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Biosynthesis And Characterization Of Gold Nanoparticles Using Pyracantha Crenulata Leaves And Evaluation Of Its Antimicrobial And Anticancer Activities

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Abstract

A green technique that enables one-pot synthesis is plant-mediated biosynthesis of nanoparticles. Because of its many uses, the synthesis of gold nanoparticles using plant extracts has drawn attention in the biomedical area. In this work, the aqueous extract of Pyracantha crenulata leaves is used to synthesize gold nanoparticles (AuNPs) via green chemistry. At room temperature, the AuNPs were created. By detecting the surface plasmon resonance at 538 nm, UV-Vis spectroscopy verified the creation of AuNPs. The crystalline character of Au nanoparticles was suggested by the XRD spectra of Pyracantha crenulata Au NP's, which revealed that the particles were cubic closed packed (ccp) phase crystals. Pyracantha crenulata Au NP's EDX spectra revealed a modest peak at about 0.25 keV and 0.5 keV that may have been caused by the biomolecules linked to the Au NPs' surface, as well as a sharp, strong signal at 2.1 keV that confirmed the existence of elemental Au. Spherical nanoparticles are visible in TEM examination. The existence of certain bioactive molecules in charge of the Au^{3+} ion reduction process was shown by FTIR analysis. Bacterial cultures were cultivated in soybean casein digest agar medium (SCDM) for antimicrobial activity analysis, while fungal cultures were cultivated in potato dextrose agar medium, which was created by a method at varying concentrations against Escherichia coli, Bacillus subtilis, Candida albicans, and Aspergillus oryzae. The zone of inhibition has diameters of 5.0 mm, 8.0 mm, 10.0 mm, and 8.0 mm, in that order. The anti-cancer capability of gold manufactured nanoparticles was assessed by both in-vitro and in-vivo experiments using two MTT and SRB assays in hepatic (hep-2) cell lines and cancer induction of cancer cell line (hep-2) in Swiss albino rat's livers. Anticancer tests were also conducted to assess many parameters, including tumor weight, haemoglobin content, viable and non-viable cells, RBC, and WBC count. It was discovered to have an impact on reducing the proliferation of cancer cell lines and providing a zone of inhibition.

Keywords: *Pyracantha crenulata, Gold nanoparticles, Anticancer, MTT assay, XRD.*

INTRODUCTION

Metallic nanoparticles can be considered one of the most versatile types of nanoparticles due to their applications in chemistry, electronics, medicine, and pharmaceutical sciences [1]. Among them, the gold nanoparticles (AuNPs) stand out for their advantages such as biocompatibility, tunable optical properties, and easily changed surface chemistry [2,3]. Because of these unique physical–chemical properties, the AuNPs are widely used as carriers of drugs and molecules to improve the diagnosis and treatment of diseases [4,5]. The synthesis of AuNPs through chemical and physical routes has been already well-established. However, these pathways generally use toxic substances and non-polar solvents, which generate hazardous impacts for the environment and requires various steps of product purification, resulting in an expensive process [6]. For overcoming the challenges related to conventional methods, a biosynthetic route has been proposed in the literature [7]. The green synthesis

uses natural compounds from plants or microorganisms (e.g., fungi, bacteria, algae) as precursors of the reaction of gold ions reduction [8,9]. Biosynthesis is considered a simple, low-cost, and eco-friendly approach since it uses non-toxic solvents, such as water [10]. The production of metallic nanoparticles using natural sources has already been reported in the literature, showing it is a potential synthetic route that should be explored [11,12]. Plant extracts are complex mixtures providing a rich arsenal of molecules with high redox potential [13], such as flavanones, flavones, flavanols and chalcones, fatty acids, amino acids, terpenoids, aldehydes, and alcohols [14]. Furthermore, biogenic synthesis produces large amounts of highly stable nanoparticles with a better-defined size than some conventional methods since phytochemicals compounds that are used in the reaction also act as stabilizing agents [15,16].

The large surface area and high proportion of surface atoms of metal nanoparticles make them extremely important. A great deal of study has been done on metal nanoparticles because of their unique chemical and physical characteristics and their scientific and technological value. Specifically, gold nanoparticles find application in biosensing [17], catalysis [18], electronics [19], enzyme electrodes [20], super conductors [21], and cancer treatment [22], among other industries.

Due to its cutting-edge nature and the effectiveness of produced nanoparticles in industrial, biomedical, and electronic applications like bioimaging [23], cancer detection [24], and catalyst [25], biological nanoscience has recently attracted a growing amount of attention. Gold nanoparticles are among the many metal nanoparticles that have a particularly broad range of applications in DNA identification, genetic medicine, and nano-catalysis. It has been noted that these nanoparticles size determines their different capacities [26, 27, 28].

Various techniques have been developed to produce noble metal nanoparticles with specified sizes and shapes based on desired outcomes. Because of the rising need to create environmentally benign technologies for material production, biosynthesis of nanoparticles has emerged as a highlight of the junction of biotechnology and nanotechnology. Because they are not biocompatible, biomolecules as reductants are shown to have a considerable advantage over chemical reductants [29].

This article discusses a straightforward biosynthetic technique for creating gold nanoparticles using *Pyracantha crenulata* leaf extract. Standard procedures were utilized to characterize the recently produced gold nanoparticles. *Pyracantha crenulata*, sometimes referred to as the ghingharu plant, is a plant with several medical uses, such as antibacterial, antiseptic, and anti-inflammatory properties. In fact, it is anticipated that gold nanoparticles made from *Pyracantha crenulata* plant leaves would have therapeutic effects. Consequently, attempts are performed to determine the therapeutic efficacy of the recently produced gold nanoparticles. Their potential to prevent liver cancer has so been investigated. Following cardiovascular illnesses, cancer is the world's biggest cause of mortality [30]. One common form of cancer that affects a sizable portion of the population is liver cancer. It has been researched and successful to use clinical trials to find novel medications and therapeutic approaches. However, side effects and the emergence of acquired drug resistance are major drawbacks of current therapies [31]. As a result, in addition to needing to be more potent and have fewer adverse effects, the new therapeutic agents also need to function via a different mechanism than the cytotoxic drugs now in use.

Pyracantha crenulata D. Don commonly known as "**Ghingharu**" belongs to Rosaceae family is evergreen, spinescent, under shrubs, or shrubs, to 5 m high, spines straight, stout, 1.5-1.3 cm long, bark ashy grey, leaves crowded at the ends of short lateral branches, shortly petioled, narrowly oblong or oblong- lanceolate, 1.5-3 cm long, crenate, apex, obtuse, shining green above, glaucous beneath [32]. It is widely distributed in mountain region of Himalaya from Himachal to Bhutan, Tibet, Myanmar, and China. The plant's fruits are helpful in treating hypertension and myocardial weakening since they contain cardiotonic qualities. Fruits are said to have hypotensive, coronary vasodilator, and cardio tonic qualities. Burger's illnesses, paroxysmal tachycardia, heart failure, and myocardial weakening have all been treated with it. Many phytochemicals like tannis, saponins [33], cyanidin, polymeric flavone, rutine, glycosides, cyanogenetic glycosides compounds were isolated from the *Pyracantha crenulata* plant extract. *Pyracantha crenulata* exhibited a variety of biological activities like antioxidant [34], antiproliferative [35], antiurolithogenic [36], anti-inflammatory activities [37, 38].

This plant's fruit has been utilized in Garhwal folk and traditional medicine to cure a number of significant illnesses, including circulatory system problems, diabetes, hypertension, heart problems, and angina [39]. In addition to being used to produce herbal tea, the leaves have been shown to have anti-inflammatory, immunomodulatory, and antioxidant properties. The orange-red pome fruit has a high sugar content [40].

Food ingredients that are utilized as nutraceuticals include dietary fibers, some phytochemicals, minerals, vitamins, probiotics, perbiotics, and polyunsaturated fatty acids [41]. According to this investigation, the fruits had a higher potency of nutrients and antimicrobials than grown fruits with berries, and 500 gm of fruits had all the nutrients an individual needs each day. April to May is when the flowers bloom, and June to September is when the fruits mature. Phytochemical constituents were investigated and the several new and known compounds namely, β- Sitosterol (a), (cis)-3,4-Dioxyethylene-5-methoxycinnamic acid (b), Cranulanostenoic acid (c) Pyracrenic acid (d)were reported from the methanolic extract of *Pyracantha crenulata* [42, 43].

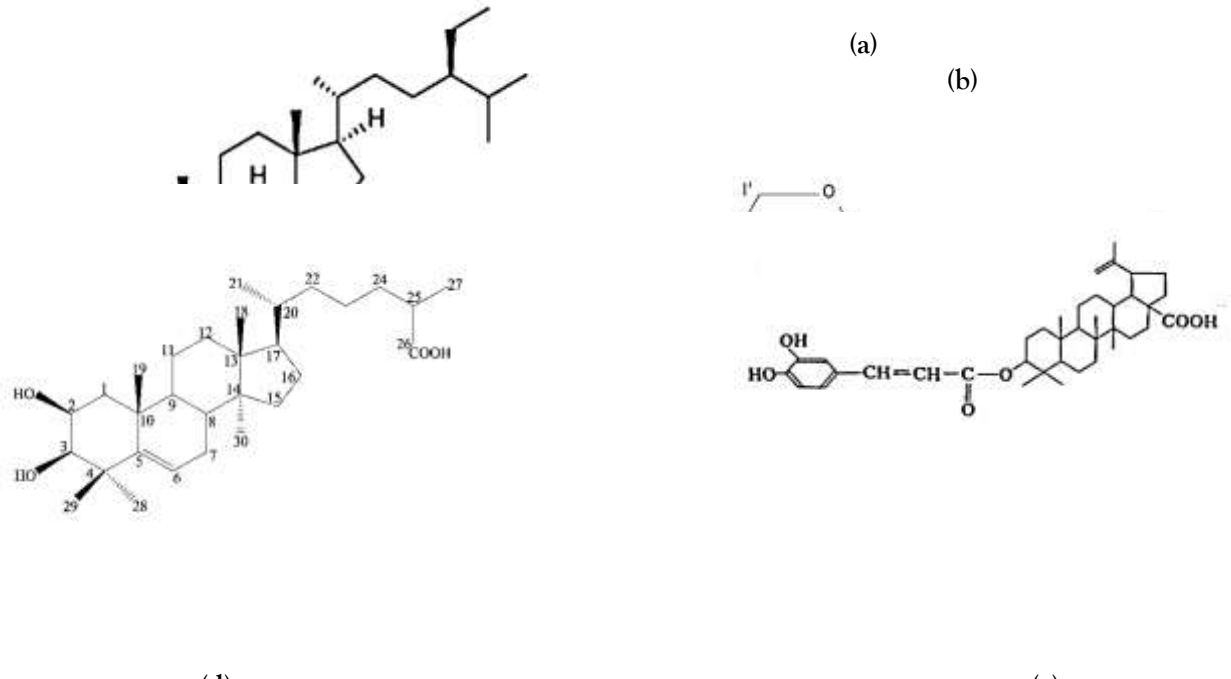

Figure 1: *Structure of several compounds isolated from Pyracantha crenulata: β -Sitosterol (a), (cis)-3,4-Dioxyethylene-5-methoxycinnamic acid (b), Cranulanostenoic acid (c) Pyracrenic acid (d)*

Figure 2: *Pyracantha crenulata*

Figure 3: *Dried leaves part of Pyracantha crenulata*

S. No.	Extracts of plants	Size and shape of nanoparticles	References
1.	Mango	6.03-18 nm; spherical	[44]
2.	*Gymnocladus assamicus*	4-22 nm; hexagonal, pentagonal and triangular	[45]
3.	*Cacumen Platycladi*	variable	[46]
4.	*Pogestemon benghalensis*	13.07 nm; cubic	[47]
5.	Coriander	6.75-57.91 nm; spherical	[48]
6.	*Nerium oleander*	2-10 nm; spherical	[49]
7.	*Butea monosperma*	10-100 nm; spherical, triangular	[50]
8.	Pea nut	110 to 130 nm; variable	[51]
9.	*Solanum nigrum*	50 nm; spherical	[52]
10.	*Hibiscus cannabinus*	10-13 nm; spherical	[53]
11.	*Sesbania grandilora*	7-34 nm; spherical	[54]
12.	*Pyracantha crenulata*	38 nm; spherical	Present Work

Table 1: *Synthesis of gold nanoparticles using extracts of different plants.*

METHODOLOGY

Resources and Methods obtaining and confirming plant samples. The Herbarium Forest Research Institute confirmed that the *Pyracantha crenulata* leaves were authentic, having been obtained from the Nagdev forest range in Pauri, Uttarakhand.

Preparation of leaves extract

To get rid of any remaining dirt, fresh, healthy *Pyracantha crenulata* leaves were properly cleansed in double-distilled water. The leaves had dried in the shade for fifteen days until they attained their steady weight. After crushing the dried leaves using a crusher and pestle, 10 g of *Pyracantha crenulata* was

added to 500 ml of double-distilled water in a 500 ml Erlenmeyer conical flask and heated to 70 °C for 20 minutes. Following a period of cooling to room temperature, the extract was filtered using Whatman filter paper no. 1 in a separate conical flask.

Figure 4: *Plant extract of Pyracantha crenulata*

Synthesis of gold nanoparticles using plant extract

Fresh/dried *Pyracantha crenulata* leaf was washed several times with deionized water. 10 g of *Pyracantha crenulata* leaves was cut and boiled with 500 ml de-ionized water and filtered to get the extract. The extract was filtered and stored at 4° C for further experiments. The extract is used as both reducing and stabilizing agent. Gold tetra choloroauric acid trihydrate ($HAuCl_4.3H_2O$) was purchased from Uttarakhand educational material centre and used without further purification. De-ionized water was used throughout the experiments. Synthesis of gold nanoparticles involved the mixing of aliquot amounts of gold chloroauric acid trihydrate, and *Pyracantha crenulata* extract in water. *Pyracantha crenulata* leaf extract and 1 mM aqueous $HAuCl_4.3H_2O$ solution were combined in a 1:9 ratio in a 2 L Erlenmeyer flask. It was kept for 72 hours in a dark place. Rapid reduction takes place and is complete in 10 min by visual color change from light yellow to stable violet color. After centrifuging the mixture for 20 minutes at 7500 rpm to remove impurities, it was washed with distilled water and acetone and it was then dried at 50 °C for 20 hours in an oven in order to characterize the gold nanoparticles and their anti-cancer effects. The final substance was crushed up in a mortar and pestle to create finely powdered golden blackish color nanoparticles.

Figure 5: *Plant parts extract and tetra chloroauric acid trihydrate solution mixture after 10 minutes and 72 h: 1:9 Pyracantha crenulata leaf extract and tetra chloroauric acid trihydrate solation mixture*

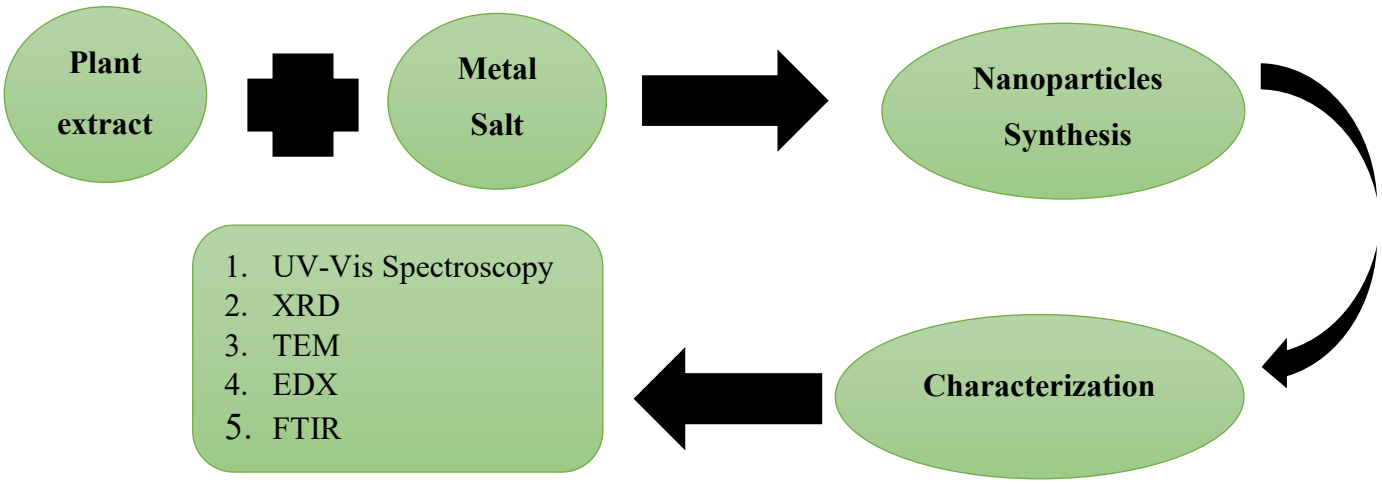

Figure 6: *General method of synthesis and characterization of nanoparticles using plants*

Characterization

The bioreduction of the $AuCl_4^-$ ions in solution was monitored by measuring the UV-vis spectra of the solution in quartz cuvettes with Varian, Cary 100 UV-Vis spectrophotometer in the range of 200–800. The morphology of the nanoparticles was analyzed using the images obtained with a Philips CM30 transmission electron microscope. Gold nanoparticles were characterized with in order to carry out the energy dispersive X-ray analysis (EDAX), analyses were performed on a MIRA3-LMU FE-SEM instrument equipped with a Thermo EDAX attachment. The FT-IR spectra were obtained on a Bruker FTIR instrument model tensor 27 with the sample as KBr pellets. X-ray diffraction pattern of dry nanoparticle powder was obtained using an X'Pert-Pro diffractometer manufactured by PAN analytical with monochromatized Cu Kα radiation (l = 1.54 Å).

RESULT AND DISCUSSION

In the current study, gold nanoparticles were produced using an aqueous leaf extract of *Pyracantha crenulata*.

UV-Visible Analysis

A fundamental aspect of nanoscience is the creation of nanomaterials with unique optoelectronic and physio-chemical properties. The ethnopharmacologically significant *Pyracantha crenulata* leaf extract's bioreduction ability of gold ions into gold nanoparticles is revealed in the current study. Gold nanoparticle synthesis was accomplished in 5 minutes of reaction time without the need for any experimental procedures like heating, stirring, or pH changes. Using *Pyracantha crenulata* leaf extract, newly produced gold nanoparticles were reduced and capped. The synthesis was effective with a concentration of 1:9 (100 mL of leaves extract and 900 mL of auric chloride) after thorough screening with various doses of auric chloride and leaves extract. The bioactive components of the leaf extract were essential to the production of gold nanoparticles. The solution changed from pale pink to dark pinkish purple, possibly due to the production of gold nanoparticles. The solution's broad absorbance peak at max 538 nm, whose estimated energy band gap is between 2.30 eV, confirmed the presence of gold nanoparticles in the mixture.

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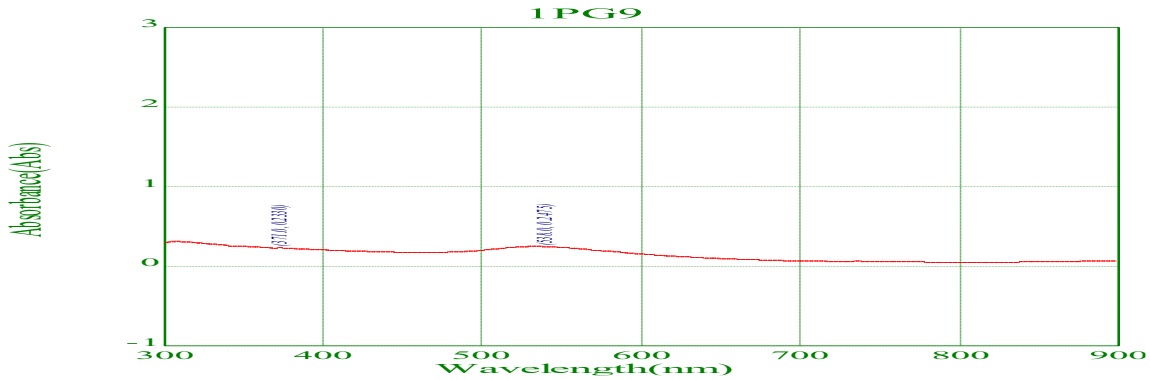


Figure 7: *UV-Visible spectra of AuNP's of Pyracantha crenulata leaves part*

XRD analysis

XRD was used to analyze the structure of gold nanoparticles that were made from the sample. Based on the angular orientations of the Bragg peaks, the gold nanoparticles were given a cubic shape. Thus, it is evident from the XRD pattern that *Pyracantha crenulata* generated crystalline gold nanoparticles. The average crystallite size of the gold nanoparticles was calculated using Debye-Scherrer's equation [29, 30, 31]. The crystalline nature of the gold nanoparticles was shown by the XRD spectrum. The synthesized nanoparticles XRD spectra showed sharp peaks at 2θ = 38.19°, 44.39°, 64.58°, 77.58°, and 81.73°, respectively. These peaks correspond to the lattice planes (111), (200), (220), (311), and (222), of cubic phase nano crystals.

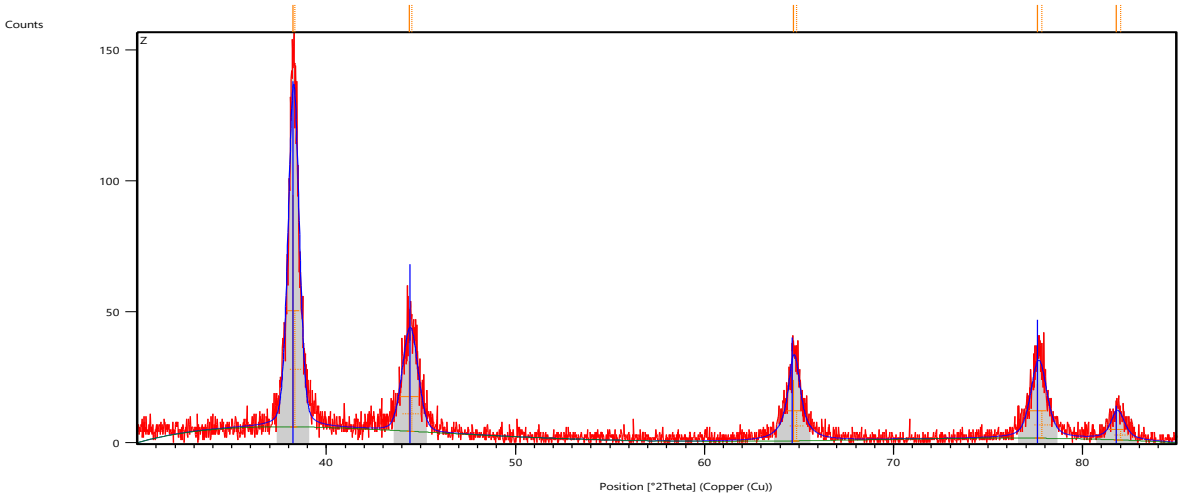


Figure 8: *XRD graph of synthesized AuNP's of Pyracantha crenulata leaves part*

TEM analysis

Through TEM analysis, the surface morphology of gold nanostructures was studied. These results validated the gold nanoparticles spherical form and average size of less than 38 nm. Lethal reducing agents are the primary means of producing metal nanoparticles. Several biomimetic strategies are being investigated for the creation of biocompatible nanoparticles. Undoubtedly, the expanding field of study on the use of nanoparticles in medicine reinforces the remarkable discoveries being made in this area. This makes the use of biologically safe nanoparticles necessary. With an emphasis on producing ecologically friendly nanoparticles, biological technologies may offer an alternative to traditional procedures. Nonetheless, the large-scale manufacturing is responsible for restrictions such pH shift and incubation. To get around the problems with microbial nanoparticle synthesis, greener technologies for nanoparticle production have promise. This study examines the biological creation of gold nanoparticles using an extract from the leaves of *Pyracantha crenulata*, a plant of therapeutic importance.

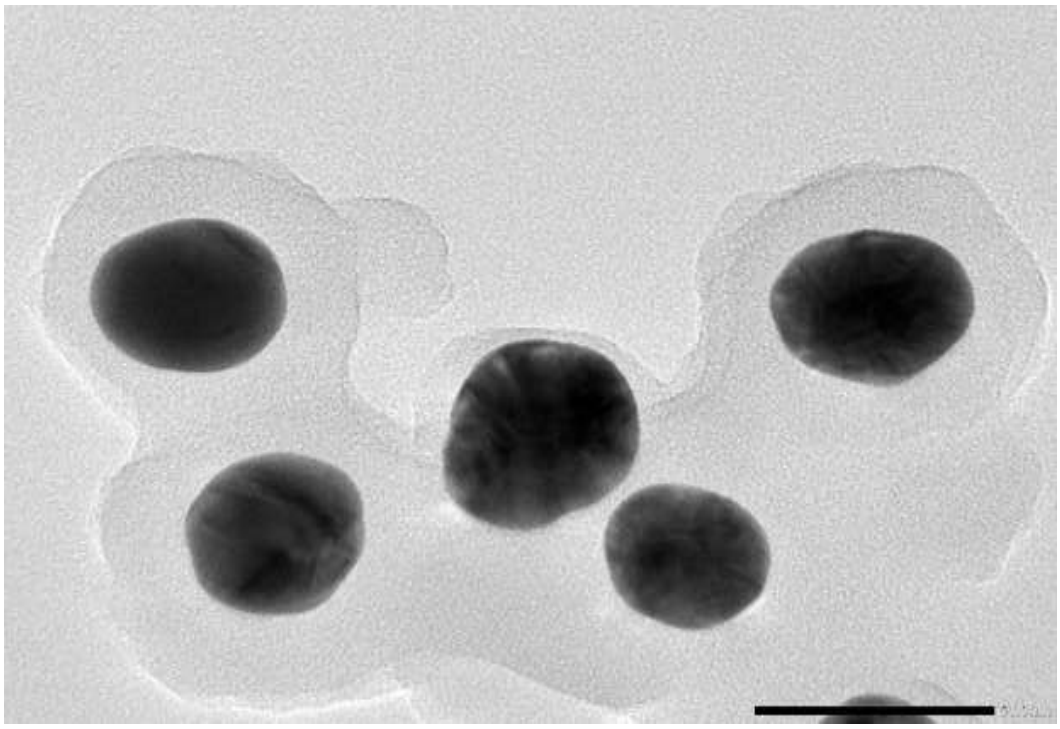


Figure 9: *TEM image of synthesized AuNP's of Pyracantha crenulata leaves part*

EDX analysis

EDX was utilized to ascertain the nanoparticles composition. A distinct signal in at around 2.1 keV confirms the presence of elemental gold, whereas a weak peak at 0.25 keV for carbon may have resulted from biomolecules bound to the surface of AuNPs. The extra signals observed in the spectra might be attributed to the bioactive compounds present in plant extract.

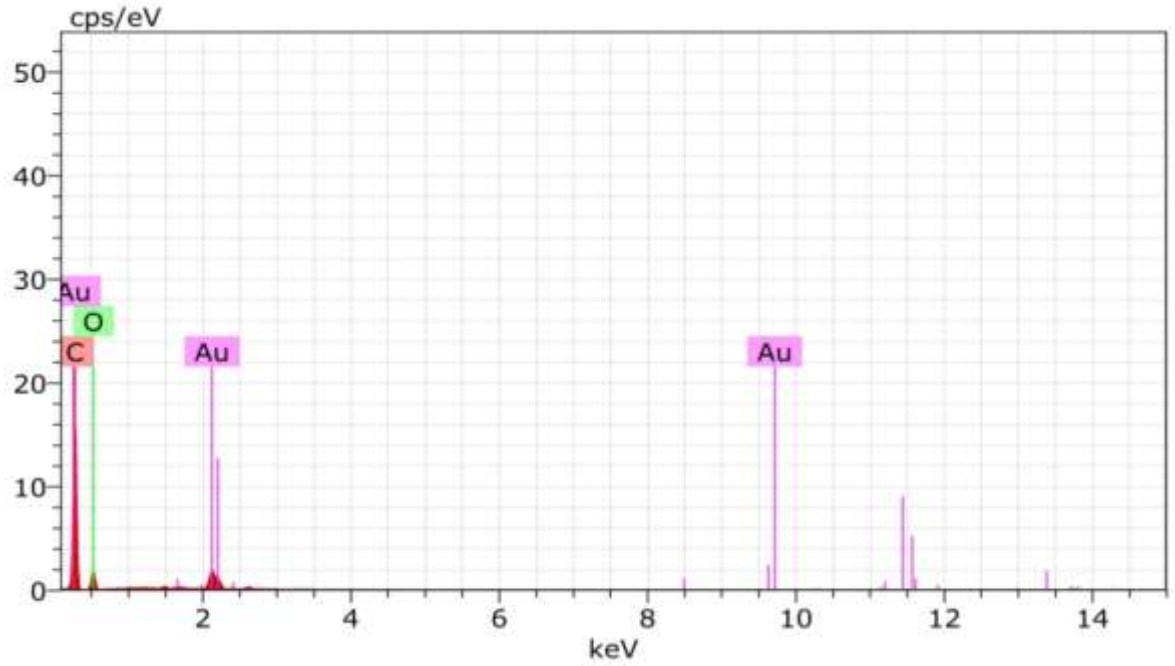


Figure 10: *EDX spectra of AuNP's of Pyracantha crenulata leaves part*

FTIR Analysis

Using Fourier transform infrared (FTIR) spectrum analysis, potential biomolecule functional groups that may have contributed to the creation of Au nanoparticles were found. Absorption peaks were

detected at 3377, 3009 and 2924 cm^{-1} in the FTIR spectrum. These peaks were associated with O-H stretch, C-H stretch in aromatic ring & Isocyanate stretch.

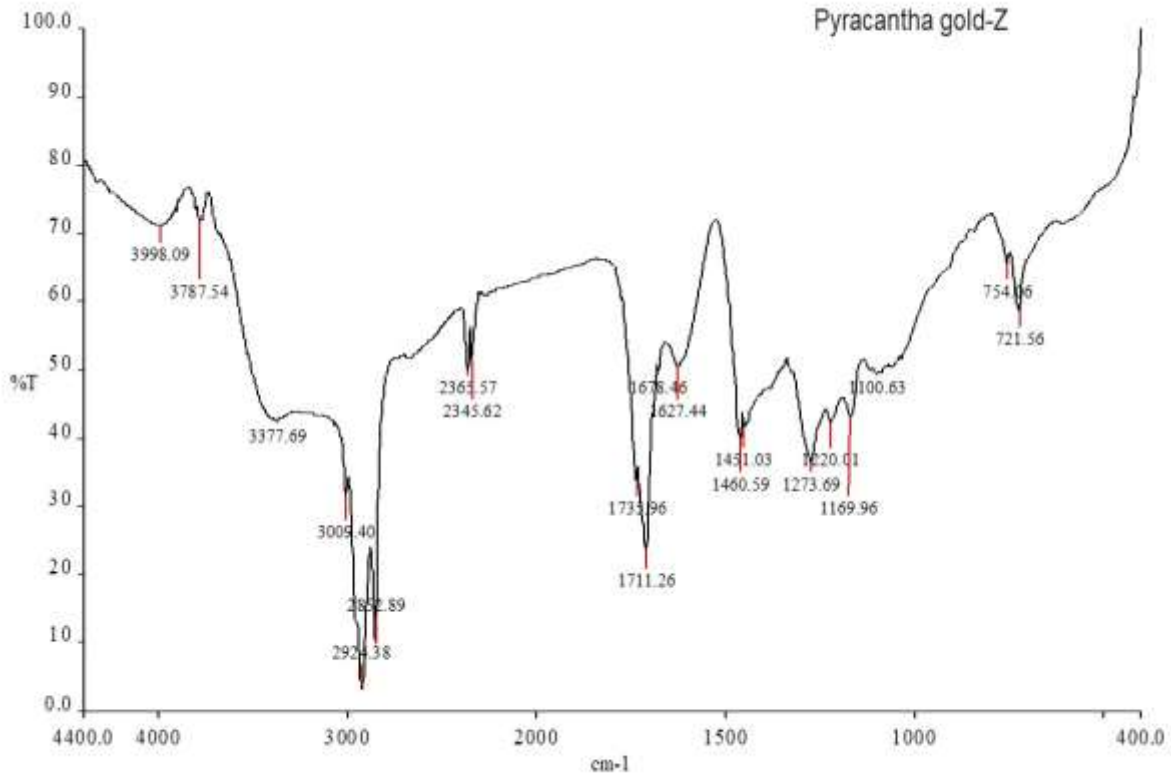


Figure 11: *FTIR spectra of synthesized AuNP's Pyracantha crenulata leaves part*

Invitro anticancer activity

A decrease in the rate of cell growth is shown by absorbance values that are less than those of the control cells. On the other hand, more cell proliferation is indicated by a greater absorbance rate. Hep-2 cell lines of liver were shown to have viable in vitro cells. Through the use of the trypan blue dye exclusion method, the percentage of viable cells was determined. The MTT test and the SRB assay were used to measure the cytotoxicity activity.

Trypan blue dye exclusion method was used to test the 85.12 cell viability of the liver (hep-2) cell line and the findings shown in **Figures 12** and **Tables 2**. After being treated with the dye, it was found that the viable cells had a pink color, while the non-viable cells displayed a blue color.

50% cytotoxicity was detected at 10 µg/ml for each of the cancer cell lines examined when the cytotoxicity assay of the nanoparticles was conducted against the liver (hep-2) cell line. Cyclophosphamide monohydrate, the standard positive medication, exhibited 50% cytotoxicity against the liver (hep-2) cell line at 5µg/ml. As a result, it was discovered that the IC_{50} values of cyclophosphamide monohydrate against the liver (hep-2) cell line were 5 µg/ml and 10 µg/ml, respectively, for *Pyracantha crenulata* gold nanoparticles and cancer cell lines. There were no cytotoxic values for DMSO. **Table 3** presents the findings.

Prior to the anticancer experiment, the cell concentration (number of cells) in both cancer cell lines was raised. The 20 µg/ml nanoparticles were tested using the MTT assay and the Sulphorodamine B assay, respectively, against the hep-2 cell line. *Pyracantha crenulata* gold nanoparticles were shown to be efficient against the cell line under investigation. The findings of Sulphorodamine B and MTT assay tests were discovered to be associated with one another.

By using the Sulphorodamine B test, it was discovered that *Pyracantha crenulata* gold nanoparticles (10 µg/ml) inhibited cancer cells in the liver (hep-2) cancer cell line by 75.02%, respectively. Cyclophosphamide monohydrate (5 µg), the positive control, demonstrated a 95.0% suppression of cancer cells in liver (hep-2) cancer cell lines, respectively. **Table 4** presents the findings.
liver (hep-2) cancer cell line by 72.23%. By using the MTT test, the positive control, cyclophosphamide monohydrate (5 mg), demonstrated 87% suppression of cancer cells in the liver (hep-2). **Table 5** presents the findings.

Cell lines	Percent viability	Live cell count	Total cell count	pH
Liver (Hep-2)	85.12	$1.42x10^5$	$2.5x10^5$	7.2

Table 2: *Percent cell viability and characterization of cell lines via Trypan blue assay (before anticancer assay)*

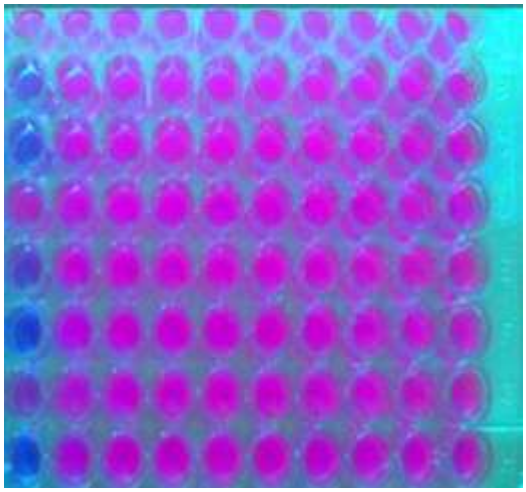


Figure 12: *Viability of cells in cancer cell lines in titter plate as determined by Trypan blue assay (Pink coloured wells shows viability of the cells; Blue coloured wells shows non-viable cells)*

Samples	IC50 values (µg/ml), Cancer cell lines used, Liver (Hep-2)
Cyclophosphamide monohydrate	5.0
Gold nanoparticles of *Pyracantha crenulata*	10.0
DMSO	0.0

Table 3: *Cytotoxicity assay of nanoparticles against Hepatic (Hep-2) cancer cell line (IC50 values determination)*

	Percent inhibition of cancer cells	
Sulphorodamine B assay In hepatic (hep-2) cell line	Gold nanoparticles of *Pyracantha crenulata* (10µg/ml)	Cyclophosphamide monohydrate (5 µg/ml)
	75.02	95.0

Table 4: *Sulphorodamine assay in hepatic (hep-2) cell line*

	Percent inhibition of cancer cells	
MTT assay In hepatic (hep-2) cell line	Gold nanoparticles of *Pyracantha crenulata* (10µg/ml)	Cyclophosphamide monohydrate (5 µg/ml)
	72.23	87.0

Table 5: *MTT assay in hepatic (hep-2) cell line*

Groups	Parameters evaluated					
	Tumor weight (g)	**Viable cell count (cellsx10^6/ml)**	**Non-viable cell count (cellsx10^6/ml)**	**RBC (cellsx10^6/µl)**	**WBC (cellsx10^6/µl)**	**Hemoglobin (g/dl)**
1st Group- Negative Control	2.23±0.86	3.45±0.67	0.55±0.85	5.67±0.94	7.12±0.88	10.58±0.65
2nd Group- Positive Control	0.52±0.53	2.56±0.48	0.67±0.53	4.54±0.75	5.57±0.64	8.37±0.48
3th Group- Test-B	0.78±0.43	1.78±0.23	2.34±0.25	5.58±0.51	6.23±0.43	13.15±0.27

Invivo anti-cancer activity

Using human cancer cell lines, namely liver (hep-2), the in vivo anticancer efficacy of gold *Pyracantha crenulata* nanoparticles (10 µg/ml) was investigated in this study. The anticancer research was also carried out to assess many criteria, including tumor mass, viable and non-viable cell counts, red blood cell and white blood cell counts, and hemoglobin content. The current study's findings imply that the nanoparticles were successful in reducing the number of cancer cells in the livers of Swiss albino mice. Cyclophosphamide monohydrate (5 µg/ml) was used as a positive control to compare the study outcomes. As investigated in the study, the hepatic cells of tumor development were further homogenized from animals (various groups: positive control, negative control, and treated). The hepatic (hep-2) cancer cell line negative control groups' tumor weight in the first group (negative control) was found to be 2.23 g. The tumor mass was determined to be 0.52 g in the second group and 0.78 g in the third.

Table 6: *Parameters determined in different sets of animal models for evaluation of anticancer activity*
* ±SD; p<0.5 (level of significance)
***Note: 1st Group- Negative Control -** Mice administered with Hepatic (Hep-2) cancer cell lines only;
2nd Group- Positive Control- Mice administered with Hep-2 cancer cell lines injected with Cyclophosphamide monohydrate (5 µg/ml);
3th Group- Test-B- Mice administered with Hepatic (Hep-2) cancer cell lines injected with gold nanoparticles of *Pyracantha crenulata* (10 µg/ml).

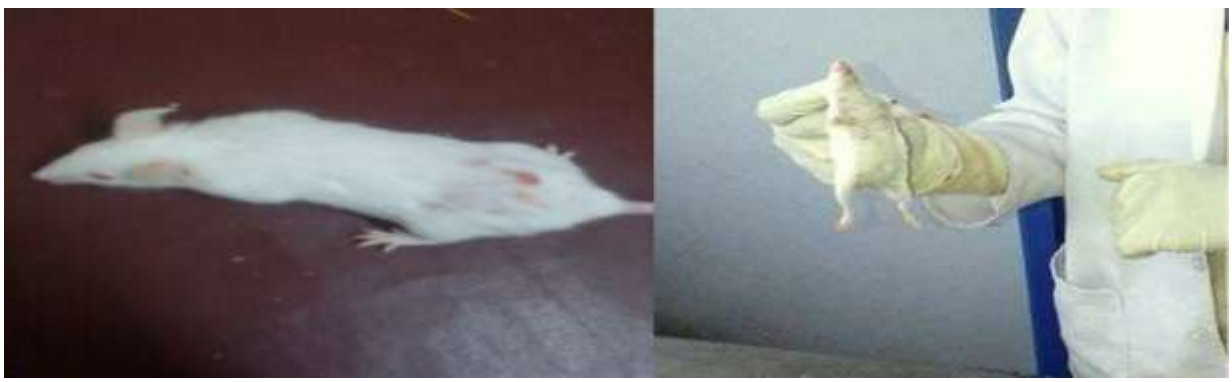


Figure 13(a): *Cancer induction of cell line (hep-2) in liver of Swiss albino rats.* ***13 (b):*** *Dosing of nanoparticles via oral route*

Invitro Anti-microbial Activity

In order to evaluate synthesized nanoparticles for anti-microbial potential, nanoparticles were tested against:

1. Two bacterial strain ***Bacillus subtilis* & *Escherichia coli*,**
2. Two fungal strains ***Candida albicans* & *Aspergillus oryzae***

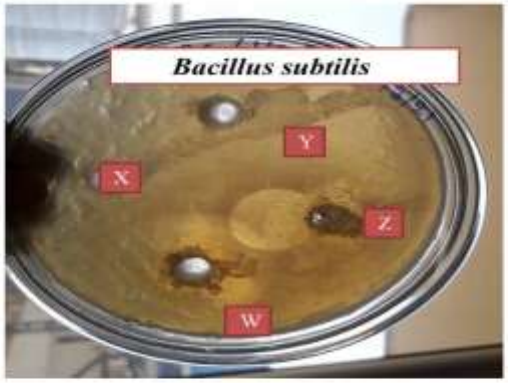
Standard antibiotic (Azithromycin and Fluconazole) was used as positive control in the present study and it was found that it effected on microbial growth and gave zone of inhibition. Au NPs of leaf extracts of *Pyracantha crenulata* showing the antibacterial results of Au nanoparticles synthesized from *Pyracantha crenulata* leaf extract. The clear zone of inhibition was observed against all the bacterial strains, with the minimum diameter of zone of inhibitions was observed for *Bacillus subtilis* (5.0 mm) while the maximum diameter of zone of inhibition was observed for *E. coli* (8.0 mm). Au NPs of leaf extracts of *Pyracantha crenulata* showing the antifungal results of Au nanoparticles synthesized from *Pyracantha crenulata* leaf extract. The clear zone of inhibition was observed against all the fungal strains, with the maximum diameter of zone of inhibitions was observed for *Aspergillus oryzae* (10 mm) while the minimum diameter of zone of inhibition was observed for *Candida albicans* (8 mm).

S. No.	Samples (100 µg/ml)	Diameter of zone of inhibition (mm)/Antimicrobial activity	
1.		*Bacillus subtilis*	*E. coli*
2.	Gold nanoparticles of *Pyracantha crenulata*	5.0	8.0
3.	Azithromycin	25.0	28.0

***Table 7**: Antimicrobial activity of gold nanoparticles against different bacterial strains.*

S. No.	Samples (100 µg/ml)	Diameter of zone of inhibition (mm)/Antimicrobial activity	
1.		*Aspergillus oryzae*	*Candida albicans*
2.	Gold nanoparticles of *Pyracantha crenulata*	10.0	8.0
3.	Fluconazole	18.0	28.0

***Table 8:** Antimicrobial activity of gold nanoparticles against different fungal strains.*



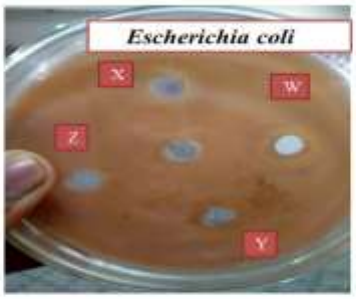
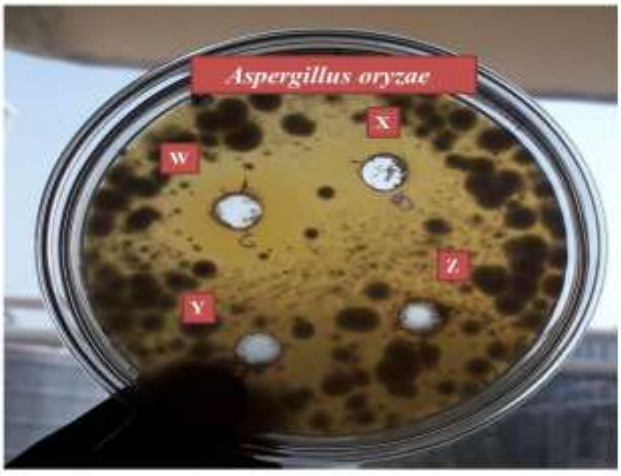


Figure 14: *Antimicrobial activity of Pyracantha crenulata (Z) of gold nanoparticles against different bacterial*



strains.

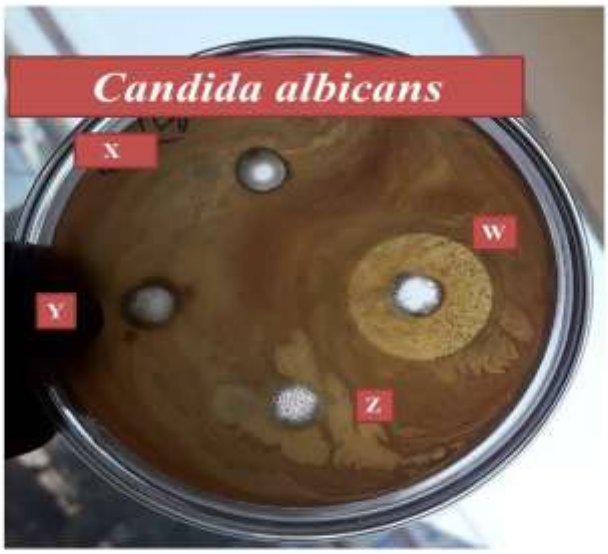


Figure 15: *Antimicrobial activity of Pyracantha crenulata (Z) of gold nanoparticles against different fungal strains*

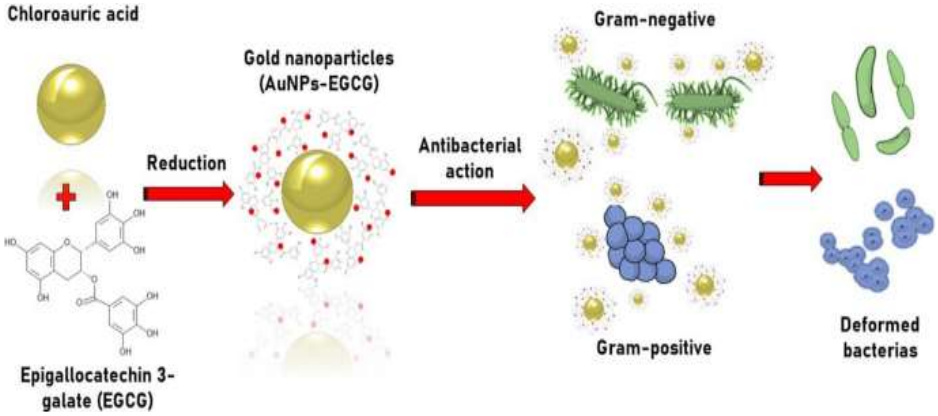


Figure 16*: Synthesis of gold nanoparticles (Avila et al., 2021).*

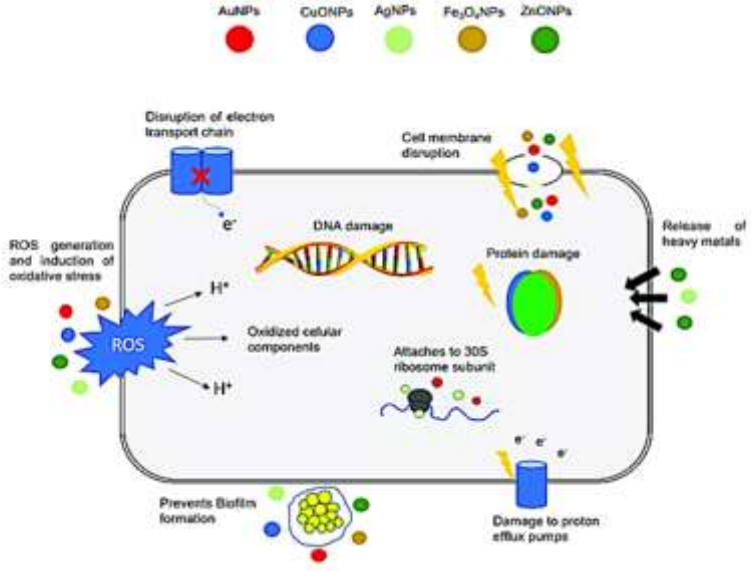


Figure 17: *Different mechanisms of action of NPs in bacterial cells: The combination in a single nanomaterial of a multitude of cellular effects may have a tremendous impact in fighting MDR bacteria. DNA, deoxyribonucleic*

acid; ROS, Reactive oxygen species; AuNPs, gold NPs; CuONPs, Copper oxide NPs; AgNPs, silver NPs; Fe_3O_4 NPs, iron oxide NPs; ZnONPs, zinc oxide NPs (Baptista et al., 2018).

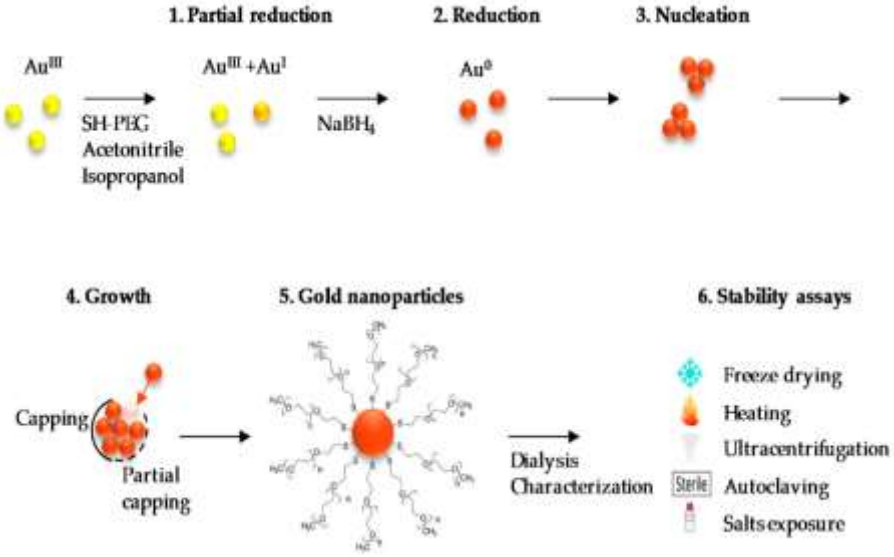


Figure 18: *Mechanism of formation of Au NPs: GNP synthesis (Masse et al., 2019)*

CONCLUSION

We successfully developed a green route to synthesis of colloid AuNPs through the aqueous leaf extract of *Pyracantha crenulata* as a potential renewable bioresource. The size, morphology and chemical composition of all nanoparticles were evaluated via a variety of techniques such as UV, TEM, XRD, EDX and FT-IR. By varying of reaction temperature, contact time and pH, the particle size and shape could be modulated and we could achieve nearly spherical nanoparticles with an average size of 38 nm. The inhibitory potencies of AuNPs of *Pyracantha crenulata* extract against anticancer and antimicrobial activities were studied and the results showed that nanoparticles have a stronger anticancer and antimicrobial activities. These results suggest that *Pyracantha crenulata* GNPs also exhibit a dual effect against anticancer and antimicrobial activities. Hence, we have established *Pyracantha crenulata* GNPs as a potentially valuable liver cancer inhibitor. Cytotoxic activity testing revealed that green nanoparticles showed effective activity against hepatic (Hep-2) cancer cell line in a dose dependent manner.

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Funding Declaration

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical standards This article does not contain any studies involving human or animal subjects.

Informed consent: Not applicable

REFERENCES

1. Murphy, C. J., Sau, T. K., Gole, A. M., Orendorff, C. J., Gao, J., Gou, L., ... & Li, T. (2005). Anisotropic metal nanoparticles: synthesis, assembly, and optical applications. *The Journal of Physical Chemistry B*, *109*(29), 13857-13870.

2. Sun, Y., Wang, Q., Chen, J., Liu, L., Ding, L., Shen, M., ... & Duan, Y. (2017). Temperature-sensitive gold nanoparticle-coated pluronic-PLL nanoparticles for drug delivery and chemo-photothermal therapy. *Theranostics*, *7*(18), 4424.

3. Nguyen, T. T., Mammeri, F. & Ammar, S. (2018) Iron oxide and gold based magneto-plasmonic nanostructures for medical applications: A review. Nanomaterials 8, 149.
4. Fazal, S., Jayasree, A., Sasidharan, S., Koyakutty, M., Nair, S. V., & Menon, D. (2014). Green synthesis of anisotropic gold nanoparticles for photothermal therapy of cancer. *ACS applied materials & interfaces*, *6*(11), 8080-8089.
5. Wang, C., Singh, P., Kim, Y. J., Mathiyalagan, R., Myagmarjav, D., Wang, D., ... & Yang, D. C. (2016). Characterization and antimicrobial application of biosynthesized gold and silver nanoparticles by using Microbacterium resistens. *Artificial Cells, Nanomedicine, and Biotechnology*, *44*(7), 1714-1721.
6. Khan, M. Z. H., Tareq, F. K., Hossen, M. A. & Roki, M. N. A. M. (2018) Green synthesis and characterization of silver nanoparticles using Coriandrum sativum leaf extract. J. Eng. Sci. Technol. 13, 158–166.
7. Ovais, M., Khalil, A. T., Raza, A., Islam, N. U., Ayaz, M., Saravanan, M., ... & Shinwari, Z. K. (2018). Multifunctional theranostic applications of biocompatible green-synthesized colloidal nanoparticles. *Applied microbiology and biotechnology*, *102*(10), 4393-4408.
8. Veisi, H., Farokhi, M., Hamelian, M. & Hemmati, S. (2018) Green synthesis of Au nanoparticles using an aqueous extract of Stachys lavandulifolia and their catalytic performance for alkyne/aldehyde/amine A3 coupling reactions. RSC Adv. 8, 38186–38195.
9. Mukherjee, S., Chowdhury, D., Kotcherlakota, R., Patra, S., Bhadra, M. P., Sreedhar, B., & Patra, C. R. (2014). Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system). *Theranostics*, *4*(3), 316.
10. Benedec, D., Oniga, I., Cuibus, F., Sevastre, B., Stiufiuc, G., Duma, M., ... & Lucaciu, C. M. (2018). Origanum vulgare mediated green synthesis of biocompatible gold nanoparticles simultaneously possessing plasmonic, antioxidant and antimicrobial properties. *International Journal of Nanomedicine*, 1041-1058.
11. Francis, S., Joseph, S., Koshy, E. P. & Mathew, B. (2017) Green synthesis and characterization of gold and silver nanoparticles using Mussaenda glabrata leaf extract and their environmental applications to dye degradation. Environ. Sci. Pollut. Res. 24, 17347–17357.
12. Cruz, D. M., Tien-Street, W., Zhang, B., Huang, X., Crua, A. V., Nieto-Argüello, A., ... & Webster, T. J. (2019). Citric juice-mediated synthesis of tellurium nanoparticles with antimicrobial and anticancer properties. *Green chemistry*, *21*(8), 1982-1998.
13. Mohammadinejad, R., Shavandi, A., Raie, D. S., Sangeetha, J., Soleimani, M., Hajibehzad, S. S., ... & Varma, R. S. (2019). Plant molecular farming: production of metallic nanoparticles and therapeutic proteins using green factories. *Green chemistry*, *21*(8), 1845-1865.
14. Borase, H. P., Salunke, B. K., Salunkhe, R. B., Patil, C. D., Hallsworth, J. E., Kim, B. S., & Patil, S. V. (2014). Plant extract: a promising biomatrix for ecofriendly, controlled synthesis of silver nanoparticles. *Applied biochemistry and biotechnology*, *173*(1), 1-29.
15. Iravani, S. (2011) Green synthesis of metal nanoparticles using plants. Green Chem. 13, 2638–2650.
16. Mittal, A. K., Chisti, Y. & Banerjee, U. C. (2013) Synthesis of metallic nanoparticles using plant extracts. Biotechnol. Adv. 31, 346–356.
17. Mirkin, Chad A., Robert L. Letsinger, Robert C. Mucic, and James J. Storhoff. (2020) "A DNA-based method for rationally assembling nanoparticles into macroscopic materials." In *Spherical Nucleic Acids*, pp. 3-11. Jenny Stanford Publishing.
18. Sen, I. K., Maity, K., & Islam, S. S. (2013) Green synthesis of gold nanoparticles using a glucan of an edible mushroom and study of catalytic activity. *Carbohydrate polymers*, *91*(2), 518-528.
19. Rao CNR, Cheetham AK (2001) Science and technology of nanomaterials: current state and future prospects. *J Mater Chem*. 11:2887–2894.
20. Crumbliss AL, Perine SC, Stonehuerner J, Tubergen KR, Zhao J, Henkens RW, O'Daly JP (1992) Colloidal gold as biocompatible immobilization matrix suitable for the fabrication of enzyme electrodes by electrodeposition. *Biotechnol Bioeng* 40:483–490.
21. Sun Y, Xia Y (2002) shape-controlled synthesis of gold and silver nanoparticles. *Science* 298:2176–2179.
22. EI-Sayed IH, Huang X, EI-Sayed MA (2006) Selective laser photothermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. Cancer Lett 239(1):129– 135.
23. Wu YL, Li YN, Liu P, Gardner S, Ong BS (2006) Studies of gold nanoparticles as precursors to printed conductive features for thinfilm transistors. *Chem Mater* 18:4627–46.
24. Huang XH, Jain PK, EI-Sayed IH, EI-Sayed MA (2007) Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostic and therapy. *Nanomedicine* 2:681–693.
25. Hashmi ASK, Hutchings GJ (2006) Gold catalysis. *Angew Chem Int* Ed 45:7896–7936.
26. Alivisatos AP (1996) Perspectives on the physical chemistry of semiconductor nanocrystals. *J Phys Chem*100:13226–13239 and its catalyic acivity. Mater Let 108: 276-279.
27. Jin, R., Cao, Y., Mirkin, C. A., Kelly, K. L., Schatz, G. C., & Zheng, J. G (2001) Photoinduced conversion of silver nanospheres to nanoprisms. *Science*, *294*(5548), 1901-1903.
28. Aizpurua J, Hanarp P, Sutherland DS, Kall M, Bryant GW, Garcia de Abajo FJ (2003) Optical properties of gold nanorings. *Phys Rev Lett* 90:057401-1–057401-4 298-309.
29. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Shao W, He N, Hong J, Chen C (2007) Biosynthesis of silver and gold nanoparticles by using novel sun-dried *Cinnamomum camphora* leaves. *Nanotechnol* 18:105104–105114.
30. Garrett MD, Workman P. (1999) Discovering novel chemotherapeutic drugs for the third millennium. *Eur J Cancer* 35(14):2010–2030.
31. Robert J, Jarry C. (2003) Multidrug resistance reversal agents. *J Med Chem* 46(23):4805–4817.

32. Gaur, R. D (1999) Flora of the District Garhwal, North West Himalaya. *Transmedia*.
33. Saklani, Sarla, and Subhash Chandra (2014) "In vitro antimicrobial activity, nutritional value, antinutritional value and phytochemical screening of *Pyracantha crenulata* fruit." *International Journal of Pharmaceutical Sciences Review and Research* 26.1: 1-5.
34. Guglani, A., Pandey, H. K., Arya, R. K. K., & Gaddam, B (2022) The Nutritional Profile, Phytochemical Investigation and In Vitro Antioxidant Activity of Leaves Extract of *Pyracantha crenulata* Collected from Middle Hill Climatic Conditions of Western Himalayas. *Indian Journal of Pharmaceutical Sciences*, *84*(1).
35. Singh, Himani, Madhuri Kaushish Lily, and Koushalya Dangwal (2015) "Evaluation and comparison of polyphenols and bioactivities of wild edible fruits of North-West Himalaya, India." *Asian Pacific Journal of Tropical Disease* 5.11: 888-893.
36. Bahuguna, Y. M., Rawat, M. S. M., Juyal, V., & Gusain, K (2009) Evaluation of *Pyracantha crenulata* Roem for antiurolithogenic activity in albino rats. *African journal of urology*, *15*(3).
37. OTSUKA, HIROSHI, SHOJI FUJIOKA, TAKEYA KOMIYA, MINORU GOTO, YASUZO HIRAMATSU, and HAJIME FUJIMURA (1981) "Studies on anti-inflammatory agents. V. A new anti-inflammatory constituent of *Pyracantha crenulata* Roem." *Chemical and Pharmaceutical Bulletin* 29, no. 11: 3099-3104.
38. Bahuguna, Y. O. G. E. N. D. R., and K. A. I. L. A. S. H. Rawat (2014) "Evaluation of anti-inflammatory effect of *Pyracantha crenulata* (D. Don) M. Roemer by in-vitro methods." *Pharmanest Int J Adv Pharm Sci* 5.4: 2248-51.
39. Spectrum, (2010). Science Reporter, Himalaya Red Berry- Wonder Heart Tonic, September, 16-18.
40. R. S. Pal, R. A. Kumar. (2013). Antioxidant capacity and related phytochemicals analysis of methanolic extract of two wild edible fruits from north westen Indian Himalaya, 4(2), April, 113-123.
41. Sarla Saklani, Subhash Chandra, (2011). Evaluation of nutritional profile, medicinal value and qualitative estimation in different parts of *Pyrus pashia, Ficus palmata* and *Pyracantha crenulata, Journal of Global Trends in Pharmaceutical Science*, 2, July-Sept, 350-354.
42. Sultana, Shahnaz, Mohammed Ali, and Showkat Rasool Mir. (2017). "Cinnamic acid and lanostenoic acid derivatives from the leaves of *Pyracantha crenulata* (D. Don) M. Roem." *Journal of Pharmaceutical and Biological Sciences* 5.3: 91.
43. OTSUKA, HIROSHI, SHOJI FUJIOKA, TAKEYA KOMIYA, MINORU GOTO, YASUZO HIRAMATSU, and HAJIME FUJIMURA. (1981). "Studies on anti-inflammatory agents. V. A new anti-inflammatory constituent of *Pyracantha crenulata* Roem." *Chemical and Pharmaceutical Bulletin* 29, no. 11: 3099-3104.
44. Yang N, WeiHong L, Hao L. (2014) Biosynthesis of Au nanoparicles using agricultural waste mango peel extract and its in vitro cytotoxic efect on two normal cells. *Mater Let* 134: 67-70.
45. Tamuly C, Hazarika M, Bordoloi M. (2013) Biosynthesis of Au nanoparicles by *Gymnocladus assamicus* and its catalyic acivity. Mater Let 108: 276-279.
46. Wu, Weiwei, Jiale Huang, Lingfeng Wu, Daohua Sun, Liqin Lin, Yao Zhou, Haitao Wang, and Qingbiao Li. (2013) "Two-step size-and shape-separation of biosynthesized gold nanoparticles." *Separation and purification technology* 106: 117-122.
47. Paul B, Bhuyan B, Dhar Purkayastha D, Dey M, Dhar SS. (2015) Green synthesis of gold nanoparicles using *Pogestemon benghalensis* (B) O. Ktz. leaf extract and studies of their photocatalyic acivity in degradaion of methylene blue. *Mater Let* 148: 37-40.
48. Narayanan KB, Sakthivel N. (2008b) Coriander leaf mediated biosynthesis of gold nanoparicles. *Mater Let* 62: 4588-4590,.
49. Tahir, Kamran, Sadia Nazir, Baoshan Li, Arif Ullah Khan, Zia Ul Haq Khan, Peng Yu Gong, Shahab Ullah Khan, and Aftab Ahmad. (2015) "*Nerium oleander* leaves extract mediated synthesis of gold nanoparticles and its antioxidant activity." *Materials Letters* 156: 198-201.
50. Patra S, Mukherjee S, Barui AK, Ganguly A, Sreedhar B, et al., (2015) Green synthesis, characterizaion of gold and silver nanoparicles and their potenial applicaion for cancer therapeuics. *Mater Sci Eng* C 53: 298-309.
51. Raju D, Vishwakarma RK, Khan BM, Mehta UJ, Ahmad (2014) A Biological synthesis of cationic gold nanoparticles and binding of plasmid DNA. *Mater Let* 129: 159-161.
52. Muthuvel, A., Adavallan, K., Balamurugan, K., & Krishnakumar, N. (2014) Biosynthesis of gold nanoparticles using *Solanum nigrum* leaf extract and screening their free radical scavenging and antibacterial properties. *Biomedicine & Preventive Nutrition*, *4*(2), 325-332.
53. Bindhu MR, Vijaya Rekha P, Umamaheswari T, Umadevi M (2014) Anibacterial acivities of *Hibiscus cannabinus* stem-assisted silver and gold nanoparicles. *Mater Let* 131: 194-197.
54. Das J, Velusamy P (2014) Catalyic reducion of methylene blue using biogenic gold nanoparicles from *Sesbania grandilora L. J Taiwan Inst Chem Eng* 45: 2280-2285.
55. Avila, S.R.R., Schuenck, G.P.D., Silva, L.P.C.E., Keijok, W.J., Xavier, L.M., Endringer, D.C., Oliveira, J.P., Schuenck, R.P. and Guimaraes, M.C.C., (2021). High antibacterial in vitro performance of gold nanoparticles synthesized by epigallocatechin 3-gallate. *Journal of Materials Research*, *36*, pp.518-532.
56. Baptista, P. V., McCusker, M. P., Carvalho, A., Ferreira, D. A., Mohan, N. M., Martins, M., & Fernandes, A. R. (2018). Nano-strategies to fight multidrug resistant bacteria “A Battle of the Titans”. *Frontiers in microbiology*, *9*, 1441.
57. Masse, Florence, Pascale Desjardins, Mathieu Ouellette, Camille Couture, Mahmoud Mohamed Omar, Vincent Pernet, Sylvain Guérin, and Elodie Boisselier. (2019). "Synthesis of ultrastable gold nanoparticles as a new drug delivery system." *Molecules* 24, no. 16: 2929.