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A novel study of pH influence on Ag nanoparticles size with antibacterial and antifungal activity using green synthesis

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ABSTRACT

In this paper, the effect of pH on nanoparticles (AgNPs) using plants extracting has been investigated. The aqueous sol of AgNPs prepared at different pH values using hydrothermal method display different Surface Plasmon Resonance (SPR) behavior and the maximum absorption values were at pH = 14. AgNPs were characterized using X-Ray diffraction, UV-Vis spectroscopy and scanning electron microscope. Face-center cubic Ag nanoparticles with crystal size about 3.86 nm have been noticed. The absorption band showed that Ag has sharp curves in the ultraviolet and at the edge of the visible region. The SEM images showed cluster shaped nanoparticles, and when increasing the pH values, the result revealed the formation of larger nanoparticles cluster with more accurate crystallite sizes. The antimicrobial and antifungal activity was performed by Agar well diffusion assay against Escherichia coli, Bacillus subtilis and Candida albicans. The diameter of the inhibition zones of Ag NPs against the bacterial strains such as, Bacillus subtilis (31 mm) and Escherichia coli (30 mm) at 200 µg/ml concentration and the diameter of the inhibition zones of Ag NPs against the fungus strains such as, Candida albicans (36 mm) at the same concentration.

***Keywords*:** Hydrothermal, Green synthesis, Zingiber Officinale (Ginger), Silver nanoparticles, pH effect, AgNPs

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1. INTRODUCTION

Nanotechnology encompasses the designing of materials at nanoscale levels to achieve exclusive properties, which can be suitably employed for the required applications [1]. Throughout the past decade, a vast potential in nanotechnology has been recognized due to the effectiveness of various metal nanoparticles against several pathogenic microorganisms such as bacteria, fungi, algae, yeast, and virus [2].

Nanoscale materials thought of unique antimicrobial agents having a high area to volume quantitative relation to unravel the murder of the emergence of microorganism multidrug resistance [3]. Several types of metal nanoparticles like magnesium, Iron, gold, copper, zinc, alginate, and silver have come up [4]. However, AgNPs have been established to be simple because they have sensed antimicrobial activity against various microorganisms. Almost 5000 years ago, Romans, Greeks, Egyptians, and Indians used silver in several forms to preserve the food products [5]. In the recent decade, Ag NPs have been received the huge attention of the scientists due to their remarkable defense against various pathogenic microorganisms.

The exceptional characteristics of AgNPs have made them applicable in various fields like biomedical, drug delivery, water treatment, agricultural [6]. AgNps are applied in inks, adhesives, electronic devises, pastes, etc. due to high conductivity [7]. AgNps have been synthesized by physicochemical techniques such as chemical reduction, gamma-ray radiation, microemulsion, electrochemical method, laser ablation, autoclave [8], microwave and the photochemical reduction [9].

These methods have effective yield, but they are associated with the limitations of use of toxic chemicals and high operational cost and energy needs. Considering the drawbacks of Physio-chemical methods, cost-effective and energy efficient new alternative for Ag NP synthesis using microorganisms [10], plant extracts [11] and natural polymers [12] as reducing and capping agents are emerging very fast.

The association of nanotechnology and green chemistry will unfold the range of biologically and psychologically compatible metallic nanoparticles [13]. Many plants such as *Pelargonium graveolens* [14], *Medicago sativa* [15], Lemongrass [16], *Aloe vera* [17], *Cinnamomum camphora* [18], *Boswellia ovalifoliolata* [19], *Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium* [20], have shown the potential of reducing silver nitrate to give formation of AgNPs.

Green synthesis of nanoparticles has been an exploring research topic in recent days due to their advanced use in biomedical, chemical and related fields. We herein report the synthesis of silver nanoparticles by the reduction of aqueous Ag^+ and with the extract of ginger (Zingiber officinale) rhizome.

Ginger is known for its medicinal values such as ginger has been used to treat skin diseases, colorectal cancer, arthritis, heart condition and also have been reported for its antibacterial properties [21]. In this study the cost-efficient and completely biogenic method for synthesizing silver nanoparticles using Zingiber officinale extract and the main aim of the present research was to study the relationship between the pH solutions and the major characteristics of nanoparticles (size, size distribution, morphology and stability).

2. EXPERIMENTAL PART

2. 1. Material and Methods

In this study, silver nitrate $AgNO_3$ (Reagent World, USA, purity 99.99 %), Sodium hydroxide and Zingiber officinal plant extract were used as the starting materials. The ginger extract solution was prepared using 60 g of Zingiber officinal rhizome that had been rinsed with deionized water and finely cut into small pieces. The chopped ginger was boiled in a 100 ml of deionized water for 30 minutes at 90 °C and allowed to cool and filtrated by What man filter paper no. 1, After filtration, it uses centrifuged at 4000 rpm for 15 min to get rid of the impurities and use the extractor for the experiment as show on Figure 1.

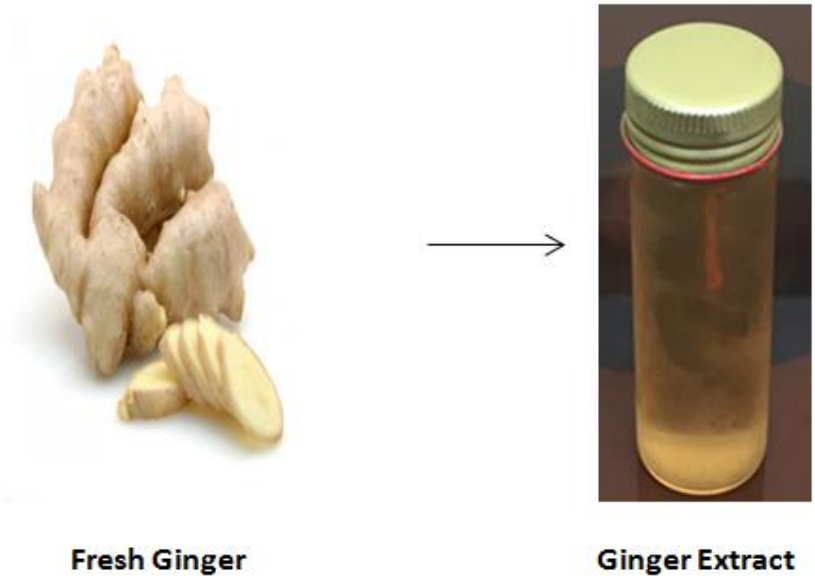


Fig. 1. The conversion of fresh ginger into a ginger extract

2. 2. Biosynthesis of Silver Nanoparticles Using Zingiber Officinal Extract

20 ml of 0.1 mM Silver nitrate solution was prepared, 25 ml of ginger extract 1 ml of 14 mM Sodium hydroxide was prepared.

25 ml of ginger extract was added to 20 ml of silver nitrate solution and put the mixture on the magnetic stirrer at 60-70 °C for 30 minutes. After that, add 1 ml of sodium hydroxide to change the pH status in pH = 6, the pH change was examined by pH test paper. The mixture was added to seal Teflon-lined vessels of 100 ml capacity (autoclave), and heated in the oven at 190 °C for one hour. A gray precipitate was collected by filtration, washed with ethanol and distilled water several times, finally dried in air at 30 °C for 24 hours. Repeat the same previous steps using (2,3,4,5) ml of NaOH were added to prepare different samples to obtain varying values of pH (8, 10, 12 and 14). The crystal phase analysis of the AgNPs powders was conducted using X-Ray diffraction (XRD). The particle sizes of AgNPs samples

were characterized using scanning electron microscopy (SEM). The Absorbance data of the AgNPs were measured using UV-1800.

2. 3. Mechanism of AgNPs Synthesis

The synthesis of AgNP by biological entities is due to the presence of a large number of organic chemicals like carbohydrate, fat, proteins, enzymes, coenzymes, phenols flavonoids and alkaloids. Capable of donating electron for the reduction of Ag^{+} ions to Ag^{0}. The active ingredient responsible for the reduction of Ag^{+} ions varies depending upon organism/extract used. For nano-transformation of AgNPs, electrons are supposed to be derived from dehydrogenation of acids (ascorbic acid) and alcohols (catechol) in hydrophytes, Quito to Enol conversions (paraquinone, dietchequinone, remain) in mesophytes or both mechanisms in xerophytes plants [22].

The microbial cellular and extracellular oxidoreductase enzymes can perform similar reduction processes. A schematic diagram showing the silver ion reduction, agglomeration and stabilization to form a particle of nano size is shown in Figure 2.

Fig. 2. Synthesis mechanism of AgNPs.

2. 4. Factors Affecting AgNPs Synthesis

The major physical and chemical parameters that affect the synthesis of AgNP are reaction temperature, metal ion concentration, extract contents, pH of the reaction mixture, duration of the reaction and agitation. Parameters like metal ion concentration, extract composition and reaction period largely affect the size, shape, and morphology of the AgNPs [23]. Small and uniform sized nanoparticles were synthesized by increasing pH of the reaction mixture [24]. The nearly spherical AgNPs were converted to spherical AgNP by altering pH [25]. The particle size of AgNPs could be tuned by varying both time and temperature. A process using a pure Ag phase could go to completion in 1h at 190 °C, whereas reactions at lower temperatures required longer times.

3. CHARACTERIZATION

The optical properties were examined via (CARY, 100 CONC plus UV-Vis-NIR, Split-beam Optics, Dual detectors) spectrophotometer equipped with a xenon lamp at a wavelength range at (300-900 nm). All films were deposited on glass substrates. The size and shape of the nanoparticles were determined by X-Ray system (Shimadzu - XRD6000, Shimadzu Company /Japan). The X-Ray source was Cu-K_{α} radiation with 0.15406 nm wavelength. The system operates at 40 KV and 30 mA emission current. The sample is scanned from (20 - 90 degree).

The scanning electron microscope, a sample of silver nanoparticles was loaded onto the sample holder. This was allowed to be analyzed in a fully automated scanning electron microscope SEM (Make – JEOL, Model – 6390).

4. RESULTS AND DISCUSSION

4. 1. X-Ray diffraction measurements

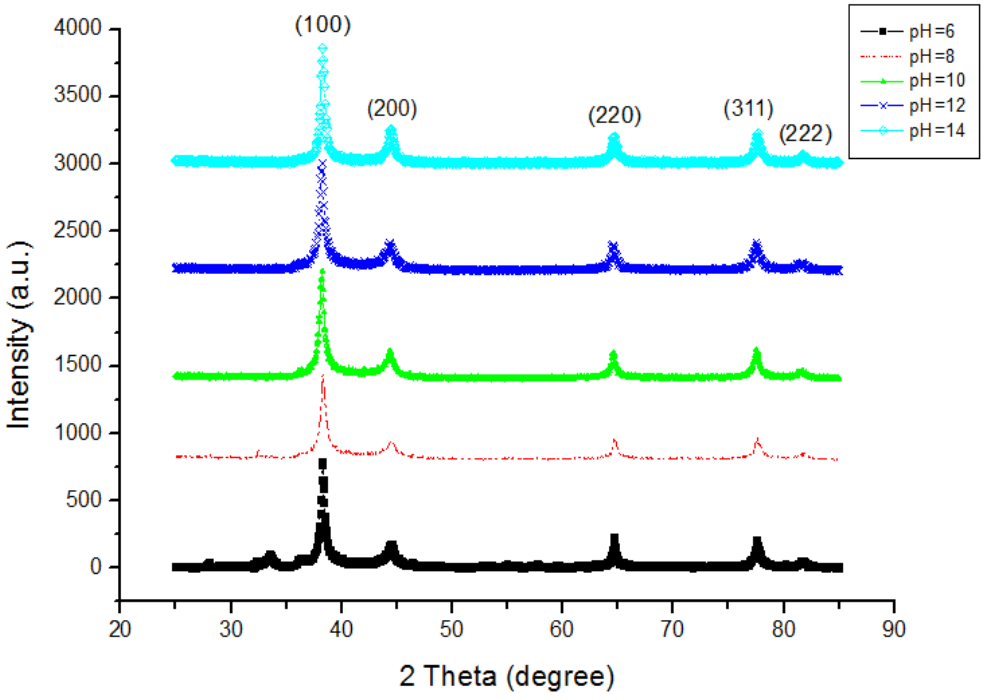


Fig. 3. XRD pattern of synthesized Ag nanoparticles using fresh ginger extract under different pH conditions.

The crystalline structures of AgNPs were confirmed by the XRD analysis as shown in Figure 3. The peaks located at 2θ° = 38.2°, 44.4°, 64.6°, 77.5° and 81.5° are indexed to the (100), (200), (220), (311), and (222) for pH = 6, 8, 10,12 and 14 respectively. The peaks placed at 2θ° = 38.2°, 44.4°, 64.6°, and 77.5° are characteristic diffractions of face-centered-cubic (FCC) structured metal Ag (JCPDS Card no. 04-0783). The average crystallite size from XRD has been estimated using Scherer formula [26]:

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

where: k = constant (0.89 < k < 1), λ = wavelength of the X-Ray, β = full width at half maximum (FWHM) of the diffraction peak, and θ = diffraction angle. The crystal size of Ag for the prevailing peak nanoparticles of (100) about 3.86 nm.

In the figure below, the peaks of AgNPs increasing when pH 14 and become more regular compare with other pH values. The position, height and width of the diffraction peaks depend on the nanocrystalline nature of the AgNPs. The sharp diffraction peaks with strong peak intensity reflect the formation of small-sized AgNP crystallites with high crystallinity. The peak intensity of the unsplit (100) peak is the strongest, suggesting that the (100) is the predominant orientation of AgNPs, followed by the (200), (220), (311), and (222) facets

4. 2. SEM analysis

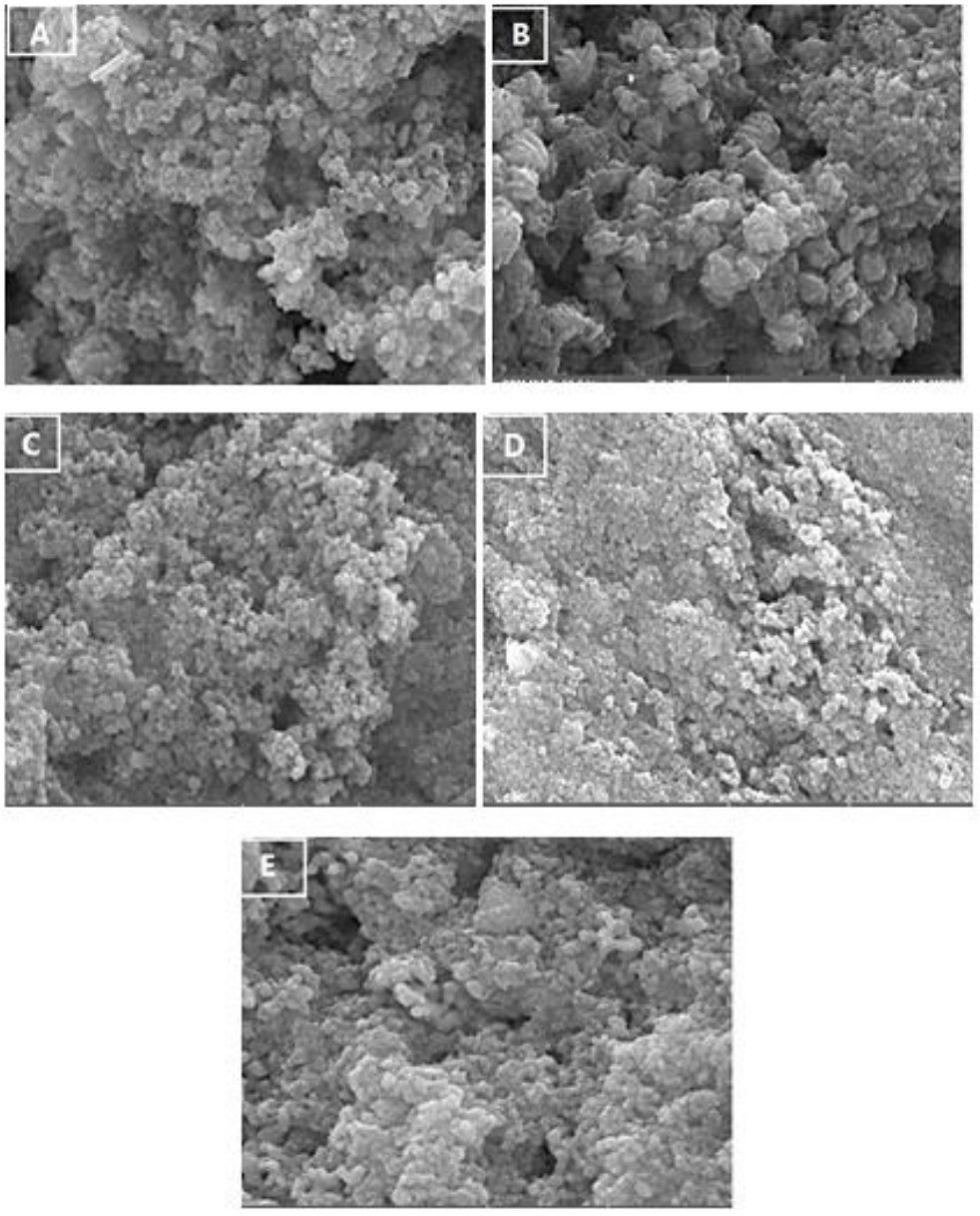


Fig. 4. SEM images of Ag Nanoparticles under different pH conditions
A) pH = 6, B) pH = 8, C) pH = 10, D) pH = 12, E) pH = 14.

Scanning electron microscopy analysis reveals the structural morphology of the synthesized silver nanoparticles. The SEM image of AgNp is as shown in Figure 4. It reveals that the synthesized nanoparticles are predominantly cluster in shape. Through the Figure.4, when increasing pH values, it observe the formation of nanoparticles more pronounced. Nanoparticles in pH = 14 are more clearly compared with other pH values.

4. 3. UV -Vis spectroscopy analysis

The reduction of silver nitrate was monitored by measuring the absorbance as shown in Figure 5. AgNPs seems to be brown color as a signed of reduction silver nitrate, the absorption maximum peak of UV-VIS spectrum was around 380 nm for pH = 6, 8, 10, 12 and 14 results showed formation of silver nanoparticles by reduction of silver ion to Ag^0 which carried out by using ginger root aqueous extract. Through the graph, it observes that the absorbance of pH = 14 has the highest value compare with others pH values. The absorption peak intensity gradually increased with increasing pH. When the pH value reached 14, the band shifted toward obviously lower wavelengths with more centralized particle size distribution. The first reason to increase absorbance of pH =14, that by increasing pH values leads to increased material thickness on the thin film glass substrate, thus increasing absorbance. The second reason is also the increase in pH leads to the reduction of the distances between the particles where happens the homogeneity and regularity for nanomaterials or so-called crystalline regeneration leads to increased absorption and reduced transmittance.

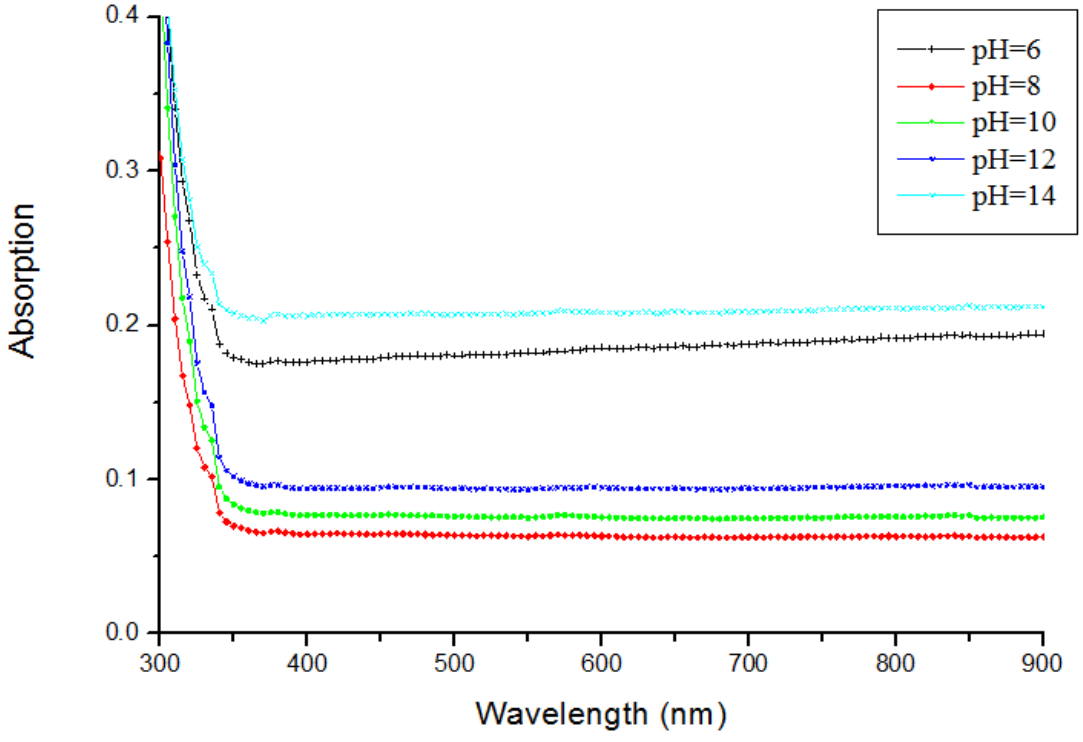
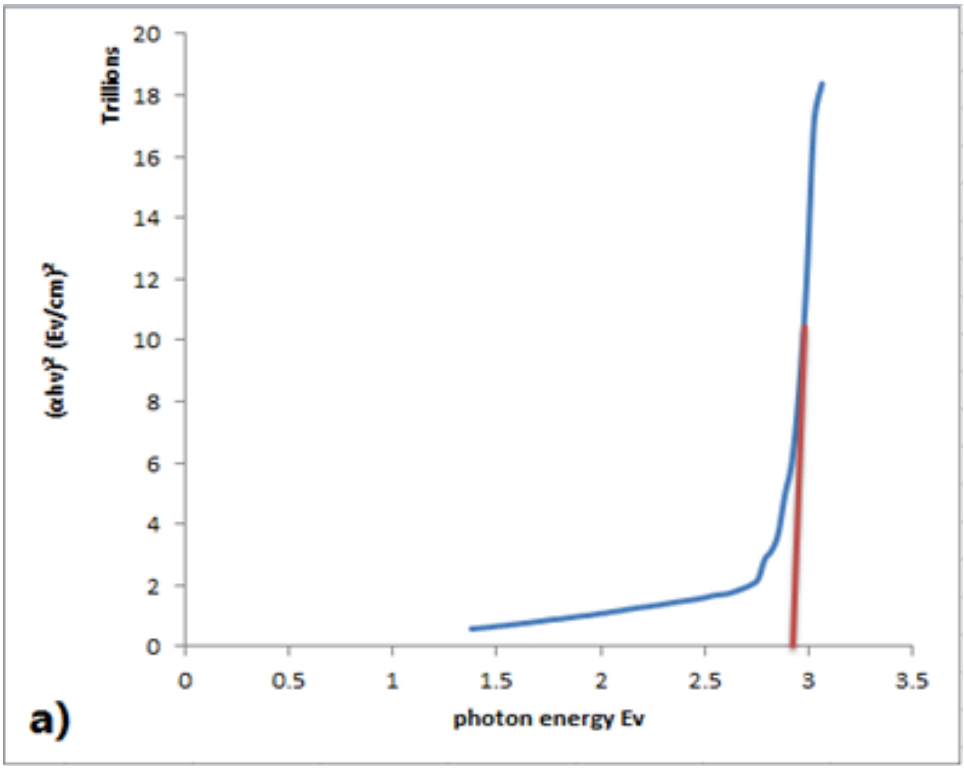


Fig. 5. UV-visible absorption spectrum of silver Nanoparticles (AgNPs) synthesized by reducing silver ion with ginger extract for different values of pH.


Trillions
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18
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14
12
10
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6
4
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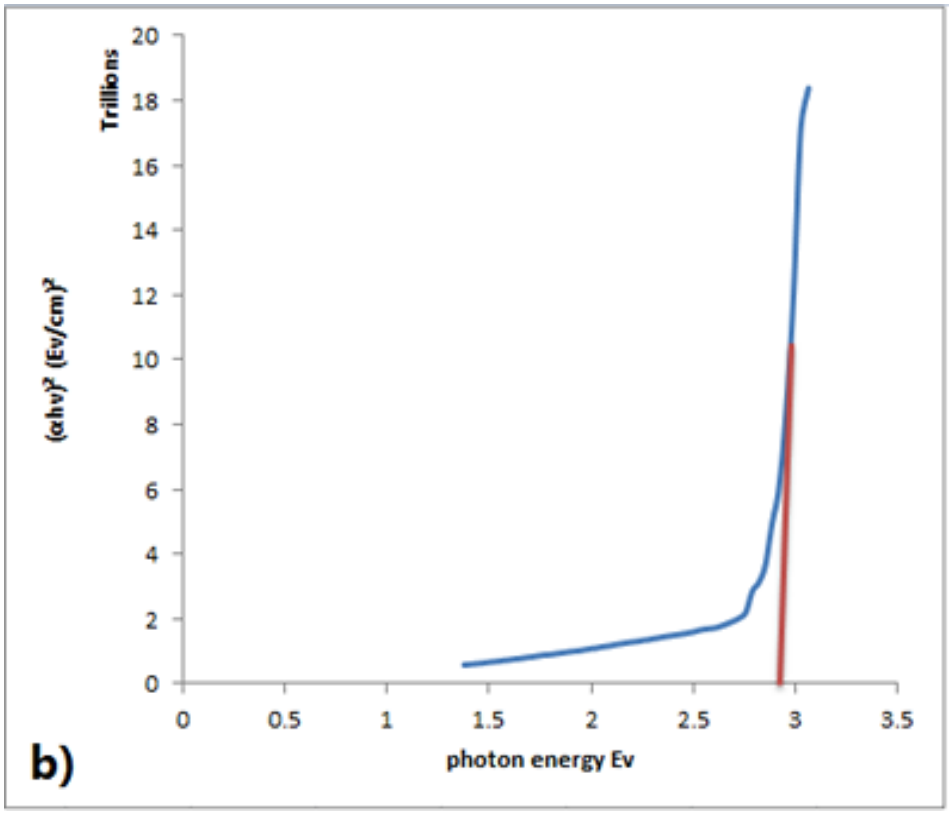
photon energy Ev
a)


Trillions
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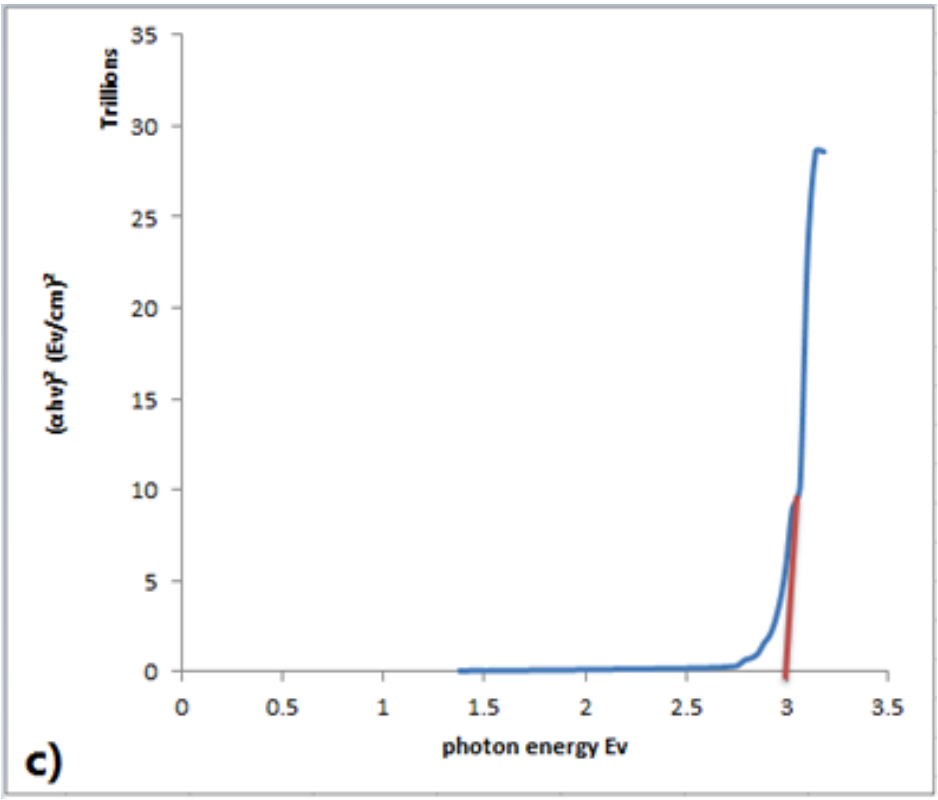
Trillions
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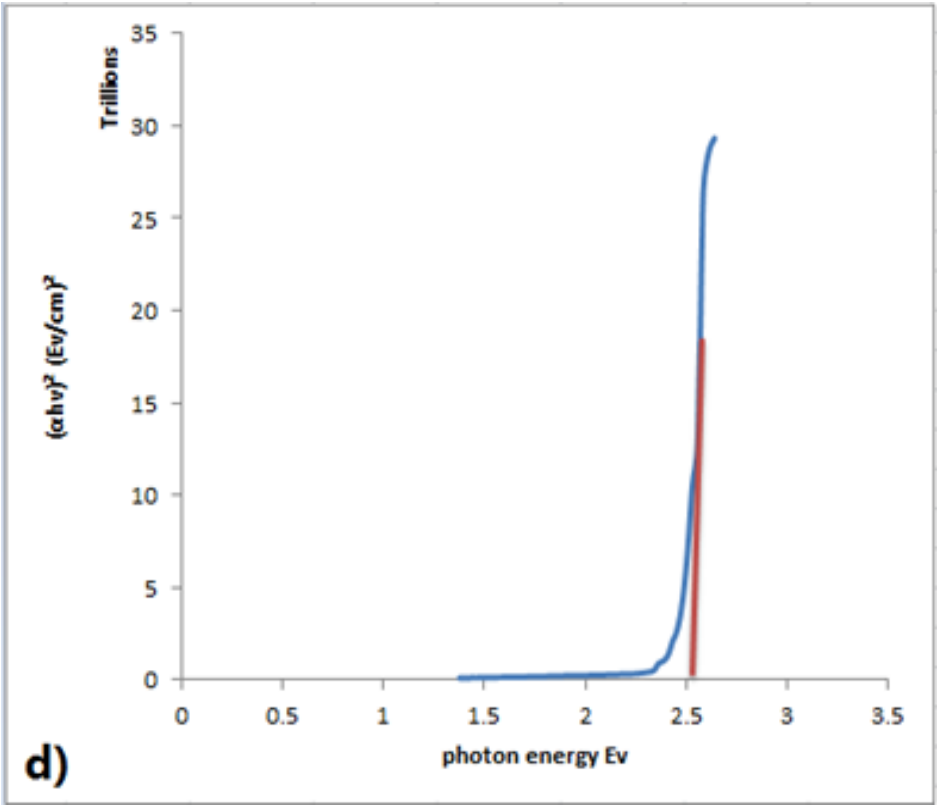
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d)

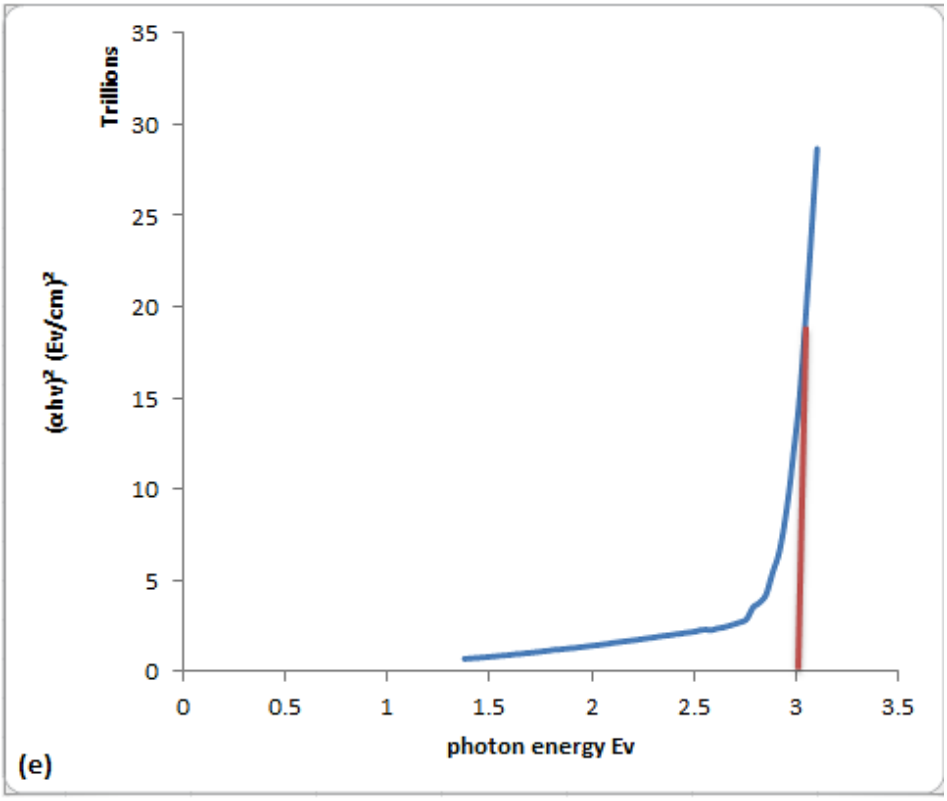

Fig. 6. The optical band gap of Ag nanoparticles under different pH conditions a) pH = 6, b) pH = 8, c) pH = 10, d) pH = 12, e) pH = 14.

The band gap can be estimated by the variety of $(\alpha h\nu)^2$ with a photon (hv) as shown in Figure 6. The band gap of silver thin film on glass substrate were 2.8 eV of pH = 6, 2.9 eV of pH = 8, 2.95 eV of pH = 10, 2.6 eV of pH = 12 and 3 eV of pH = 14. The Energy gap increase with with increase pH that due to quantum size confinement. The energy gap is proportional to r^{-1} according to the law of particle radius: [27]

$$E_g = \frac{z\, e^2}{r}$$

where: E_g - energy gap, z - Atomic number, e - charge of the electron, r - radius particles

5. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES FOR pH = 14

Kirby-Bauer method is used to explore the antibacterial activity of AgNPs. Gram positive bacteria, such as Bacillus subtilis and Gram negative bacteria, such as *E*scherichia coli were chosen for the analysis and chosen fungi, such as Candida albicans. The action of silver nanoparticles on microbes has the ability to anchor to the bacterial cell wall and

subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell [28].

The effects of the Zingiber officinale-synthesized nanoparticles were tested for antimicrobial activity against pathogenic strains. The silver nanoparticles obtained from ginger extracts showed antimicrobial activity against two laboratory pathogens such as *Escherichia coli*, *Bacillus subtilis* and antifungal activity such as *Candida albicans*.

The synthesized AgNPs colloidal solution has shown better antibacterial activity against both Gram-positive and Gram-negative bacterial strains and they showed the inhibition zone on the petri plates using the agar diffusion method in Figure 7. The diameter of the inhibition zones of AgNPs against the bacterial strains such as, *Bacillus subtilis* (31 mm) and *Escherichia coli* (30 mm) at 200 µg/ml concentration and The diameter of the inhibition zones of AgNPs against the fungus strains such as, *Candida albicans* (36 mm) at the same concentration as shown on Table 1.

Table 1. Zone of inhibition of Antimicrobial and Antifungal activity of silver nanoparticles (Zingiber Officinale) for pH = 14.

S. No	Test pathogens	Zone of inhibition (mm) at 200 µg/ml concentration	Control (Dmso)
1	*E. coli*	30	-
2	*B. subtilis*	31	-
3	*Candida albicans*	36	-

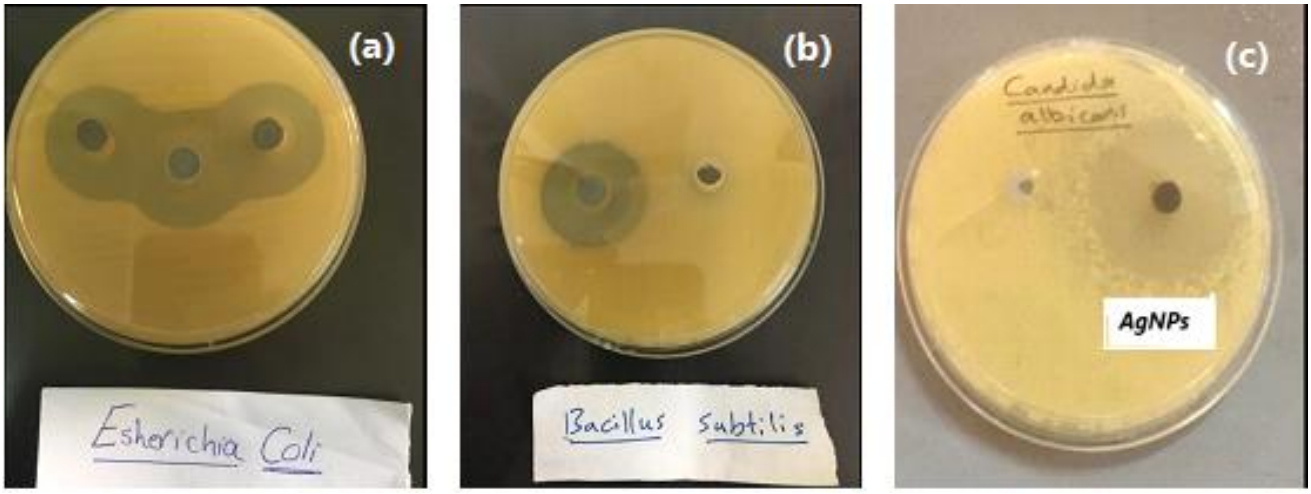

Fig. 7. Antimicrobial and antifungal activity of synthesized silver nanoparticles from Zingiber officinale **(a)** *E. coli*, **(b)** *B. subtilis* and **(c)** *Candida albicans*.

6. CONCLUSIONS

The Ag nanoparticles have been synthesized by a green method using an extract of ginger.. This is an advantage for ginger and other plants extract containing similar functional groups which can potentially contribute in the fabricating of nanoparticles and be converted into valuable nanomaterials. The synthesis of such nanoparticles has been carried at different pH conditions brought about by the addition of NaOH. This paper described a facile method to synthesize AgNPs in large quantities. Silver nitrate was reduced in an ginger plant-extract solution under a hydrothermal conditions. Zingiber Officinale plant extract solutions were used as both reducing and stabilizing agents. Fine cluster shaped nanoparticles were obtained. The particle size of AgNPs can be tuned by varying the hydrothermal temperature and by changing pH values. When increasing the pH values will result in the formation of larger nanoparticles but with more accurate crystallite sizes. The peaks of AgNPs increasing in pH 14 and become more regular compare with other pH values and the crystal size of Ag nanoparticles in higher peak is (100) about 3.86 nm.

The absorbance of pH = 14 has been higher than the absorbance of pH = 6, and the energy gap of the AgNPs increased with increased the pH value. The antimicrobial and antifungal activity was performed by Agar well diffusion assay against *Escherichia coli, Bacillus subtilis* and *Candida albicans*. The result of the studied showed that the green synthesized silver nanoparticles possessed to have significant antimicrobial and antifungal properties. Thus, the green synthesized silver nanoparticles from Zingiber officinale can be used as a curative agent for targeted drug delivery to cure diseases. This may be due to the mode of action of silver ions against the bacteria. These silver ions can cause a destruction of peptidoglycan cell wall and lysis of cell membrane. The silver ions bind to DNA bases, causes and condense the DNA to lose its ability to replicate, thereby prevents replication via binary fission. Also, it leads to induction of ROS (Reactive Oxygen Species) synthesis, thereby forming highly reactive radicals that destroy the cells.

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