueous and ethanol leaf extract of *G. gummi-gutta* before and after the addition of silver nitrate solution

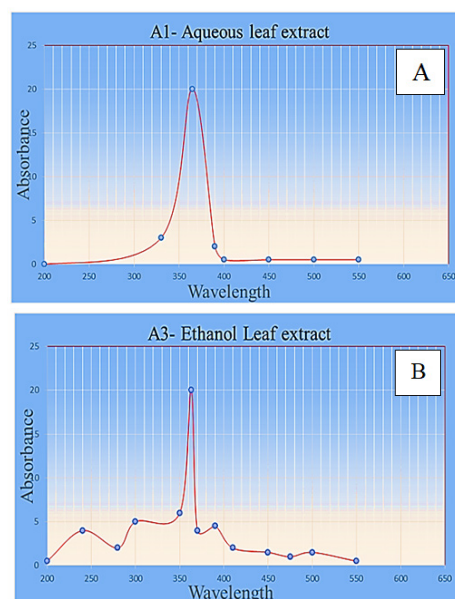


Fig. 2: UV-vis spectrum of silver nanoparticles synthesized from aqueous and ethanol leaf extracts (A&B) of *G. gummi-gutta*

prominent peaks around 873 to 876 cm^{-1} assigned to -CH bending, 1638 to 1648 cm^{-1} strong bond corresponded to C=C stretching of alkenes and 3445 to 3455 cm^{-1} strong band attributed to -OH bending of alcohol. Also, the peak at 2900 to 2985 cm^{-1} corresponded to -CH stretching of alkenes and at 2079.60 cm^{-1} showed -NH₃ stretching.^[37] Shanmugam *et al.*, in 2014 have reported that these peaks are due to the stretching of bonds in proteins, enzymes or polysaccharides present in the extracts.^[38] The FTIR

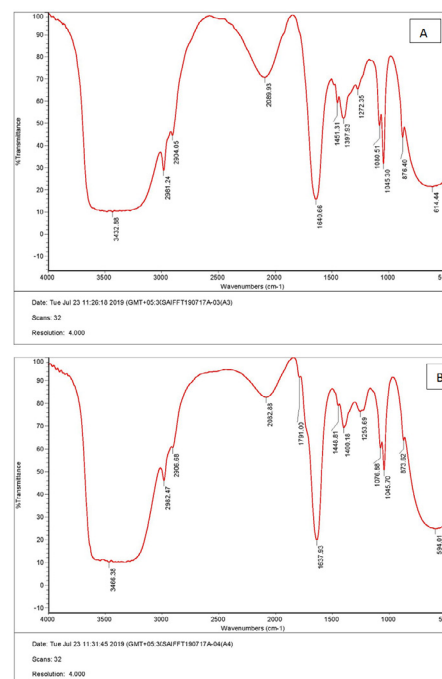


Fig. 3: A&B FTIR graph of silver nanoparticles from aqueous (AL) and ethanol (EL) leaf extracts of *G. gummi-gutta*

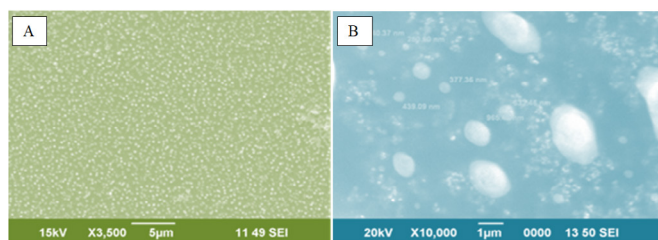


Fig. 4: SEM images (A&B) of silver nanoparticles from aqueous (AL) and ethanol (EL) leaf extracts of *G. gummi-gutta*

spectra provided information about the local molecular environment of the organic molecules on the surface of the nanoparticle. The FTIR reading suggests that each peak represents a functional group that is responsible for the formation of nanoparticles in the samples. XRD analysis gave the idea of crystallinity, purity, and size of the synthesized metal nanoparticles. The XRD pattern of the two samples AL and EL are shown in Fig. 4 respectively. Sample AL showed three characteristic peaks at 2θ values of 38.88° , 31.35° and 33.23° corresponding to 224, 164 and 88 crystalline planes. The shapes of the nanoparticles formed were cubic, hexagonal and tetragonal. The average size of the nanoparticles formed was 25 nm. Plant sample EL showed three characteristic peaks at 2θ values of 46.95° , 38.89° and 33.07° corresponding to 162, 224 and 224 crystalline planes of face-centered cubic (FCC) indicating the crystalline nature of the silver nanoparticles. This showed that the average size of the nanoparticles was 23 nm. The findings of the XRD data were compatible with the database of the Joint Committee on Powder Diffraction Standards (JCPDS) file no 04-0783. In addition to these peaks, there were other unassigned peaks weaker than those of silver were formed. This may be due to the bioorganic compounds occurring on the surface of the silver nanoparticles.^[39-41] The SEM analysis was done to analyze the size and shape of the nanoparticles. The SEM images of AL and EL clearly indicated the presence of well-dispersed spherical and hexagonal-shaped nanoparticles (Fig. 5). Nanoparticles may be aggregated due to the cross-linking.^[42] The particle size obtained from SEM images is well correlated with the particle size determined from XRD using the Scherrer formula and the average size of the synthesized nanoparticles was in the range of 20 to 25 nm.

Antimicrobial and Cytotoxicity Studies

Pseudomonas aeruginosa, *S. typhi*, *B. subtilis*, and *S. aureus* were selected for the assay of antimicrobial activity of the silver nanoparticles synthesized from aqueous and ethanol leaf extracts of *G. gummi-gutta*. The results of the study are shown in Table 1. The studies revealed that the silver nanoparticles (100 µg/ml) synthesized from the aqueous and ethanol leaf extract of this plant labeled as AL and EL, respectively showed a good zone of inhibition when compared to the antibiotic taken as the positive control. Here gentamycin (160 µg/mL) was used as the positive

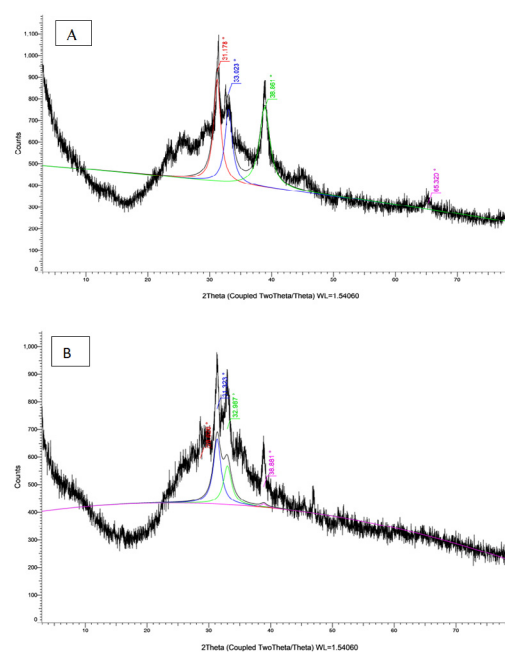


Fig. 5: XRD pattern of silver nanoparticles obtained from *G. gummi-gutta* aqueous and ethanol leaf extracts

Table 1: Antimicrobial activity of silver nanoparticles synthesized from aqueous and ethanol leaf extracts of *G. gummi-gutta*

Zone of inhibition in mm (Including well diameter)			
Test organisms	AL	EL	Positive control
<i>Staphylococcus aureus</i>	18.24 ± 0.54	19.66 ± 1.5	23.00 ± 0.23
<i>Bacillus subtilis</i>	13.5 ± 0.82	17.19 ± 0.93	21.00 ± 0.6
<i>Salmonella typhi</i>	15.76 ± 1.31	23.45 ± 1.87	22.00 ± 1.92
<i>Pseudomonas aeruginosa</i>	15.48 ± 1.79	20.52 ± 2.14	17.00 ± 1.57

Values are expressed as mean ± SD, AL: silver nanoparticles synthesized from aqueous leaf extract (100 µg/mL), EL: silver nanoparticles synthesized from ethanol leaf extract (100 µg/mL), Gentamycin (160 µg/mL) taken as positive control.

control. The results showed a maximum zone of inhibition of 23.45 ± 1.87 mm by silver nanoparticles synthesized from ethanolic leaf extract (200 µg/mL) against *S. typhi*. A minimum zone of inhibition was observed for the silver nanoparticles synthesized from aqueous leaf extract, AL against *B. subtilis* (13.5 ± 0.82 mm). Silver nanoparticles synthesized from *G. gummi-gutta* showed a good antimicrobial effect against all the gram-negative bacteria taken for this study.^[43] The two samples AL and EL were taken at a concentration of 25 to 200 µg/mL and tested for the trypan blue dye exclusion test on DLA cell lines (Table 2). It was found that the sample EL showed more cytotoxicity towards the DLA cells; hence the sample EL alone was taken for trypan blue dye exclusion test on rat spleen cells. The results of the tests are shown in



Table 2: Trypan blue exclusion test in synthesized silver nanoparticles of *G. gummi-gutta* aqueous and ethanol leaf extracts on DLA cell line

Drug concentration ($\mu\text{g/mL}$)	Percentage cell death in DLA cells	
	AL	EL
25	62.19 $\pm$ 0.82	76.41 $\pm$ 1.33
50	70.03 $\pm$ 1.29	78.97 $\pm$ 1.64
100	79.6 $\pm$ 0.56	81.78 $\pm$ 2.32
200	82.54 $\pm$ 0.37	86.12 $\pm$ 1.75

Values are presented as mean $\pm$ SD, AL: the nanoparticles synthesized from aqueous leaf extract, EL: the nanoparticles synthesized from ethanolic leaf extract.

Table 3: Trypan blue exclusion test of silver nanoparticles synthesized from ethanolic leaf extract of *G. gummi-gutta* on rat spleen cells

Sample EL ($\mu\text{g/mL}$)	Percentage of cell death
25	4.05 $\pm$ 0.84
50	4.13 $\pm$ 0.36
100	6.26 $\pm$ 0.11
200	15.81 $\pm$ 0.29

Values are presented as mean $\pm$ SD, EL, nanoparticles reduced from ethanol leaf extract of *G. gummi-gutta*

Table 3. The sample EL was also subjected to MTT assay using MCF-7 cell lines. The results clearly stated that the sample EL has remarkable anticancer properties towards MCF-7 human breast cancer cells (Table 4). The drug was found non-toxic towards the normal spleen cells. The nanoparticles synthesized using the ethanol leaf extracts (200 $\mu\text{g/mL}$) had more cytotoxic effects on DLA cells. The percentage of cell death was found to be $86.12 \pm 1.75\%$ whereas it showed a very minimum effect on the normal spleen cells extracted from rats with $15.81 \pm 0.29\%$ cell death at the same concentration. A dose-dependent increase can be observed in the percentage of cell death of both the trypan blue exclusion test and MTT assay (Fig. 6).

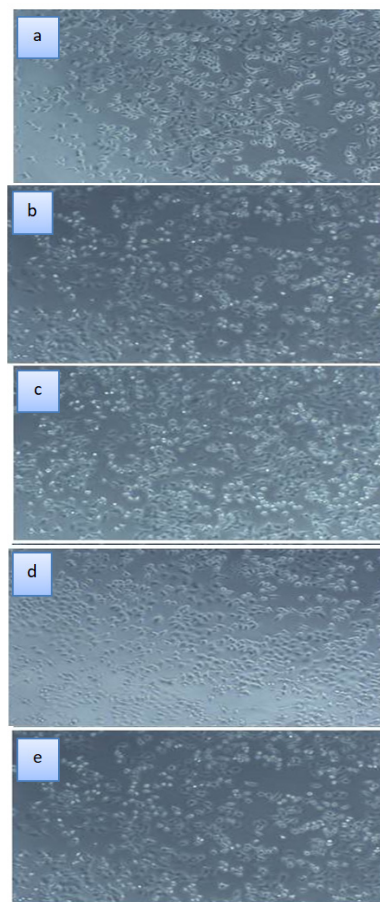
DISCUSSION

In green synthesis, several factors lead to the formation of silver nanoparticles like temperature, pH, and selection of the plant material. In this study, the formation of

Table 4: MTT assay of silver nanoparticles from ethanolic leaf extract of *G. gummi-gutta* on MCF-7 cell lines

Volume of the sample EL ($\mu\text{g/mL}$)	Percentage of cell viability (%)
25	66.17 $\pm$ 0.99
50	65.50 $\pm$ 1.24
100	58.94 $\pm$ 2.28
200	43.10 $\pm$ 1.65

Values are presented as mean $\pm$ SD, EL, nanoparticles reduced from ethanol leaf extract of *G. gummi-gutta*

**Fig. 6:** Images of loss in cell viability of MTT assay conducted on MCF-7 breast cancer cells (a)-Control; (b)-25 $\mu\text{g/mL}$; (c) - 50 $\mu\text{g/mL}$; (d)-100 $\mu\text{g/mL}$; (e)-200 $\mu\text{g/mL}$

silver nanoparticles (AgNPs) from aqueous (AL) and ethanol leaf extracts (EL) of *G. gummi-gutta* was clearly observed by the change in color of the solution from green color to yellowish brown. The change in color was due to the excitation vibration of SPR that occurred due to the interaction of light with the nanoparticle.^[44] UV-vis spectrophotometric analysis of synthesized silver nanoparticles of *G. gummi-gutta* leaf extracts showed an absorption spectrum ranging between 360 to 370 nm wavelengths. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical surroundings.^[45] Previous studies on the preliminary phytochemical screening revealed that the aqueous and ethanolic leaf extracts of *Garcinia gummi-gutta* showed the presence of some important secondary metabolites like phenolics, flavonoids, alkaloids, carbohydrates, proteins, terpenoids, etc.^[46] FTIR is a simple technique to identify the role of biological molecules in the reduction of silver ions to nanoparticles. Here FTIR study reveals that the $-\text{CH}_2-$, $-\text{CO}-$ and $-\text{NH}_2-$ groups present in different secondary metabolites are responsible for capping, stabilization, and reduction of silver ions and formation of the NPs in both aqueous and ethanol leaf extracts of *G. gummi-gutta*. The

synthesized silver nanoparticles interact with the cell membrane of the bacteria and induce oxidative stress with the formation of reactive oxygen species which can damage the bacterial membranes, mitochondria, and DNA leading to the death of the bacterial cell.^[47] Smaller nanoparticles can affect the larger surface area of the bacteria and hence it has more bactericidal activity than the large-sized nanoparticles. From SEM and XRD results it is clear that the sample EL, silver nanoparticles from ethanol leaf extract contains smaller and spherical silver nanoparticles. Nanoparticles synthesized from the ethanol leaf extract of *G. gummi-gutta* exhibited a great antibacterial effect against selected gram-negative bacteria. The results of the trypan blue exclusion test of different samples (AL and EL) on DLA cells showed that the biosynthesized silver nanoparticles are cytotoxic against these cancerous cell lines. The sample EL, which showed the highest activity, was subjected to a trypan blue exclusion test on normal rat spleen cells and an MTT assay on MCF-7 cell lines. The study showed that the sample EL, synthesized silver nanoparticles from ethanolic leaf extract indicated less toxicity impact on normal cell lines than cancerous cell lines. Similar studies reported in green tea, *Abelmoschus esculentus*, *Cassia alata* and walnuts etc. emphasize that the sample containing smaller nanoparticles has a greater impact.^[48-51] The present study underlines the significance of the green synthesized silver nanoparticles of the plant *G. gummi-gutta* as a potential tool in modern medicine due to their antimicrobial efficacy and cytotoxicity functions. The biosynthesized silver nanoparticles could be of greater scope in modern medicine especially in targeted drug delivery and controlled release of therapeutic agents.

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