Synthesis and Characterization of Silver Nanoparticles using Green Route

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ABSTRACT: The motto of the work investigates the synthesis of silver nano-particles by simple and economic green synthesis. With aid of citrus fruit that is lemon peel extract under different conditions, Characterization of silver nano-particles was carried out by using ultra-violet visible spectroscopy, X-ray diffraction, Fourier transferred infrared spectroscopy. The antimicrobial activities of synthesized silver nano particles (AgNPs) characterized by well diffusion method (WDM). Lemon peel extract mediated silver nano particles showed significant antimicrobial activity and have attention of pharmacists in order to design medicines. The results of antimicrobial test on the lung cancer cell has been witness the synthesis of drugs using extracted lemon peel is emerged as an eco-friendly, cost effective and most attentive synthesis.

KEYWORDS: silver nano particles, Green synthesis, X-ray diffraction spectra, biological activity

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1. INTRODUCTION

Now a day's Nanotechnology is flourishing tremendously in each and every field of science and technology corresponding physical science, electronic [1], medical and chemical, drug delivery sciences [1-2]. The materials (or devices) evolving within nano size [3] nanoparticles possess unique electrical, optical, biological properties which are employed in bio sensing, catalysis, medicine, nano device fabrication and imaging drug delivery (Nair and Laurencin 2007). AgNPs have earned interest antimicrobial properties, [4] and representative as future generation antimicrobial agents [2-5]. Development of reliable and eco-friendly process for synthesis of metallic nanoparticles done by physical, chemical, and biological method, but biosynthesis of nanoparticles demonstrates immense potential using biological waste.

The chemical and physical methods are costly as they are lethal perilous chemicals which are directly damage the living organisms. Synthesis of silver and gold nanoparticles using biological methods [5-6] and metal nanoparticles can be synthesized by using various plants and also bio-organisms (bacteria, fungus, and yeast) reported [7-12].

Citrus lemon is a medicinal plant is rich in citric acid and ascorbic acid while flavonoids and polyphenols of disorders including headache

nervousness gastrointestinal disorders and rheumatism [13-14]. The peel is significant as reducing agents like bio reductive synthesis of metal nanoparticles citrus lemon like anticancer, antidiuretic, antioxidant, anti-inflammatory, antifungal, and antiviral activities [6].

The present study illustrates the bio fabrication using microwave assisted synthesis of silver nanoparticles from citrus fruit peel extracts (lemon peel extract) materials especially. Characterization of silver nanoparticles was done by using XRD, UV - visible spectroscopy, FTIR spectroscopy. After confirmation of silver nanoparticles an attempt has been made to compare the MTT and antimicrobial activity of AgNPs prepared from citrus fruit peel (lemon).

2. PREPARATION METHODS

2.1 Preparation of lemon peel and powder preparation

The *citrus sinensis* (citrus lemon) and citrus limetta were received from the market and lemon peel were removed, cleaned thoroughly in double distilled water. The lemon peels were kept for drying in microwave oven and the other is under sunlight also [9,13-14]. In microwave oven it was dried at 60 degree Celsius for five minutes and under sunlight it was dried for of 5- 6 days. The dried peels were powdered and extract develop for each type of lemon peels and mixed lemon peel

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extract was also prepared in both the cases. Nine grams of powdered peel of each type of citrus fruit was added to 300ml of double distilled water and heated in electric furnace for one hour at 90 degree Celsius and extract was filtered. Synthesis of silver nanoparticles (Shown in Fig.1) prepared by taking 2ml of 1M silver nitrate solution to 8ml of lemon peel extract and was kept under dark condition for 24 hours at room temperature [15-18].

Fig.1 Schematic diagram of proposed work

2.2 Synthesis of silver nano-particles: Biological method.

The fabrication of metal nanoparticles done by biological method in which extracts from biological agents (microbes and plants) can be engaged either as reducing or protective agent [18-20]. Microwave assisted synthesis is a promising method for synthesis of silver nanoparticles and better due to good yielding of nanostructures with smaller sizes, narrower size distributions and a higher degree of crystallization. Silver nanoparticles with different shapes can be synthesized by microwave irradiation of silver nitrate ethylene glycol hydrogen (platinum chloride) poly(vinylpyrrolidone) solution within three minutes. Moreover the use of microwave irradiation to produce mono dispersed silver nanoparticles using basic amino acids (as reducing agents).

We have employed factorial design "one factor at a time" was employed, varied at a time while all parameters remain the same, 30ml of freshly prepared 1mM silver nitrate solution was added to 5ml of lemon peel extract and then boiled for 10 minutes for the synthesis of silver nanoparticles. For maximum yield of silver nano- particles the pH, lemon peel extract volume at different times and concentration of silver nitrate was varied, thus optimize the conditions. The pH of 5ml lemon peel extract was adjusted as 6, 7,8,9 and co-separately then silver nitrate (30ml)(0.1M

concentration) was added and boiled for 30mins. Similarly in next step 1,3,5 and 7ml of lemon peel extract was mixed with silver nitrate solution (30ml) and boiled and then for similar 8ml of sample and 8ml of copper sulphate solution then heated in a microwave the color changes after 5minutes that is from pale yellow to brownish color. In the third and last step the silver nitrate concentration and copper sulphate solution was altered that is 0.5, 1, 2, 3nm silver nitrate and copper sulphate was used and added to 5ml and 7ml of extract separately and boiled stability of synthesized silver nanoparticles was checked at different days after the reaction and UV visible spectrometer was used to analysis the synthesis of silver nanoparticles and copper sulphate solution nanoparticles.

2.3 Purification and drying of silver nanoparticles of lemon peel extract.

The silver nanoparticles solution was kept in microwave oven at 60 degree Celsius for 5minutes process of microwaves drying was repeated two times. By immersing the resultant pellet in deionized water followed by immersion of washed pellet of silver nanoparticles and copper sulphate solution in methanol oil other conditions kept the same [21-22]. Alcohol was evaporated and dried powdered silver nanoparticles were carried for further analysis.

Fig.2. For synthesis of silver nanoparticles: Sample + silver nitrate solution + 5mins heat = pale yellow

Fig.3. Sample + copper sulphate solution + microwave heat at 5 mins = brownish colour

3. RESULTS AND DISCUSSION

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3.1 XRD, FT-IR and UV- visible spectroscopy analysis

Figure 4 XRD pattern of silver nanoparticles

Figure 4 shows the XRD pattern obtained for silver nano-particles synthesized using biological method (biosynthesis). Various Bragg reflections representing to the (111), (200), (220), (311), and sets of lattice planes are observed which correspond to 37.90° , 44.55° , 64.81° and 77.43° , 2θ values respectively. From the XRD studies the particle size was calculated by FWHM method and Tabulated in Table.No.1. Using Scherer's formula [23-24] the crystallite sizes of sample calculated (Eqn No.1)

$$D = \frac{K \cdot \lambda}{\beta \cdot \cos \theta} \text{ -----(1)}$$

It is found to be (28.27) to (13.41) nm. The obtained XRD data for 2θ positions identifies the sample as silver crystalline particles having (hkl) values matching to silver (FCC) and results are in good agreement with 43-0649 standard JCPDS. The average crystalline size of the silver nanoparticles using Debye-Scherrer's formula [23-24] is found to be 20 nm and lattice constant found to be $4.0676 \text{ \AA}$. XRD analysis has evidenced that well-defined silver nanoparticle by reduction of metal ions due to extract of lemon peel.

The micro- strain and dislocation density are important parameters of crystal. Dislocation density (ρ_D) is a evaluates of the number of dislocations in a unit volume of a crystalline material, which signifies one-dimensional crystalline defects marking the boundary between a slipped and an unslipped region of a material. The amount of the defects in the as deposited film was resolved by evaluating the dislocation density [7].

$$\text{Dislocation Density } (\rho_D) = \frac{1}{D^2} \text{ ---- (2)}$$

The micro strain in the sample is calculated by (equation 3)

$$\text{Micro-strain } (\epsilon) = \beta \cos \theta / 4 \text{ ---- (3)}$$

The dislocation density (ρ_D) of nano samples is $2.5 \times 10^{15} \text{ m}^{-2}$.

All the parameters like crystallite-size, strain, lattice parameter, volume and dislocation density tabulate in Table.2

Silver nanoparticles are soluble in double distilled water and the dark brownish coloration suggests the formation of silver nanoparticles was monitored by measuring the UV spectrum of the reaction medium after overnight incubation after diluting a small aliquot of the sample in double distilled water. The silver nanoparticles synthesized were analyzed with help of UV visible spectrometer with a range of 200nm - 600nm.

Fig.5. UV visible spectra of silver nanoparticles.

Fig. 5 depicts bio-reduction of Ag^+ ions (UV-visible spectroscopy) in aqueous extracts of citrus lemon. Its observed in the range of 330- 380nm and sharp peaks were observed at 330nm, 340nm, 350nm, 360nm, 370nm, 380nm for the silver nanoparticles at pH 7, 8 and 9 respectively. At pH 8 different volumes of lemon peel extract were also used and peak was examined just after the reaction. At pH 8 the absorption of silver nanoparticles was increased gradually for 1,3 and 5ml of lemon peel extract but decreased for 8ml of extract while at pH 9 the absorbance was increased sharply at 3ml and almost remains same for 5 and 8ml of extract [25].

In order to check this abnormal behaviour of 5ml and 8ml extract, we have varied the reaction conditions that different concentrations of silver nitrate solution. At pH 8 using 5ml and 8ml of extract the trend of silver nanoparticles synthesis was normally goes on enhancing as the concentration of silver nitrate increases. While at pH 9 the trend was irregular for 3mM silver nitrate solution initially silver nanoparticles yield

was increasing up to the 2mM concentrations of silver nitrate but yield was low at 3mM silver nitrate. The stability of silver nanoparticles was

also checked up to the 30days and it showed the gradual increase in absorption.

Table.1 Detailed XRD analysis of Silver nano particle

Sl no	2 θ	θ	cos θ	sin θ	FWHM	β radian	Crystalline size 'D' in nm	d_{cal} Å	d_{obs} Å	hkl	Lattice constant 'a' Å
1	37.90	18.95	0.9455	0.3255	0.341	0.0059	24.68	2.3683	2.356	111	4.0677
2	44.55	22.27	0.9253	0.3790	0.640	0.0111	13.41	2.0340	2.034	200	4.0638
3	64.81	32.40	0.8442	0.5359	0.603	0.0105	15.59	1.4385	1.429	220	4.0648
4	77.43	38.71	0.7803	0.6253	0.361	0.0063	28.27	1.2326	1.232	311	4.0740

Table. 2 Various parameters of silver Nano particle from XRD analysis

Sample	Crystal size (nm)	Micro strain	Lattice Constant Å	Volume (V) Å ³	Dislocation Density (m ⁻²)
Silver nano particle	20 nm	0.0158	4.0676Å ⁰	67.299	2.5 X10 ¹⁵

Fig.6. FT-IR spectroscopy of silver nano particles (AgNPs)

The extract (dried silver nanoparticles and lemon peel) was subjected to FT-IR, AgNPs prepared (Fig. 6). FTIR analysis helps in functional groups and stabilization of the bio reduced AgNPs, bands at 1591.88 cm⁻¹, 1393.30 cm⁻¹ and 1011.32 cm⁻¹ confirm the amide I, II and III bands [26]. The stretch mode of the carbonyl group coupled to the amide linkage (NH)-C=O represents amide band and amino acid residues and peptides have a strong affinity to bind with metals and act as encapsulating agent, thus forestalling the nanoparticles from agglomeration [27]. The band at 1591.88 cm⁻¹ due to methylene scissoring vibrations from the proteins and all three bands directed the presence of peptide moiety probably as capping agent in bio-reduced AgNPs and strong

absorption band at 3280.59 cm⁻¹ may be due to presence of polyphenolic hydroxyl group. The bands 1591.88 and 1393.30 cm⁻¹ are characteristics of amide II and are assigned to N-H stretching modes of vibration in the amide linkage all bands proposes the AgNPs in the aqueous medium, biological molecules performs dual functions of stabilization and reduction.

3.2. MTT and Kirby-Bauer Disk Diffusion Susceptibility Test results:

Fig.7. Microscopic images of MCF-7 cell line after treatment

The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability [28-30]. The reduction of tetrazolium salts to examine cell proliferation, using yellow

tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH[28-30].

Procedure:

The cells were trypsinized and aspirated into a 15ml centrifuge tube and cell pellet was obtained by centrifugation at 300 x g. Dulbecco's Modified Eagle Medium (DMEM) used to adjust cell count, such that 200µl of suspension confined approximately 10,000 cells. To each well of the 96 well microtitre plate, 200µl of the cell suspension was added and the plate was incubated at 310 K and 5% CO₂ atmosphere for 24 h. After 24 h, the spent medium was aspirated and 200µl of 20, 40, 60, 80 and 100 µg/ml from stock of test drugs were added to the respective wells. The plate was then incubated at 37°C and 5% CO₂ atmosphere for 24 h. The plate was removed from the incubator and the drug containing media was aspirated. 200µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and the plate was incubated at 37°C and 5% CO₂ atmosphere for 3 h. The culture medium was removed completely without disturbing the crystals formed. Then 100µl of Dimethyl sulfoxide (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. Using a microplate reader at two different wavelengths ($\lambda = 570\text{nm}$, 630 nm) absorbance was evaluated and percentage growth inhibition was computed, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) was yielded from the dose-response curve for the cell line.

Table 3. The IC₅₀ values of the test compounds for A549 cell-line for 24 hour treatment were found to be:

Sample name	A 549 cell line IC ₅₀ (in µg/ml) 24hr
AgNps	6.2275 µg/ml

The Inhibitory Concentration (IC₅₀) of the phyto-mediated AgNPs was recorded against MCF7 and 50 µg.ml⁻¹ A549 cells. For IC₅₀ values outside of the experimental concentration range taken, the values provided above are only expected values, re-testing may be required at the higher/lower concentration range for confirmation.

The synthesized silver nanoparticles and lemon peel extracts were compared and showed promising cytotoxicity activity against MCF-7 and A549 cell line. Thus, silver nanoparticles may

stimulate reactive oxygen species and effect in damage cellular components which lead to cell death. From the analysis, we can conclude that the silver nanoparticles prepared using lemon peel, possess high cytotoxicity activity against cell lines and intimates as high potential therapeutic.

Fig.8 Cytotoxicity evaluation of lemon peel

extract and biosynthesized AgNPs at various concentrations against cancer (MCF7) and (A549) cell line

Inoculation of the Mueller-Hinton agar plate Step 3. (A) Inoculate the plate with the test organism by streaking the swab in a back-and-forth motion very close together as you move across and down the plate. Rotate the plate by 60° once more and perform again (repeat) the streaking action [31]. This guarantees an even distribution of inoculum which consequence in a confluent lawn of growth. (B) Diagram exemplifying the perceptual structure the swab should follow as it is drawn across the plate. The agar plate surface is inoculated by known *bacteria and fungi* [32,33]. Five set of dilutions (5, 25, 50, 100, and 250 µg/ml) for *Bacillus cereus*, *E.coli* and *Aspergillusniger* added to disk and transversed to petriplate and plates were incubated for 24 to 48 hrs at 37 degree centigrade in an invere position

(1,2).(Clinical Laboratory Standards Institute. 2016).

Fig. 9 Antibacterial activity of sample for *Escherichia coli*, *Bacillus cereus* and *Aspergillus niger*

The antibacterial activity (Table.4, Table 5 and Table 6) was confirmed based on zone size

Table 4. Antimicrobial activity of Nano ferrate samples against *Escherichia coli*

Sample no.	Zone of inhibition(mm)			
	25µg/ml	50µg/ml	100µg/ml	250 µg/ml
1.	0	0	0	0

Table 5. Antimicrobial activity of Nano ferrate samples against *Bacillus cereus*

Sample no.	Zone of inhibition(mm)			
	25µg/ml	50µg/ml	100µg/ml	250 µg/ml
1.	0	0	0	0

Table 6. Antimicrobial activity of Nano ferrate samples against *Aspergillus niger*

Sample no.	Zone of inhibition(mm)			
	25µg/ml	50µg/ml	100µg/ml	250 µg/ml
1.	0	0	0	0

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