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IN VITRO ANTICANCER ACTIVITY OF SILVER NANOPARTICLES BY *CHLOROPHYTUM COMOSUM* LEAF EXTRACTS ON HUMAN LIVER CARCINOMA (HEPG2) CELL LINES

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ABSTRACT:

In the present study, green synthesis of silver nanoparticles (AgNPs)was performed using leaf extracts of *Chlorophytum comosum* from Aspargaceace family. The size, shape and elemental composition of silver nanoparticles (AgNPs)was studied using UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), Zeta potential, Particle size analysis and dye degradation. Zeta potential contributing to the stability of silver nanoparticles was recorded as -23. 8mV.The particle size of the synthesized nanoparticles ranged from 108.5. UV-Visible spectroscopy peak for AgNPs was observed at 260nm.The biosynthesized silver nanoparticles displayed antimicrobial activity of gram +ve and gram-ve bacteria and the antifungal activity against Aspergillus Niger. The hydrogen peroxide scavenging assay revealed antioxidant potential of silver nanoparticles in the concentration of 100µg/ml. The ethanolic extract showed anti-cancer activity (94.05%) at 5µg/ml exposed to $HepG_2$ cell lines. The anticancer activity was compared between the ethanolic extract and silver nanoparticles. These findings suggest that the ethanolic extract of C. comosum contains medically relevant bioactive compounds that have the potential to treat serious human health problems.

KEYWORDS: Chlorophytum comosum, silver nanoparticles (AgNPs), $HepG_2$ cell line, cytotoxicity, anti-microbial activity, dye degradation.

INTRODUCTION:

Cancers are a group of disorders in which the body's cells grow out of control and spread to other areas of the body. Metastasis, which cannot be stopped, is the term for the spread of cancerous cells. Many internal and external variables can contribute to cancer. External elements like tobacco, chemicals, radiation, and infectious diseases and internal variables include inherited mutations, hormones, immunological disorders, and mutations. Cancer has a wide range of complex and poorly understood causes. Many factors, such as dietary elements, specific illnesses, a lack of exercise, obesity, and environmental elements are known to

raise the risk of cancer. In India, cancer is now among the leading causes of mortality [1]. In India, 14,61,427 incident cases are anticipated to occur in 2022[2]. Hepatocellular carcinoma (HCC) is a primary tumour of the liver which occurs in approximately 85% of patients diagnosed with cirrhosis. Significant risk factors of HCC include viral hepatitis, alcoholic liver disease and non-alcoholic steatohepatitis [3]. Microbial infections include bacterial and fungal infections. The internal toxins that bacteria create are excreted or secreted, which causes sickness. Illnesses caused by bacteria, including gonorrhoea, syphilis, tetanus, diphtheria, cholera, Lyme disease, and TB, among others. Certain bacteria can be advantageous; for example, the bacteria in stomach aid with digestion [4]. Aspergillus Niger and candidiasis are two of the more typical fungal species. Athletes' foot and onychomycosis are examples of fungus infection. Antibiotics are drugs that treat infections brought on by bacteria and fungi. These antibiotics work by preventing or killing bacteria growth or multiplication. In every region of the world, antibiotic resistance is alarmingly increasing. There is a global spread of new resistance mechanisms. Overmedication and drug abuse both hasten the development of resistance. Drug resistance makes antibiotics ineffective and makes treating illnesses challenging or impossible.[5] While chemically produced medications may have a speedy action, they also have side effects that can be harmful to the body. So, in order to overcome the drawbacks of synthetic antibiotics, it is required to produce medications made from natural resources such as plants or medicinal goods. Medicinal plants provide a variety of phytochemical components that aid in healing.[6] Phyto therapeutics need a scientific approach to deliver the components in a sustained manner to increase patient compliance [7]. One such approach can be nanoparticles or nanotechnology or green synthesis of nanoparticles where we can have advantages like reduced dosing frequency and controlled delivery of drugs. *chlorophytum comosum(thumb)Jacques* belongs to the family Aspargaceace. The common names are spider plant, ribbon plant, airplane plant and bracketed plant. The reported pharmacological properties of *chlorophytum comosum* are anti-tumour promoter activity [8], anti-oxidant activity [9], anti-proliferative activity [10], hepatoprotective activity [11], influence on intestinal microflora [12] and indoor air purifier [13]. The different phytochemical constituents in *Chlorophytum comosum* are identified in the fractions of methanolic extract of leaves of *chlorophytum comosum* (green type) are phenolic compounds, flavonoids, carotenoids, tannins and belongs to chemical class like diterpene alcohol, isoprenoid hydrocarbon, aromatic aldehyde and sulfone glycoside [14]. The main aim of the present study was synthesis, characterization and evaluation of *in vitro* anti-bacterial, antifungal, antioxidant and anti-cancer activity of *Chlorophytum comosum* nanoparticles.

MATERIALS AND METHODS

1.COLLECTION AND AUTHENTICATION OF THE PLANT:

The leaves were collected from the Sri Venkateshwara veterinary college, Tirupati, Balaji district, Andhra Pradesh, India. It has been recognized and authenticated by Prof.N. Savithramma professor of Botany, Sri Venkateshwara University, Tirupati, Balaji district, Andhra Pradesh, India. The plant code no was SVUH-2890.

2.PREPARATION OF PLANT EXTRACT:

The leaves were cleaned, shade dried and ground into coarse powder which was extracted using ethanol in Soxhlet extractor. The resulting extract was concentrated and stored in scintillation flask which was used for further analysis.

3.SYNTHESIS AND CHARACTERIZATION OF AgNPs:

Fresh leaf juice was prepared using distilled water. To 10ml of fresh leaf juice add 90ml of 1Mm AgNo3 and was incubated overnight at room temperature and observes for colour change which indicates formation of AgNPs of plant. The characterization of AgNPs was performed using UV-Visible spectroscopy [15], FTIR analysis [16], particle size and zeta potential measurement [17]. Preliminary phytochemical screening was performed using industry -standard procedures [18].

DEGRADATION OF METHYLENE BLUE DYE:

10 ppm methylene blue dye solution was prepared by adding 10 mg of dye in 1 L distilled water. To study the catalytic degradation of methylene blue by silver nanoparticles, different concentrations of AgNPs such as 0.2, 0.4, 0.6, 0.8, 1 ml mixed with prepared dye solution separately. Then the flasks were placed in an orbital shaker. A control was also maintained without addition of nanoparticles. The degradation of the dye was observed by the reduction in the colour of the dye. The absorbance values were taken at 660 nm for 5 days with an interval of 24 hours to calculate the reduction of methylene blue dye in solution at room temperature [19]. Percentage of dye degradation was estimated by the following formula:

% Decolourization = ($C0$ - C)/C0 X 100

Where $C0$ is the initial concentration of dye solution and C is the concentration of dye solution after catalytic degradation.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF AgNPs:

The antimicrobial activity of silver nanoparticles was tested against the fungus – Aspergillus Niger and bacteria – Staphylococcus aureus and E. coli using standard disk -diffusion method [20]. For antifungal activity, 0.1 mL of Aspergillus Niger spores were aseptically added into Czapek-Dox agar plates. Cavities of 5 mm were made and were filled with various concentrations of silver nanoparticles. The plates were incubated at 28 ± 4 ◦C for 7 days. The antibacterial activity was carried out with 24-h active cultures of the

chosen bacterial strains. The bacterial strains were seeded into nutrient agar medium. Central cavity of 5 mm size was made in plates and filled with various concentrations of silver nanoparticles. The plates were incubated in an incubator at 37°C overnight. Then plates were observed and (ZOI) zone of inhibition was measured.

ANTIOXIDANT ACTIVITY:

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch et al. Aliquot of 0.1 mL of extracts (25-400micro gram/mL) was transferred into the Eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 (2 Mm) [21]. The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

H_2O_2 scavenging activity percentage=

$$[(A0-A1)/A0] \times 100$$

Where: A0=Absorbance of control,

A1=Absorbance of sample.

Determination of cytotoxic activity by MTT assay:

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37°C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test extract in represented wells in 96 plates. After 48 hrs discard the extract solution and add the fresh media with MTT solution (0.5 mg / mL-1) to each well and plates were incubated at 37°C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a micro plate reader. The percentage growth inhibition was calculated using the following formula [22]: % Inhibition=100(Control-Treatment)/control

RESULTS AND DISCUSSION

CHARECTERIZATION:

Phytochemical screening was done to confirm the presence of secondary metabolites like alkaloids, flavonoids, saponins, phenols, tannins and carbohydrates. These compounds may be actively involved in reduction of silver ions to nanoparticles. Change in colour confirmed the presence of nano particles. The colour of the solution changed from yellow to brown colour when silver nitrate was added to the plant extract solution. Fig 1a and 1b indicates before and after AgNPs synthesis.

UV-VISIBLE SPECTROSCOPIC ANALYSIS:

The synthesized AgNPs were confirmed by UV-visible spectroscopy. Change in colour confirms the formation of AgNPs. The peak for AgNPs was observed at 260 nm. Formation of AgNPs was confirmed visually from the colour change after the addition of *Chlorophytum comosum* extract into silver nitrate solution. The formation of silver nanoparticle was due to surface plasmon resonance (SPR). In UV-visible spectrum, the shift for the AgNPs was observed due to the presence of secondary metabolites such as alkaloids, flavonoids, saponins, steroids and Phenolic compounds in the plant extract in fig 2.

FTIR ANALYSIS OF AgNPs:

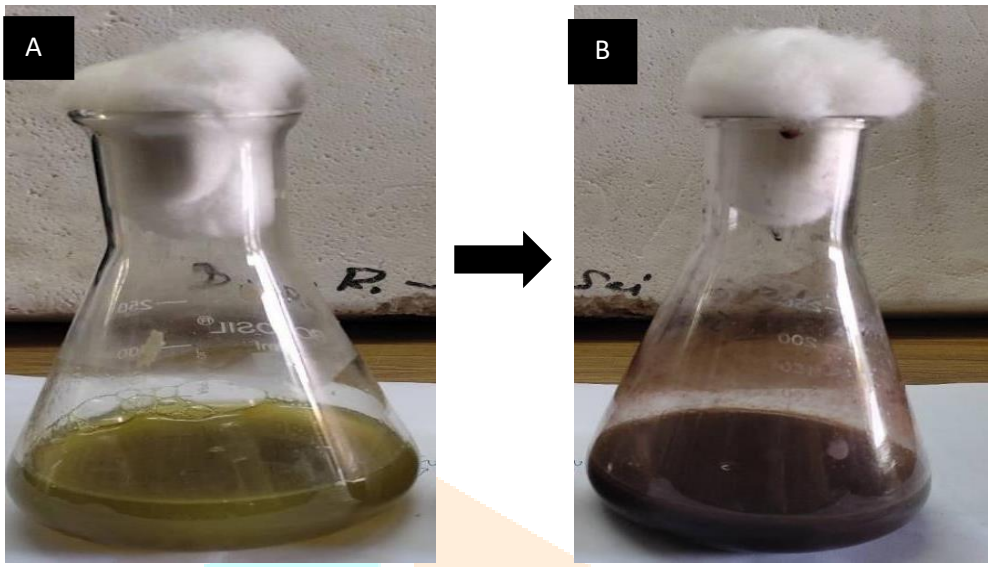
The FTIR spectra of silver nanoparticles prepared from the *Chlorophytum comosum* plant leaf extract. The FTIR analysis was carried out to identify the potential biomolecules in Chlorophytum comosum responsible for capping of the silver nanoparticles. FTIR signal at 1630.00 cm^{-1} c=c was attribute to c=c stretching of amide group which is responsible for reduction of Ag+ to Ag^0.The band at 3413.63 cm^{-1} is attributed to OH stretch of hydroxyl group. The peak at 1383.95 cm^{-1} may be attributed OH bending of tertiary alcohol. The bands at 826.61 cm^{-1} and 1050.45 cm^{-1} attributed to C-H out plane bend and aromatic C-H in plane bend respectively. The peaks at 490.28 cm^{-1} and 467.25 cm^{-1}may be attributed to S-S stretch of polysulphides and 439.03 $cm^{-1}$is attributed to S-S stretch of aryl disulphides. The absence of peak at 1256.00 cm^{-1} may be due to the capping of C-O group in the synthesis of AgNPs. These various peaks indicated the presence of biomolecules which are responsible for the reduction and stabilization AgNPs fig 3.

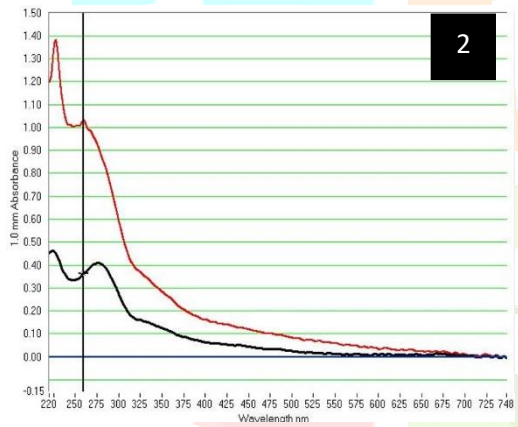
ZETA POTENIAL ANALYSIS

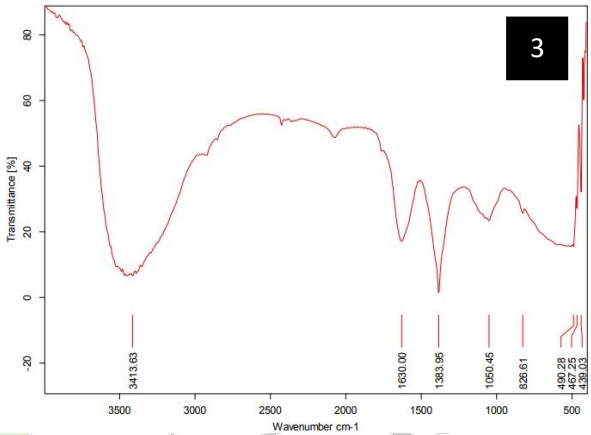
The electrostatic repulsive force between the nanoparticles depends on the charge present on the particles surface. The negative zeta potential value confirms the repulsion among the particles, thereby increasing the formulations stability and preventing the nanoparticles from agglomeration in the medium, leading to long term stability. The zeta potential of the AgNPs of *Chlorophytum comosum (Thumb) Jacque's* leaf was found to be -23.8mV in fig 4

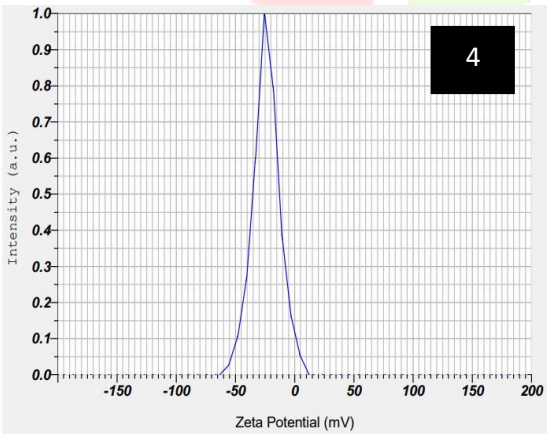
PARTICLE SIZE DETERMINATION:

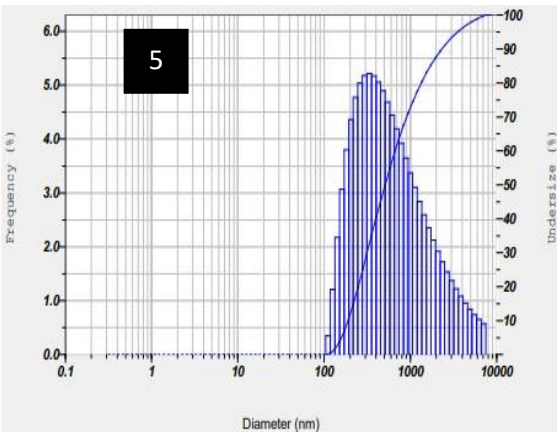
The particle size analyser is a scientific tool that measures the particle size distribution of synthesized nanoparticles. The particle size of the synthesized nanoparticles ranged from108.5 in fig 5.











Characterization of silver nanoparticles AgNPs: Fig (1a) colour change before and (1b) after synthesis of silver nanoparticles AgNPs. Fig (2) UV Spectrum Fig (3) FTIR Spectrum (4) Zeta potential and (5) particle size determination of sliver nanoparticles.

Degradation of methylene blue dye:

Catalytic activity of photosynthesized silver nanoparticles on degradation of dye was demonstrated by using methylene blue at room temperature. The degradation of methylene blue was noted by colour change from the initial first day deep blue colour to light blue at the end of 5th day. For every 24 hours for 5 days, a small volume of filtered aliquot was used to take absorbance value at 660 nm. The absorption spectrum peaks were decreased gradually with the increase of the day exposure which indicates the catalytic degradation reaction of methylene blue dye by photosynthesized silver nanoparticles. The percentage of dye degradation efficiency of silver nanoparticles was calculated for every 24 h for 5 days. Of various concentrations of AgNPs, 1 ml was found effectively degrading the dye at the end of 5th day (65%).

Table 1: Dye Degradation of methylene blue by photosynthesized AgNPs

	Methylene blue dye degradation ((%)				
AgNPs(ml)	Day 1	Day 2	Day 3	Day 4	Day 5
0.2	4.46±0.34	9.3±0.24	18.39±0.43	24.93±0.24	36.88±0.42
0.4	17.2±0.56	21.35±0.25	25.06±0.24	35.95±0.59	40.84±0.23
0.6	35.76±0.87	39.24±0.16	40.94±0.24	44.11±0.23	45.78±0.23
0.8	42±1.63	41.3±0.49	44.43±0.4	46.8±0.58	56.78±0.23
1	45.6±0.46	51.83±0.23	52.46±0.4	62.33±0.94	65.8±0.29

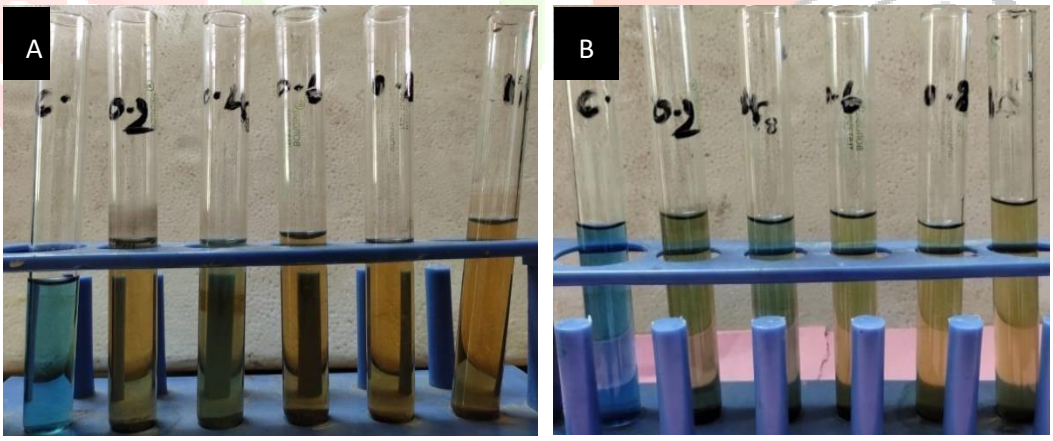


Figure 6: Visual observation of methylene blue dye degradation at different day exposure (A) dye degradation at first day (B) day degradation at fifth day.

ANTIOXIDANT ACTIVITY:

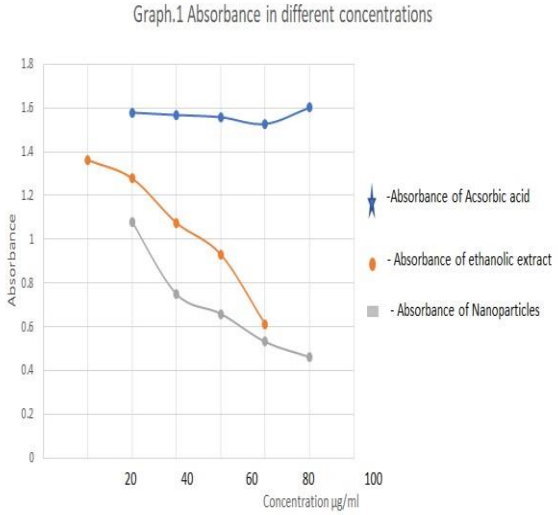
The redox properties of antioxidants play an important role in absorbing free radicles, quenching singlet and triplet oxygen or decomposing peroxides. The antioxidant properties of *Chlorophytum comosum* was evaluated by H_2O_2 scavenging assay. The nanoparticles and ethanolic extract were taken in a different concentration varying between 20-100µg/mL, and result showed the antioxidant activity, the percentage inhibition was 61.79٪ for ethanolic extract and 71.22٪ for nanoparticles. The maximum percent antioxidant potential was exhibited by nanoparticles. Both ethanolic extract and nanoparticles have shown the dose dependent antioxidant activity. The IC_{50} value of nanoparticles was 47.87 µg/ml and IC_{50} value of ethanolic extract was 89.43µg/ml.:

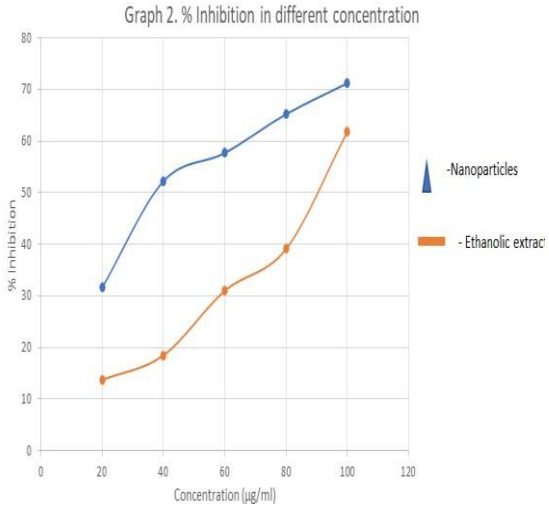
TABLE 2: Antioxidant activity of *Chlorophytum comosum* ethanolic extract

Concentration(µg/ml)	Absorbance of ascorbic acid	Absorbance of ethanolic extract	٪ inhibition	IC50(µg)
20	1.579	1.362	13.74	89.43
40	1.568	1.278	18.49	
60	1.558	1.075	31.00	
80	1.527	0.930	39.09	
100	1.602	0.612	61.79	

TABLE 3: Antioxidant activity of *Chlorophytum comosum* AgNPs

Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of nanoparticles	٪ inhibition	IC50(µg)
20	1.579	1.080	31.60	47.87
40	1.568	0.749	52.23	
60	1.558	0.659	57.70	
80	1.527	0.531	65.22	
100	1.602	0.461	71.22	





ANTI MICROBIAL ACTIVITY:

The antibacterial activity of *Chlorophytum comosum (Thunb) Jacques* leaf nanoparticles was studied against *Staphylococcus aureus* and *Escherichia coli*. It was conformed that the nanoparticles showed antibacterial activity as that of standard antibiotic (Amoxicillin). The zone of inhibition was measured and calculated. At high concentration AgNPs shows the better antibacterial activity. When compared to gram positive *Staphylococcus aureus* AgNPs shows better antibacterial activity against gram negative bacteria *Escherichia coli.*

TABLE 4: Zone of inhibition (cm) for green synthesized AgNPs from *Chlorophytum comosum*

Clinical isolates	Zone of inhibition (cm)				
	25(µl)	50(µl)	75(µl)	100(µl)	Standard(amoxicillin)
Escherichia coli	0.7cm	0.9cm	0.12cm	0.18cm	0.20cm
Staphylococcus aureus	0.4cm	0.5cm	0.7cm	0.14cm	0.20cm

The colloidal silver nanoparticles inhibited the growth of the fungus *Aspergillus Niger* which was seeded in the nutrient agar plate and formed a zone of inhibition around the central cavity. The zone of inhibition with diameter was recorded in respect of *Aspergillus Niger* the anti-fungal activity is due to the formation of insoluble compounds by inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane bound enzymes and lipids which causes cell lysis.

TABLE 5: Zone of inhibition (cm) for green synthesized AgNPs from *Chlorophytum comosum (Thunb) Jacques*

Test organism	Zone of inhibition(cm)			
	15(µl)	25(µl)	50(µl)	Control 50(µl)
Aspergillus Niger	0.5cm	1cm	1.3	0.3

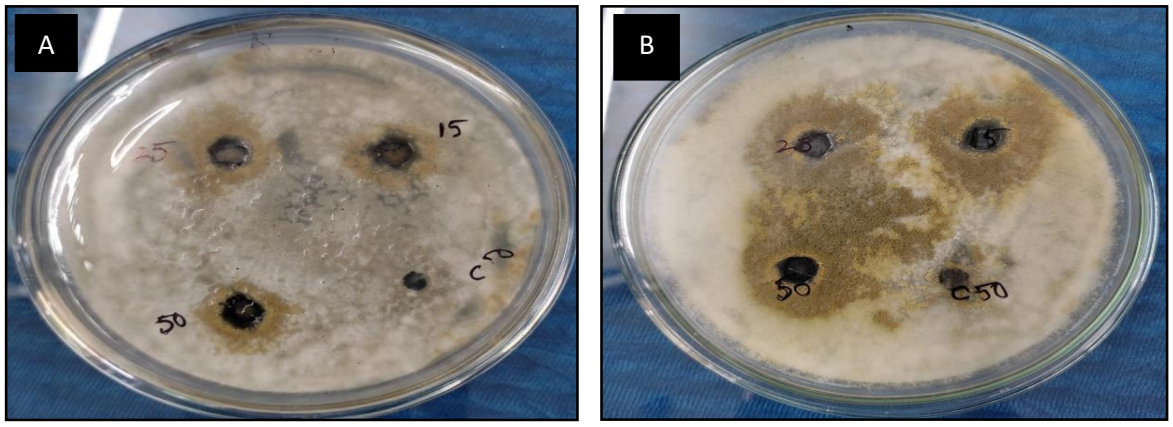


Figure-7: **A.** Represents the Zone of inhibition on 1 day **B.** Zone of inhibition on 5 th day of *Chlorophytum comosum* AgNPs against *Aspergillus Niger*

CYTOTOXIC EFFECT:

AgNPs and an ethanolic extract of *Chlorophytum comosum* were tested for anticancer activity against Hep-G2 cancer cell lines. The viability of liver cancer cells was reduced in a concentration-dependent manner by AgNPs and an ethanolic extract of Chlorophytum comosum. Higher extract concentrations demonstrated greater anticancer activity. On liver cancer cells, the IC50 values of AgNPs and ethanolic extract were calculated to be 77.07 g/ml and 98.07 g/ml, respectively, while the standard doxorubicin was 50.301.73 g/ml. Ethanolic extract inhibited more than the AgNPs. Dr. K Padmalochana reported that the difference in inhibition percentage is due to the presence of phytochemical constituents in a similar experiment on Hep-G2 cell line. Previous research concluded that phytochemical constituents found in Chlorophytum comosum were responsible for the inhibition of Hep-G2 cell lines.

Table 6: Hep-G2 cells were treated with ethanolic extract of *Chlorophytum comosum* and IC $_{50}$ values

Concentration (µg)	**Absorbance at 570nm**	**% Inhibition**	**% Viability**	**IC_{50} (µg)**
5	0.584	5.5	94.5	
10	0.561	9.22	90.78	
25	0.476	22.97	77.03	
50	0.394	36.24	63.76	
100	0.329	46.76	53.24	
Untreated	0.618	0	100	98.07

Table 7: Hep-G2 cells were treated with AgNPs of *Chlorophytum comosum* and IC $_{50}$ values

Concentration (µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
5	0.57	7.77	92.23	
10	0.546	11.65	88.35	
25	0.451	27.02	72.98	
50	0.366	40.77	59.23	77.07
100	0.254	58.89	41.11	
Untreated	0.618	0	100	

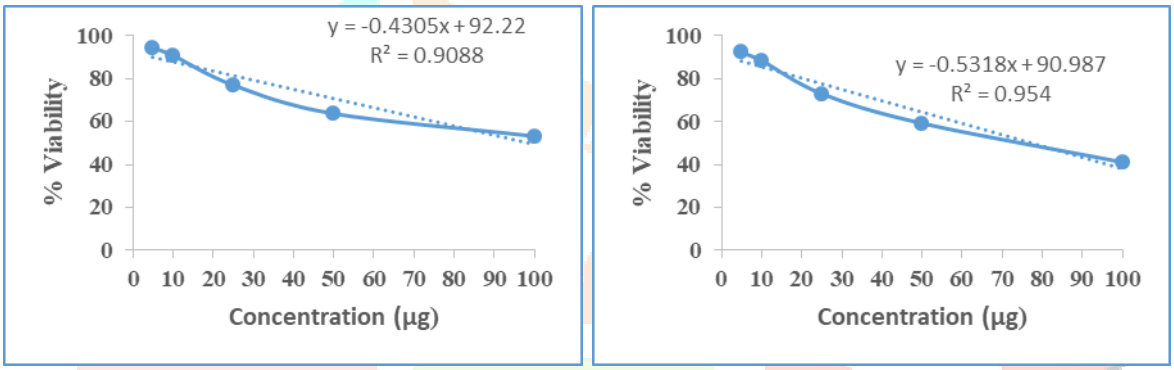


Figure 9: ٪ viability of ethanolic extract and AgNPs of *Chlorophytum comosum*

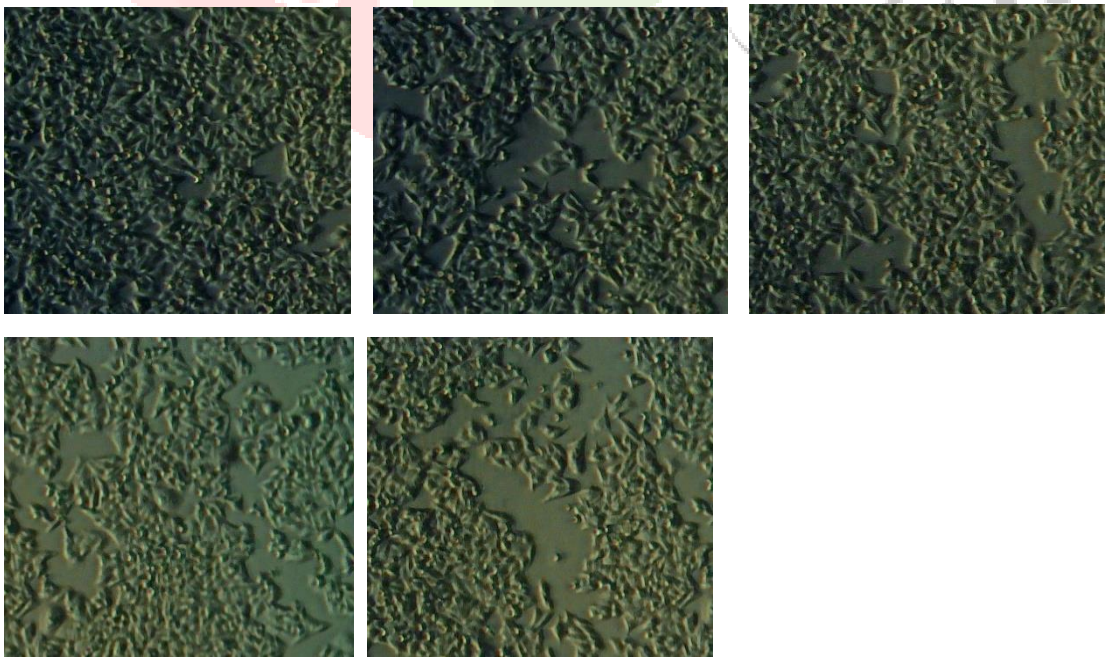


Fig 10: Cell viability of ethanolic extract of *chlorophytum comosum* against HepG2 (5µg, 10µg,25µg,50 µg,100 µg)

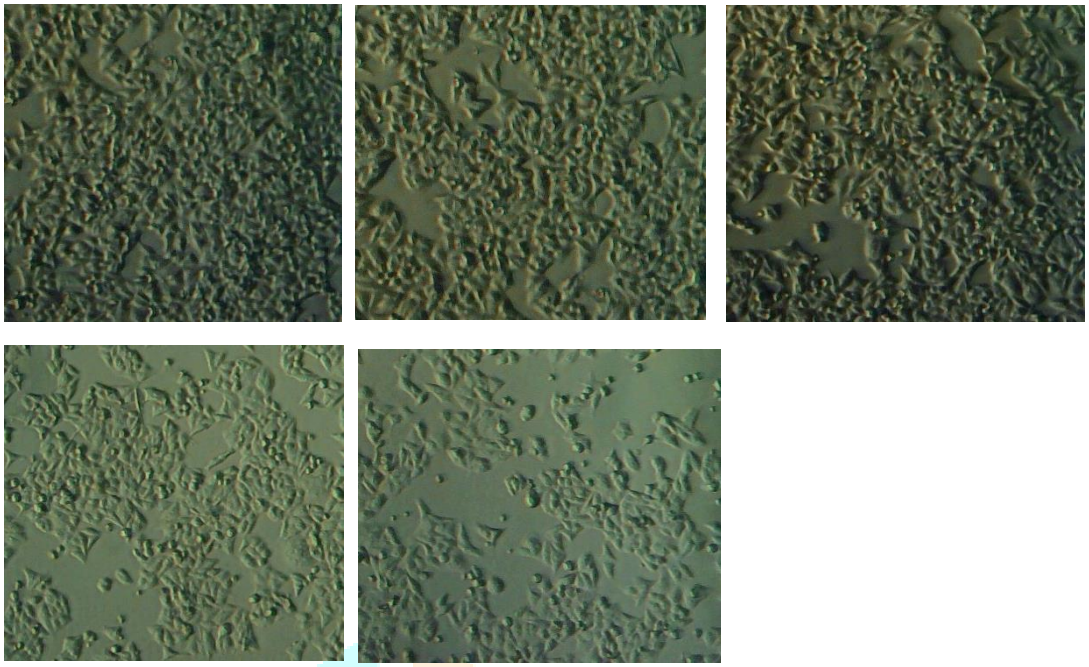


Fig 11: cell viability of AgNPs of *chlorophytum comosum* against HepG2 (5µg,10µg,25µg,50µg,100µg)

CONCLUSION

In this study, AgNPs were synthesised using the leaves of *Chlorophytum comosum* in a straightforward manner and phytochemicals in the plant extract work well as reducing and stabilising agents. UV-visible spectroscopy, FT-IR spectroscopy, Particle size, Zeta potential were used to characterise the synthesised nanoparticles. According to the particle size, nanoparticles range in size from 108.5 nm. AgNPs demonstrated broad antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. The zone of inhibition clearly indicated the antibacterial activity of silver nanoparticles and superior antifungal activity against *Aspergillus Niger*. This method of producing AgNPs is both cost-effective and environmentally friendly. AgNPs proved superior photocatalytic activity against methylene blue. The antioxidant and anticancer activity of *Chlorophytum comosum* ethanolic extract and AgNPs. Ethanolic extract has the antioxidant and anticancer activity, as evidenced by hydroxyl scavenging and reducing activity, and it has the strongest activity against Hep-G2 cell lines compared to nanoparticles. The study's findings provide a scientific basis for using the plant to control Hep-G2 cells in traditional medicine. More validation studies are needed to determine the cytotoxicity of the extract, nanoparticles, and optimal dosage for traditional medicine applications**.**

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