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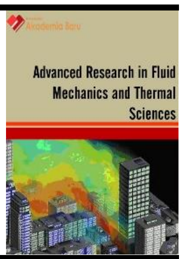

Green Synthesis of Silver Nanoparticles from Natural Compounds: Glucose, Eugenol and Thymol

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ABSTRACT

Currently, renewable energy, clean technology, recycling and green synthesis are the main fields of researchers who are interested in saving our planet. The work is mainly aimed at the use of phytochemical compounds of high purity to prepare silver nanoparticles. These compounds work as a reducing agent of aqueous silver nitrate to define the most appropriate compounds which are in charge of this chemical reaction. The compounds (eugenol, D-glucose, coumarin, thymol, sucrose, scopolamine, L-asorbic, khelin, and citric acid) are separately added to 1m M $AgNO_3$ at various pH. Only the first three compounds exhibited a significant color change to dark brown or gray as reduction indicators. UV-visible spectroscopy was utilized as a preliminary investigation of AgNP formation during the experiments. Morphology, elemental analysis and crystallinity were investigated with SEM, EDS, particle size analysis and XRD. The UV-visible spectra revealed a characteristic band at 400-450 nm. The spherical shape of the silver nanoparticles derived from all natural compounds was observed by scanning electron microscopy. The NPs synthesized in glucose solution had an average size that varied from 30 to 80 nm and contained a large portion of NP aggregates, those in eugenol solution had an average size of 150 nm, and those in thymol solution had an average size of 230 nm and contained a small portion of NP aggregates. A higher yield of NPs resulted in increased pH and concentration in a richer ion solution and more alkaline medium, respectively, due to the increased reducing power of the compound; however, this was not seen for the eugenol agent, which did not exhibit a significant effect of pH.

Keywords:
Green synthesis; Biogenic synthesis; Silver; Nanoparticles

1. Introduction

Nanotechnology is a newly discover field, which describes and manipulates the unique properties of matter, especially metals at the nanoscale, to develop new capabilities with applications across all fields of science, engineering and medicine [1,2]. In the past decade, researchers have utilized chemical methods to synthesize metallic nanoparticles as a reductant, which become toxic to biological environments. For this reason, in recent years, researchers have engendered serious

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concern for developing environmentally friendly processes [3,4]. Thus, biological approaches have been created as green synthesis processes using agents extracted from plants that are superior to chemicals. The advantages of green synthesis over chemical and physical approaches are eco-friendliness, low cost, low energy and simple technique [5]. Biogenic synthesis of metallic NPs can be divided into two mechanisms: bioreduction and biosorption. The first occurs when metal ions are chemically reduced into more stable forms due to the oxidation of enzymes, which exist in many organisms and plants [6]. However, biosorption involves the binding of metal ions onto the organism itself, such as certain fungi, bacteria, and plants, without the need for energy. Biosorption of metal ions occurs on the cell wall or even on the modified cell wall. These organisms have the ability to form nanoparticles as stable complexes [7].

Numerous studies have been carried out to synthesize green nanoparticles using different parts of plants, such as the bark, stems, leaves, pods, flowers, and fruits. Silver nanoparticles (AgNPs) were synthesized using the leaf extracts of *Bruguiera cylindrical*, *Tragia involucrata, Cymbopogon citronella, Solanum verbascifolium* and *Tylophora ovata* [8,9]. The fruit extract of *Cleome viscosa L.* was also used to synthesize silver nanoparticles [10]. Padalia *et al.* [11] prepared silver nanoparticles from marigold flowers as a source of flavonoids, alkaloids, tannins, triterpenes, cardiac glycoside. They supposed that flavonoids had a main role in forming silver nanoparticles by reducing silver nitrate. However, the probable mechanism is unclear and requires further investigation [11]. Other researchers have utilized different plant extracts, which are rich in effective compounds, such as eugenol [12-18], glucose [19-22], and thymol [23-26], with the highest nutritional contents to synthesize silver nanoparticles.

The entirety of these studies utilized a crude plant extract solution that is comprised of a diversity of natural compounds with varying compounds concentrations. The studies in general only mentioned the concentration of the entire extract and its mixing ratio with the silver nitrate. The Magdalena and *et al.* [13] utilized the clove flowers to prepare the crude extract at 0.167 mg\l concentration as a source of eugenol, afterward; 500 ml of 1 Mm silver nitrate solution was mixed with 5 ml of extract. Vijayaraghavan *et al.* used the *Syzygium aromaticum* bud powder to facilitate the crude extract, the powder worked as a source of eugenol which acts as a reducing and coupling agent. A solution of 50 ml of 1 Mm $AgNo_3$ was mixed with 500 µL of extract [14]. The study of Ashwani K. Singh *et al*. utilized a solution of 0.01 M $AgNo_3$ mixed with various quantities of clove extract as a source of eugenol (5-50 ml) [15]. The major component of clove is eugenol but it is not the only one. In order to prepare AgNPs, a 5 ml Krishna tulsi (*Ocimum sanctum*) leaf extract is added to 5-25 ml aqueous solution of $AgNO_3$ (10^{-3} M) to prepare AgNPs This plant leaves contain complicated chemicals like pegenin, ursolic acid, carvacol, acid, orientin, luteolin, apigenin, moludistin, rosmarinic, eugenol oleonic acid, and caryophyllene. Triterpenes, eugenol, and flavonoids are the major ingredients contained in the leaf and also for the preparation of the silver NPs. Also 5 ml of Krishna tulsi (*Ocimum sanctum*) leaf extract is added to 5-25 ml aqueous solution of $AgNO_3$ (10^{-3} M) to prepare AgNPs [17]. This plant leaves contain chemicals like ursolic acid, rosmarinic acid, oleonic acid, pegenin, orientin, apigenin, luteolin, moludistin, carvacol, eugenol and caryophyllene. The important ingredients in the leaf extract are triterpenes, flavonoids and eugenol. Also, for the preparation of silver NPs, ethylene glycol (EG) and glucose were utilized as reducing agent with presence of poly [*N*-vinylpyrolidone] (PVP) stabilizer. $AgNO_3$ (157 mg) and PVP (5g) were dissolved in 100 ml of 40% (w/w) of glucose syrup [19]. Others used dextran (condensed glucose) as auxiliary capping agent for grafting polyacrylamide polymer to prepare AgNPs [21].

Soluble starch was also used as polymer of glucose; 10 ml of 0.01 g/ml of soluble starch was added to 50 ml of 1 mM silver nitrate [22]. Javad Baharara *et al.* prepared AgNPs using crude extract of *Zataria multiflora, which* main constituents of this plant are phenolic compounds such as carvacrol, thymol and eugenol [23].

Thyme leaf extraction (*T. vulgaris*, *T. zygis* and *T. citriodorus*) [24], Origanum vulgare [25], were used as a crude source of thymol reducing agent. Trachyspermum ammi was extracted to reduce silver nitrate to silver nanoparticles. The main constituents in T.ammi are thymol, p-cymene and terpinenethymol, p-cymene and terpinene.

Most studies have utilized different plants with various parts [27-56] to synthesize noble metal nanoparticles, such as gold, palladium, silver, and platinum. In nature, various parts of plants contain versatile natural compounds with different concentrations that vary with the seasons. In spite of these, most of the mentioned studies and others are interested in plant extracts regardless of which effective compounds are in them. The study mainly worked with a variety of pure forms of natural compounds that contained eugenol, D-glucose, kellin, sucrose, scopolamine, thymol , L-ascorbic acid ,coumarin, and citric acid.

Eugenol is a pale, yellow, oily liquid extracted as an essential oil from clove oil, cinnamon [57,58], nutmeg [59], basil and bay leaf [60]. Thymol is a phenol obtained from thyme plants [61]. Glucose is a simple sugar made during photolysis in all plants. Sucrose is composed of glucose and fructose and is obtained from sugarcane, sugar beet and other plants. Coumarin is a natural volatile found in many plants, such as amburana, apricot, guaco, strawberry, watercress, cinnamon and cherry [62]. Scopolamine alkaloid is derived from plants of the nightshade family, such as *hyoscyamus niger* [63]. Khellin is a major constituent of the plant *Ammi visnaga* [64]. Vitamin C is found in *Kakadu plum*, *Camu camu*, *Acerola*, and *Seabuckthorn* and all citrus plants. Nevertheless, the nanoparticles produced from the utilization of natural chemicals and pure compounds is highly pure something that is very much preferable in multiple ways such as the reduction mechanism, economical purposes, commercial purposes, and even scientific purposes [65-67]. Thus, the present study aims at producing AgNPs by utilizing pure natural compounds rather than crude plant extracts to arbitrate the most appropriate compounds responsible for the process of production.

2. Methodology

2.1 Materials

Silver (I) nitrate ≥ 99.0%, thymol ≥ 98.5%, eugenol ≥ 98.5%, scopolamine alkaloid, coumarin ≥ 98%, and khellin ≤ 100% were purchased from Sigma – Aldrich, USA. D-glucose, sucrose, vitamin C (L-ascorbic acid) and citric acid were obtained from HiMedia (Mumbai, India) and were of analytical grade.

2.2 Green synthesis of silver nanoparticles

This work was performed at the Biotechnology Research Center/Al-Nahrain University and Department of Materials Engineering/University of Technology, Baghdad, Iraq. Eight different natural reducing agents were utilized: eugenol, thymol, D-glucose, sucrose, coumarin, scopolamine, kellin, and L-ascorbic acid and citric acid. Their concentrations and conditions are illustrated in Table 1. The schematic procedure is shown in Fig. 1. First, 1 mM $AgNO_3$ was dissolved in distilled water [68]. Then, 50 ml of each concentration of the dissolved agents was adjusted to pH 7.5 and poured into 50 ml of silver nitrate. In this work, the concentrations of the agents were determined according to the availability in plants and the effectiveness of the natural compound as a reducing agent. In nature, these agents were formed in plants at different concentrations.

Glucose and sucrose are synthesized by plants in large quantities during photosynthesis as primary metabolites, whereas others, such as eugenol and thymol, are synthesized by plants in smaller quantities and lower concentrations as secondary metabolites. The final solutions were placed on a shaker and heated to 60°C for 2 hr and then 40°C for 1 hr [10]. However, the glucose- and sucrose-based solutions were stirred and heated to a higher temperature of 80°C [20]. Silver nitrate and all used natural compounds were in a colorless solution before adding each compound to the first one. After adding the natural compounds to the $AgNO_3$ solution, all samples showed a color change due to the reducing reaction. Different colors of the samples are evidence of different sizes of the formed nanoparticles. Only D-glucose-, eugenol-, and thymol-based solutions exhibited a significant color change to dark colors (brown, gray, and greenish gray, respectively) to indicate the reaction of silver nanoparticle formation, as shown in Fig. 2. The fastest reaction was that for eugenol due to a change in color from gray to grayish brown directly after adding eugenol to the $AgNO_3$ solution even before heat exposure. This coincides with [15]. The slowest reaction was that for glucose and then thymol. The obtained solution was aged overnight in a dark place to reduce the silver ions to nanoparticles. The solution color was observed to change from colorless to reddish dark brown. Afterwards, the nanoparticles were collected and centrifuged (4000 rpm/min for 25 min) at room temperature [22]. The colors of sucrose, kellin, coumarin, scopolamine alkaloid, and citric acid-based solutions did not change even after 24 hr. The last compounds were neglected in this work because they did not give clear peaks in the UV-visible spectrum. Only D-glucose, eugenol and thymol exhibited surface plasmon resonance (SPR) in UV-visible light, and so only these compounds were taken into consideration.

Table 1
Natural compound as reducing agents and their concentrations

Natural compounds as reducing agents	Concentration	Details
D-glucose	10,20,30,40,50,60 g/l	Dissolved directly
Sucrose	30 and 50 g/l	in distilled water
L-ascorbic acid and citric acid	150 and 300 mg/l	
Thymol	200, 300 and 400 mg/l	Dissolved separately
Eugenol	100 and 200 mg/l	in 1/9 volume of
Scopolamine	75 and 150 mg/l	ethanol/distilled water
Coumarin	150 and 300 mg/l	
Khellin	150 and 300 mg/l	

2.3. Effect of pH

Due to the significant change in solution color with changing alkalinity during the experiments, two different levels of pH were used (pH 8 and pH 10) for the optimum concentration of the reducing agent depending on the UV spectra. At pH 8, the solution color changed to yellowish brown. At pH 10, dark brown and gray solutions were observed distinctly, as shown in Fig. 3.

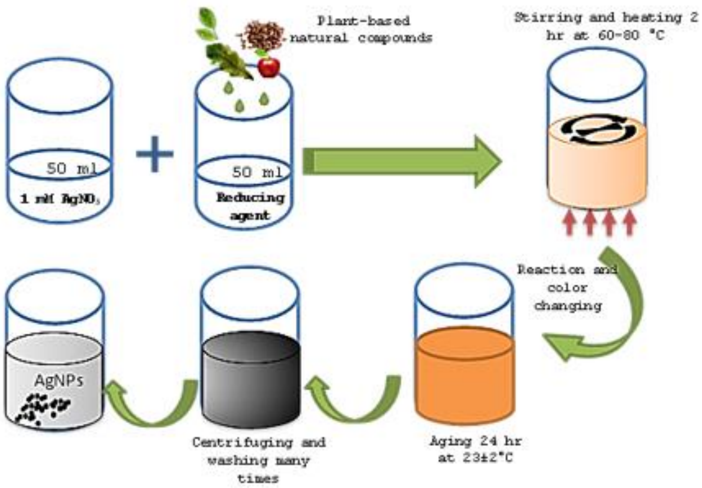
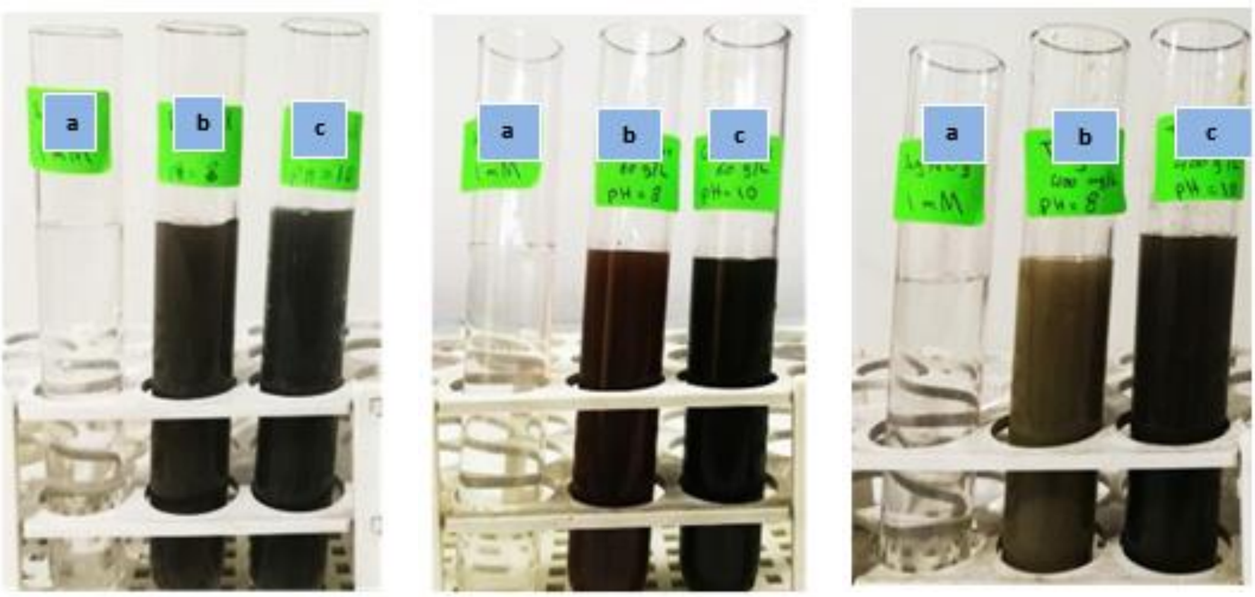


Fig. 1. Schematic diagram of AgNP green synthesis in this work

Fig. 2. Control solution ($AgNO_3$) and different natural compounds added to $AgNO_3$ as reducing agents (from left to right): eugenol, thymol, D-glucose, sucrose, coumarin, scopolamine, kellin, L-ascorbic acid and citric acid



a) Control; ($AgNO_3$ solution)
b) Eugenol+ $AgNO_3$ solution at pH 8
c) Eugenol+ $AgNO_3$ solution at pH 10

a) Control; ($AgNO_3$ solution)
b) Glucose+ $AgNO_3$ solution at pH 8
c) Glucose+ $AgNO_3$ solution at pH 10

a) Control; ($AgNO_3$ solution)
b) Thymol+ $AgNO_3$ solution at pH 8
c) Thymol+ $AgNO_3$ solution at pH 10

Fig. 3. pH effect on AgNP synthesis

2.4 Characterization
2.4.1 UV spectroscopy

UV spectroscopy was utilized as a preliminary mode of examination of AgNP formation after 24 hr of reaction. A Nano Optima UV spectrophotometer SP3000 (Japan) was used. The scanning speed was 4000 nm/min, the resolution was 0.5 nm, and the range of spectra was 200 to 600 nm.

2.4.2 SEM and EDS

One drop of ultrasonicated AgNPs in ethanol was placed on an SEM sample holder and dried by air. A scanning electron microscope (TESCAN-VEGA III, Czech Republic) was used to observe the morphology of the nanoparticles. Electron dispersive spectroscopy was utilized to identify the silver element.

2.4.3 Particle size analysis

A few milliliters of washed AgNPs were ultrasonicated in ethanol and then analyzed directly by a Brookhaven Nano Brook 90 plus (USA) to determine the particle size and distribution.

2.4.4 X-ray diffraction

Powder X-ray diffraction (XRD) was performed using an X-ray diffractometer (Rigaku model MiniFlex**,** Japan) with nickel filtered CuK*α* (1.5405 Å) radiation.

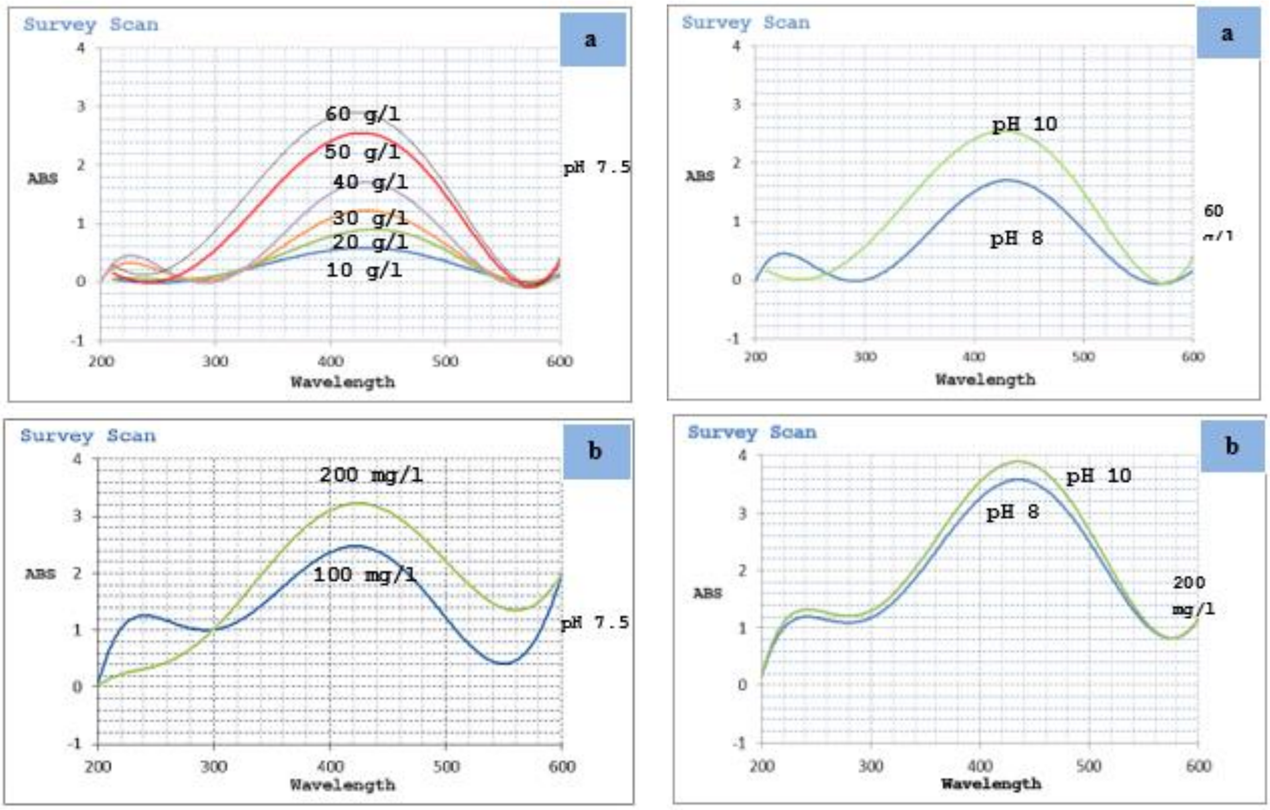
3. Results and Discussion

The reducing agents clearly do not possess absorption signatures in the visible region of the spectrum where silver nanoparticles absorb strongly. Fig. 4 (a, b, c) shows the UV visible spectra of eugenol-, D-glucose- and thymol-based solutions at different concentrations.

The obtained data reveal some important results as follows: (i) the absorption intensity observed at 410 nm and 430 nm reveals the resonance in the UV-vis spectrum of AgNPs due to the excitation of surface plasmon vibrations in the silver nanoparticles and is responsible for the brown color of the solution in visible light ranging from 400-700 nm [14]. This result agrees with those of G. Lakshmanan *et al*. [10-14]. The increasing of Plasmon intensity indicates that large amounts of silver ions are reduced at higher concentration forming more AgNPs clusters [68]. The UV-vis spectrum demonstrates that the intensity of absorption peaks rises with the increasing of all agents concentrations. By depending on these results, the highest possible concentration of each agent was chosen to examine the pH effect. Consequently, D-glucose (60 g/l), thymol (400 mg/l) and eugenol (200 gm/l) were chosen.

Figures 5(a)-(c) show the effect of solution pH on the AgNPs as seen in the UV-visible spectra of the mentioned compounds. It can be observed clearly that the pH raises the absorption peaks for the same concentration of reducing agent. This is because of alkalinity, which promotes the nucleation and subsequent formation of a large number of nanoparticles with smaller diameters [30]. When pH is low, the interaction among the functional groups of compounds through the hydrogen bonds is not anticipated to be fully active alongside the OH bonding, which consequently results in a less effective compound in the reduction process due to the stabilization of the structures. However, the action is reverted when the pH rises towards the base. With the increase of pH up to 10, it resulted in an increase of the peak intensity at 430 nm for d-glucose and eugenol, and 420 nm for thymol based solution. The increase denotes that the processes of generation of nanoparticles have progressed. The data shows that: (i) the increasing of pH solution brings with it changes to the electronic absorption spectra (ii)) band at 420 to 430 nm appear and the absorption peak that appeared is attributed to the surface Plasmon excitation of silver particles and reaches upper value of intensity at pH 10; (ii) the band becomes stronger at higher pH which refer to the Plasmon resonance of AgNPs; (iii) the pH changing affects distinctly upon D-glucose and thymol based solution whereas eugenol is weakly affected. That means eugenol is very active compound which act even alkalinity condition absence which was really noticed during experiments.

Scanning electron microscopy (SEM) images with different magnifications (20 and 50 kx) are illustrated in Fig. 6. They reveal that the AgNPs prepared using D-glucose, eugenol and thymol are spherical and nanoscale in average size. A similar morphology was reported by [35, 69]. The homogeneity in size and topography can be noticed in the lower magnification (20 kx) images in Fig. 6 (a, b, c). At higher magnifications (50 kx), the AgNPs prepared using D-glucose are smaller in size, followed by that prepared using eugenol and thymol, respectively (see Fig. 6 (d, e, f)). Agglomeration is a common issue in most nanoparticles, and the AgNPs prepared using D-glucose have the highest affinity for agglomeration, followed by that prepared using eugenol and thymol, respectively. Phenolic compounds might protect the AgNPs from aggregation, thereby retaining their long-term stability [10]. Additionally, agglomeration might be due to differences in the capping agent and the electrostatic effect between the particles. A small amount of residual thymol agent could be observed to cap the AgNPs, which made these nanoparticles isolated and less aggregated (see Fig. 6(f)).



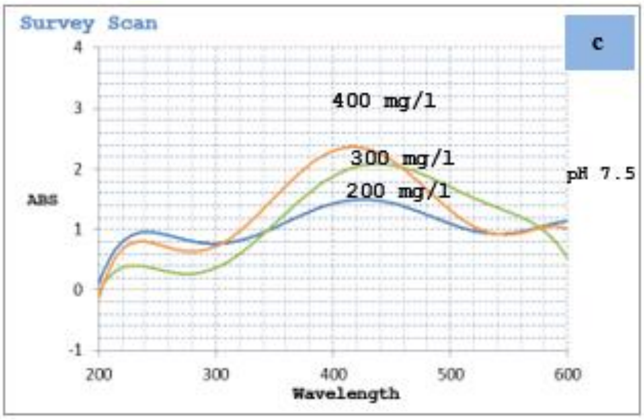


Fig. 4. UV-visible spectra of AgNPs prepared at different concentrations from the reduction of $AgNO_3$ at pH 7.5 by: a) D-glucose (10, 20, 30, 40, 50, and 60 g/l), b) eugenol (100 and 200 mg/l) and c) thymol (200, 300, 400 mg/l)

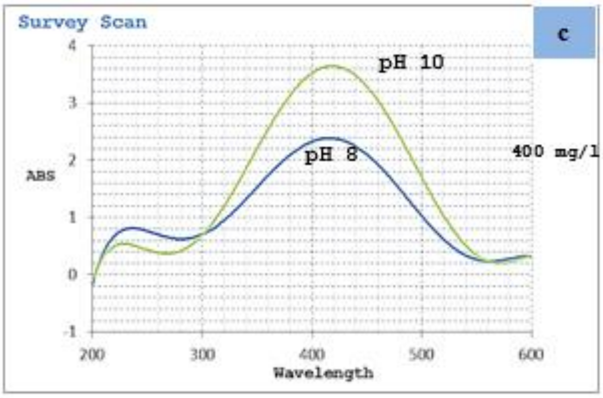
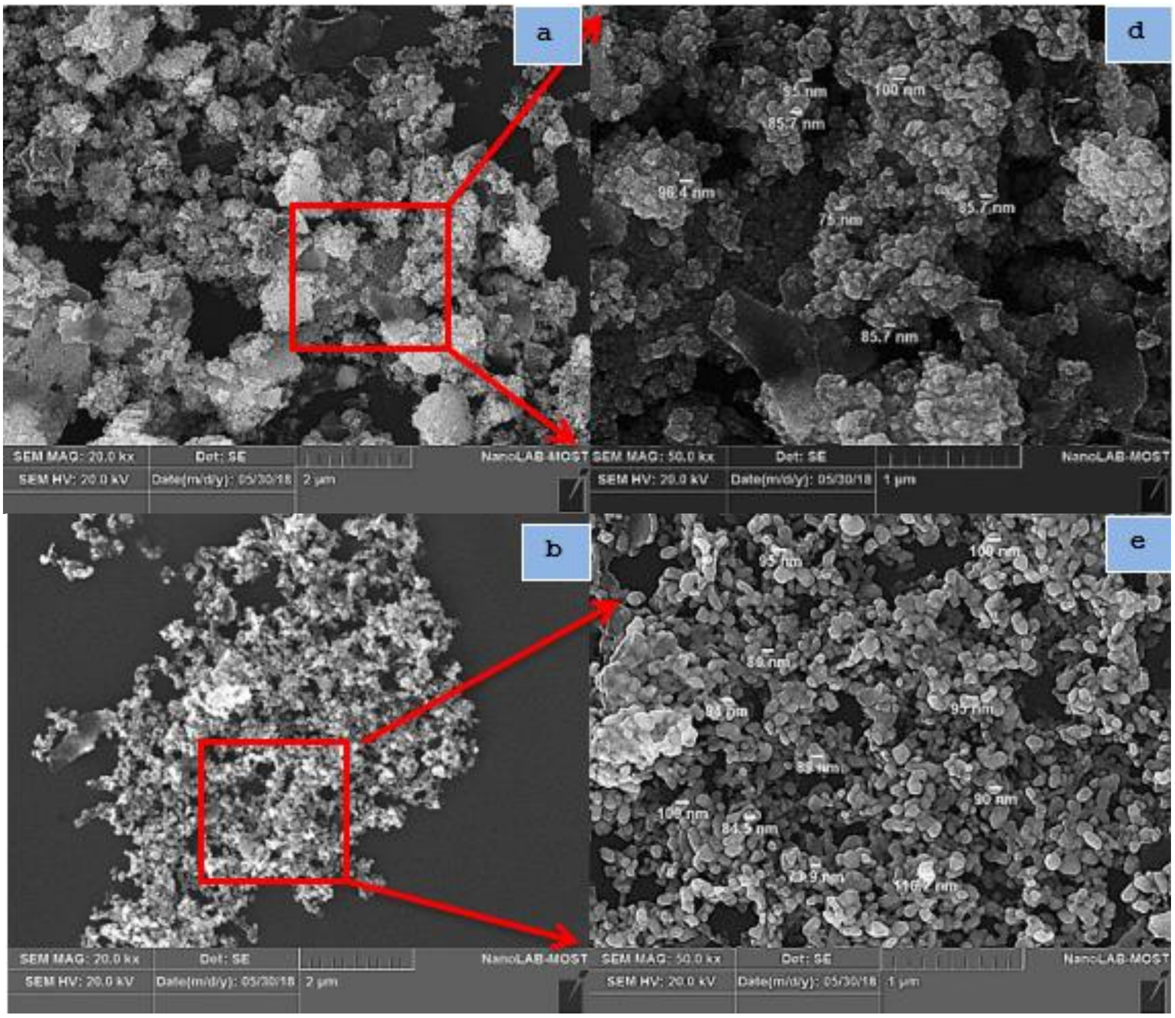


Fig. 5. UV-visible spectra of AgNPs prepared at different pH values from the reduction of $AgNO_3$ at pH 8 and 10 by: a) D-glucose (60 g/l), b) eugenol (200 mg/l) and c) thymol (400 mg/l)



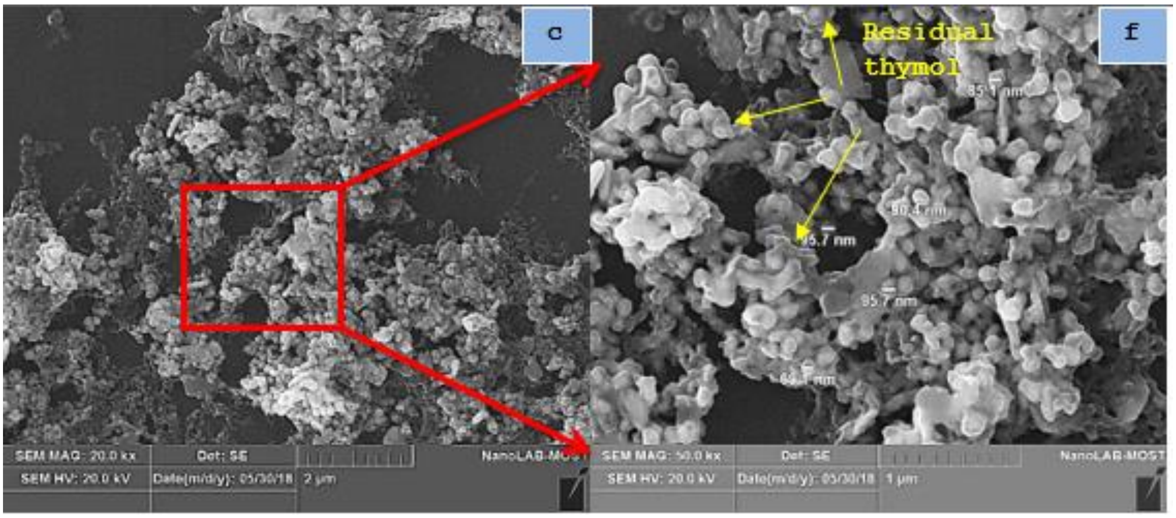


Fig. 6. SEM images of AgNPs prepared from the reduction of $AgNO_3$ at pH 10 by D-glucose (60 g/l) at different magnifications of (a) 20 kx and (d) 50 kx; eugenol agent (200 mg/l) at different magnifications of (b) 20 kx and (e) 50 kx; thymol agent (400 mg/l) at different magnifications of (c) 20 kx and (f) 50 kx

Figure 7 demonstrates the energy dispersive spectra of the revealed AgNPs. The silver element has the highest relative concentration in all samples, at approximately 93.5, 57.9, and 92% for the glucose, eugenol- and thymol-based solutions, respectively. The AgNP peak is observed at 3 keV, which is the absorption of metallic AgNPs; a similar result was reported in [69]. Oxygen is revealed as the second most abundant element after silver, and its concentrations are approximately 4, 36 and 7%. This means that eugenol compounds may reduce silver nitrate and then oxidize approximately a third of the AgNPs to silver oxide. Thymol also reduces silver nitrate to AgNPs, but it oxidizes a small amount to silver oxide. In glucose-based solutions, where less oxygen is found, glucose is the best reducing agent.

A particle size analyzer was also utilized to investigate the particle size distribution of the AgNPs. Fig. 8 clarifies the lognormal distribution and multimodal size distribution of AgNPs prepared by reducing $AgNO_3$ using D-glucose, eugenol and thymol. The effective diameters were 88, 101 and 152 nm, respectively. This means that the glucose agent promotes smaller nanoparticle formation even at a higher concentration of glucose (60 g/l) compared with small amounts of eugenol (200 mg/l) and thymol (400 mg/l). These results are similar to the ImageJ software analysis of the SEM images. However, the AgNPs obtained by using thymol as the $AgNO_3$-reducing agent are larger than those revealed in the SEM images due to a residual amount of thymol capping the nanoparticles.

Figures 9(a)-9(c) display the XRD patterns of AgNPs prepared using D-glucose, eugenol and thymol as reducing agents in the green synthesis of the AgNPs. Bragg reflections corresponding to the (111), (200), (220), and (311) lattice planes related to angles (38°, 44°, 64° and 76°) respectively are observed. These peaks matched that of the cubic (fcc) structure of silver [70]. The lattice parameter a = 4.086 Å, and the unit cell volume V = 68.20 Å. The broadened peaks of the XRD patterns of the AgNPs compared with those of bulk silver reveal the formation of silver nanoparticles. These results agree with [71-73]. These data clearly show that silver formed by reducing $AgNO_3$ with D-glucose, eugenol and thymol is crystalline in nature. Additionally, it can be observed that the small peaks at approximately 27°-32° are due to silver dioxide formation in eugenol rather than in thymol or glucose, which coincides with the EDS analysis illustrated earlier.

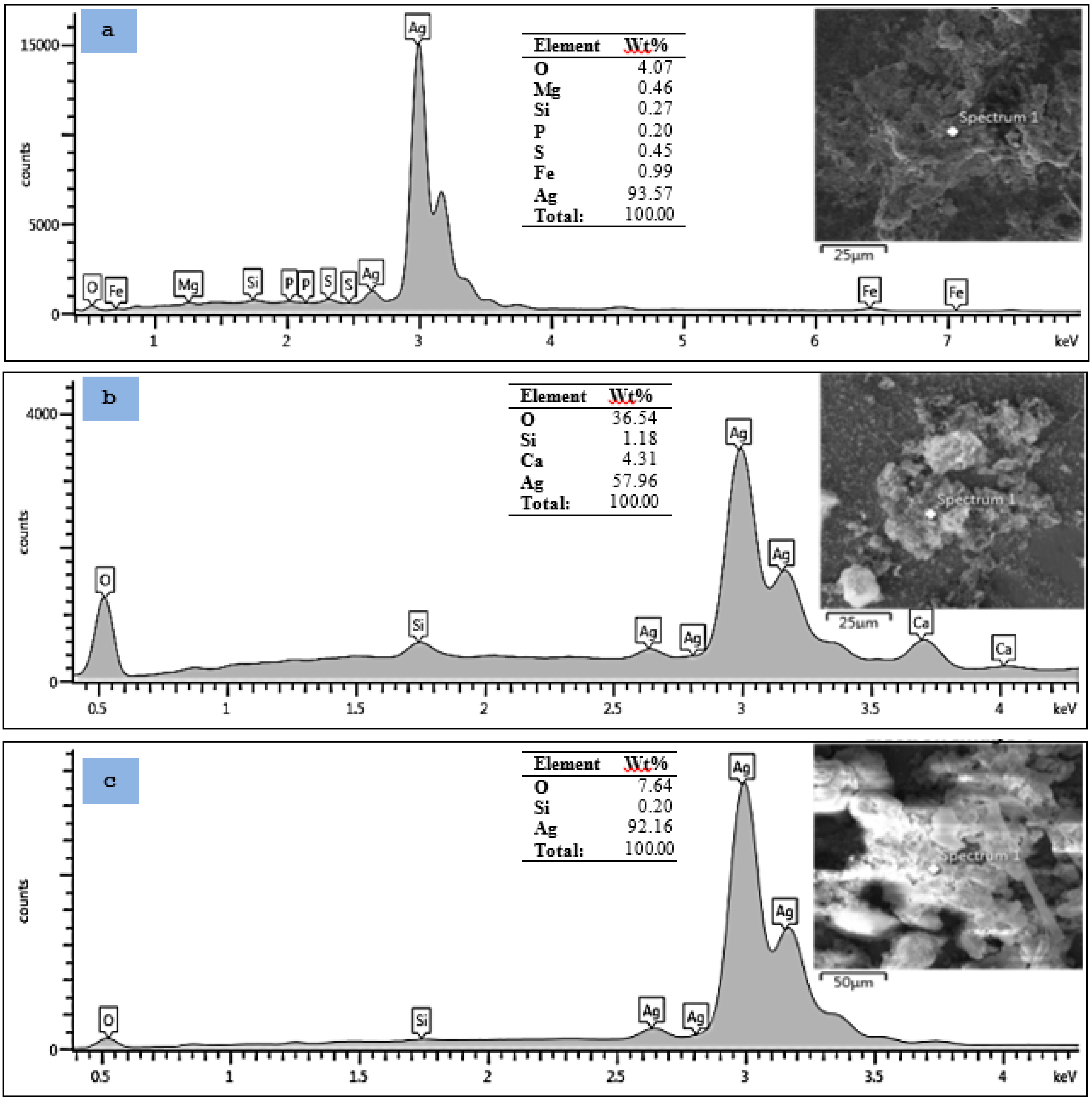


Fig. 7. Energy dispersive spectroscopy EDS of prepared AgNPs from the reduction of $AgNO_3$ at pH 10 by: a) D-glucose agent at 60 g/l ; b) Eugenol agent at 200 mg/l; c) Thymol agent at 400 mg/l

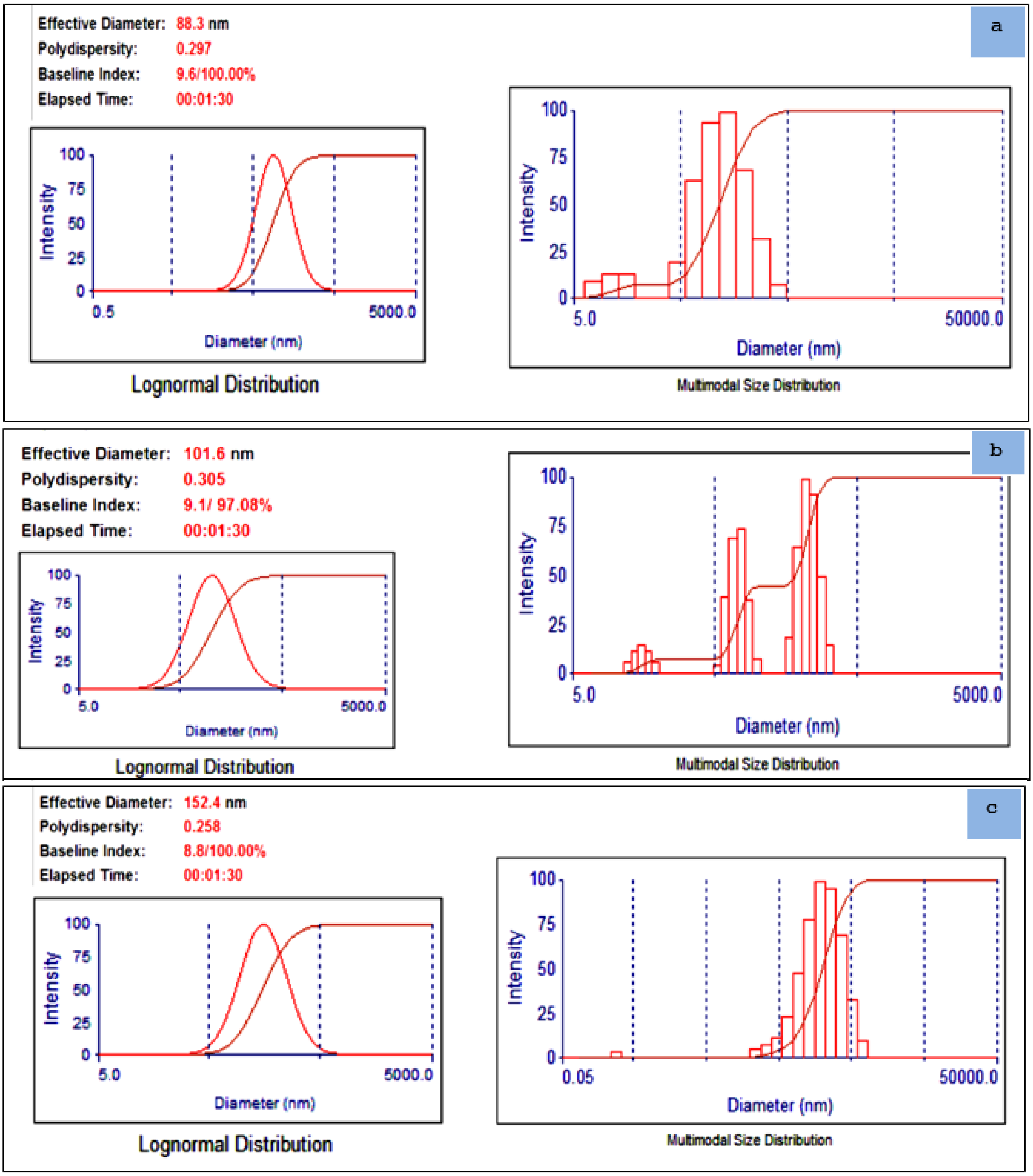


Fig. 8. Particle size analysis of AgNPs prepared from the reduction of $AgNO_3$ at pH 10 by (a) D-glucose agent at 60 g/l ; (b) eugenol agent at 200 mg/l; (c) thymol agent at 400 mg/l

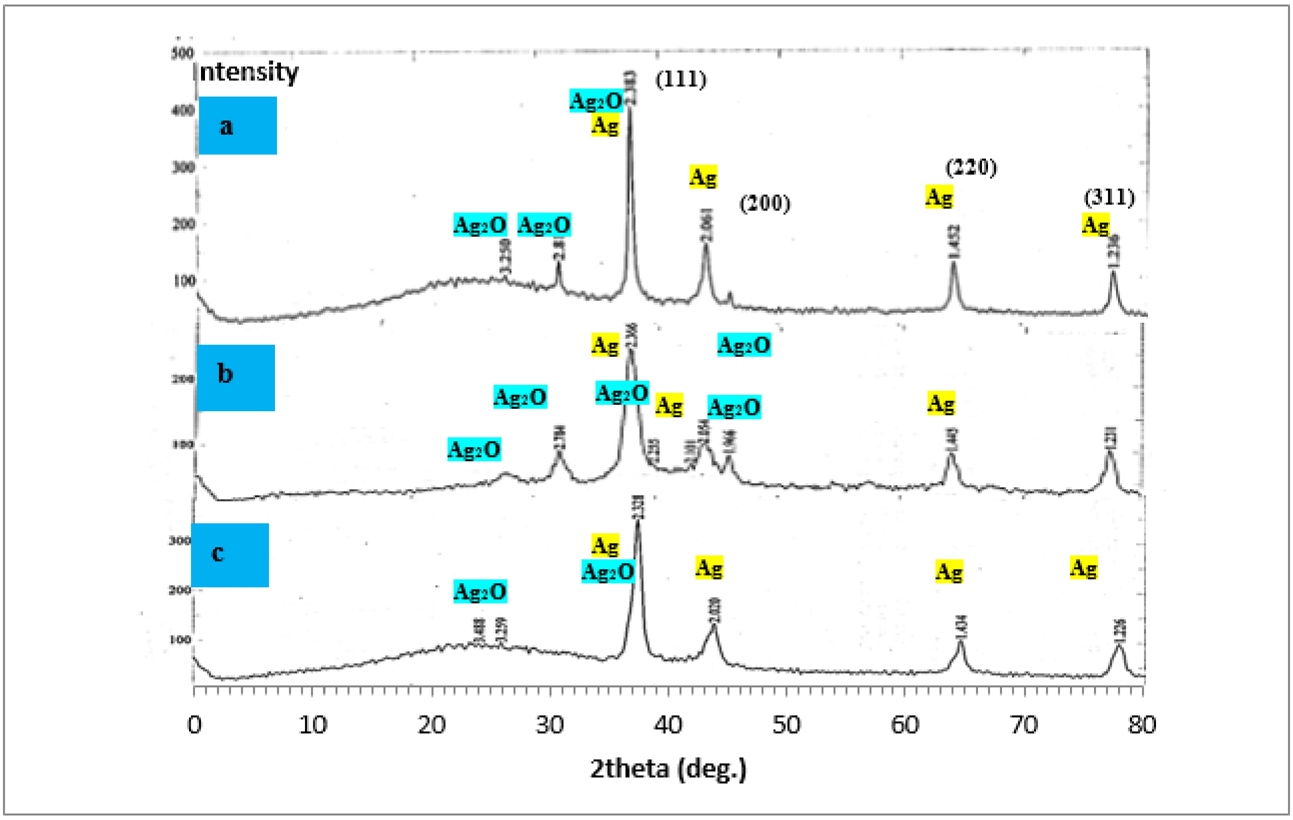


Fig. 9. XRD pattern of prepared AgNPs from the reduction of $AgNO_3$ at pH 10 by (a) D-glucose agent at 60 g/l; (b) Eugenol agent at 200 mg/l; (c) Thymol agent at 400 mg/l

4. Conclusion

The utilization of plant extracts to synthesize silver nanoparticles is considered a fast, ecofriendly, nontoxic, economic, and simple technique. This research focuses on the use of specific compounds (eugenol, thymol, D-glucose, sucrose, coumarin, scopolamine, kellin, and L-ascorbic acid and citric acid) to reveal the roles of effective substances instead of the crude plant extract. This work aimed at the production of nanoparticles from of natural chemicals and pure compounds to achieve higher levels of purity something that is very much preferable in multiple ways such as the reduction mechanism, economical purposes, commercial purposes, and even scientific purposes. For example; most plants which contain essential oils like eugenol mostly have thymol and vice versa. So it is difficult to recognize the effect of each component. Then, it would be determined which plants and plant parts are more effective and can achieve better control of the size and shape of the particles. The following points can be concluded.

i. Hydroxyl groups in phenols (eugenol and thymol) promoted the reaction and gave the solution a dark color and clear surface plasmon resonance. Glucose has neutral pH and good affinity for forming nanoparticles. Additionally, the acidic compounds (L-ascorbic acid and citric acid) have a limited affinity for forming AgNPs even when the pH is 7.5 and the temperature is increasing. The carboxylic acids in commarin flavonoids also partially prevent or delay the reaction even when both pH and temperature are increased. Sucrose is considered a weak acid, and the sucrose-based solution exhibited a weak reaction, and surface plasmon resonance was not identified during UV-visible spectroscopy. Scopolamine alkaloid has a complex structure consisting of ketone, alcohol, amine, benzene ring, ester, and a carboxylic ring, and it is difficult to determine which functional group is effective.

ii. Glucose is highly sensitive to heating and pH. Hydroxide ions in alkaline medium promote ring opening of glucose which in turn results in accelerated silver ions reduction. Additionally, alkaline medium facilitates formation of gluconic acid converting it to sodium gluconate. Also, this mechanism is accelerated by heating.
iii. Finally, three compounds (D-glucose, eugenol and thymol) exhibited significant color changes to dark brown or gray as reduction indicators. Therefore, only these compounds were selected to study the effects of varying parameters (compound concentration and pH) on the formation of AgNPs.
iv. Glucose gives the finest AgNPs, followed by eugenol and thymol.
v. The fastest reduction compound was eugenol even when there was no heating and the effect of pH on reduction efficiency was insignificant. . The use of pure eugenol as a reducing agent decreases the time taken in the reduction process to few seconds instead of minutes or hours without the need of heat or an increase in pH level. This is due to the fact that each eugenol molecule accompanied by the presence of effect of methoxy and allyl groups releases two electrons. Which is a lined with [15].
vi. This led to that eugenol reduces silver nitrate to AgNPs more than glucose and thymol.
vii. The particles obtained from the three selected compounds are spherical in shape and homogenous in size on average.
viii. Furthermore, according to the EDS data, glucose and thymol give higher-purity silver (93.57 and 92.16%, respectively), whereas eugenol produces AgNPs at the lower purity of 57.96%.
ix. Silver nanoparticles produced by eugenol reducing agent are more separated, those in by thymol and glucose. Thymol still covered the nanoparticles even after several times of washing and centrifuge. Glucose based solution have affinity to aggregate the particles in noticeable matter.
x. Most plants which contain phenolic essential oils like eugenol mostly have thymol and vice versa. So the scientists in [23] that utilized plants of crude extract will not be able to ever tell the effect of thymol and eugenol separately. Moreover, this study found that these compounds had different behaviors like in speed of reduction, darkness of colloidal, responses with pH. Even more, these compounds produced AgNPs with varying sizes of particles, agglomeration form and even residual capping agents.

The present study recommends utilizing the produced nanoparticles as antibacterial, anticancer, antifungal or for ink with conductive properties [20, 74-79]. The residual thymol may act as an antibacterial agent beside the AgNPs. Further studies could investigate the syntheses of green silver nanoparticles with the same natural compounds at varying conditions of heat, time, light, and pH or other conditions.

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