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Effect of Green synthesis silver nanoparticles from five fruits peel on protein capped and anti-fungal properties

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Abstract

In current study, synthesis of AgNPs through the reduction of aqueous silver nitrate (AgNO3) by using different peel of the fruits (Banana, pear, orange, mandarins and kiwi).The synthesis of nanoparticles by pear peel and kiwi peel has been monitored by UV–Visible spectroscopy (UV–Vis) and the characteristic surface plasmon resonance peaks were identified to be 417 and 422 nm. respectively. The morphology of the silver nanoparticles was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) , Fourier transform infrared spectroscopy (FTIR), was used to key out the specific functional groups responsible for the reduction of silver nitrate to form silver nanoparticles and the capping agents present in the waste extract. The stability of the silver nanoparticles was analyzed by zeta potential measurements synthesis of silver nanoparticles by pear peel and kiwi peel were negative eta potential value -15.6 and -20 mV respectively, showed the stability of the silver nanoparticles. The size of silver nanoparticles synthesized by pear peel and kiwi peel were ranged from 5-10 and 18-35 nm respectively by zeta size estimation. These nanoparticles were found to be naturally protein coated. SDS-PAGE analysis displayed the presence of many proteins such as 54, 39 and 11-kDa have responsible for capping and stability of the SNPs. Our results showed that the effect of different size of silver nanoparticles on growth diameter of fungal. It was noticed that the smallest-sized spherical AgNPs demonstrated a better antifungal activity three against tested fungi.

Keywords: Biosynthesized AgNPs, fruits peel extract, characterization, protein capped, antifungal.

Introduction

Varieties of synthetic method has been utilized for the synthesis of silver-based nanoparticles include chemical, biochemical techniques and physical which may have adverse impacts in the medical applications and risks on environment (Sinha et al., 2009). In contrast, green synthesis of AgNPs has many advantages as it is environmental friendly, devoid of contaminants, less cost and no toxic chemicals are required (Mittal et al., 2013; Thirumurguan et al., 2009; Yugang et al., 2003)**.**Silver nanoparticles were synthesized by using biological materials have many properties like a high surface area, smaller in size and high dispersion. At the present time, metal nanoparticles are synthesized using natural sources like honey, plant extracts, fruits and microorganisms (Philip and Rapid 2010)**.** The diverse biomolecules are present in the plant extracts like proteins, enzymes, flavonoids, and cofactors play pivotal role in reducing and capping agents (Tavakoli et al., 2015)**.** The plant-mediated synthesis of nanoparticles is relatively fast as there is no need of maintaining specific media and culture conditions, unlike microbial synthesis.

The recent reports include the biosynthesis silver nanoparticles by plant parts *like Punica granatum* peel (Ahmad et al., 2012), *Citrus sinensis* peel (Kaviya et al., 2011; Kokila et al., 2015), *Annona squamosa* peel (Kumar et al., 2012)**,** Lemon Peel (Nisha et al., 2014),Banana peel(Bankar et al., 2010) and Mango peel Yang and Li 2013). Inasmuch only few reports were present on the biosynthesis of silver nanoparticles from waste plant products. In this study we will synthesis silver nanoparticles by using peel of five different fruits and characterization . The mechanism of metallic nanoparticles biosynthesis using various biological systems have been documented (Harris and Bali 2008). In general biosynthesis of silver nanoparticles depend on the presence silver ions (Ag^+) and reduction of Ag^+ by many enzymes have reducing power inside the cell cytoplasm (Duran et al.,2011). However, many researchers reported that mysterious and knowledge limitations to molecular machinery of proteins for SNPs biosynthesis but produce nanomaterials included various oxidoreductive proteins in the cells (Duran et al., 2005; Ingle et al., 2008; Gade et al., 2008).Identification of proteins responsible for the SNPs biosynthesis would clear mechanism of SNPs production in biological systems (Baker and Tatum 1998).

Materials and Methods

Fruits peel

Collected different fruits peel (Banana, pear, orange, mandarins and kiwi) from Riyadh market.

Preparation of peel extract

Different fruits peels were washed thorough distilled water and incised into small pieces then dried in oven at 70 °C to 72h. Finely 10g were grinded and transferred to 100mL flask containing100mLof deionized water, mixed well and boiled for 5min.The extract was filtered through a cheese cloth to remove insoluble fractions and macromolecules, then filtered throughWhatmanNo.1 filter paper.

Synthesis of AgNPs by using the peel extract:

To synthesize AgNPs, 10 mL of fruits peel extract was mixed with 100mL of aqueous solution ofAgNO3 (1 mM) . Rapidly reduction occurs as indicated by brown color after 60 min at room temperature indicating the formation of the AgNPs. The 1 mM silver nitrate solution without any addition of extract was used as control.

Characterization of synthesized silver nanoparticles

The UV-Visible spectra of silver nanoparticles were recorded as a function of wavelength using UV Vis spectrophotometer (Victoria, Australia)) and compared with 1 mL of distilled water as a blank over the range 200 - 1000 nm operated at resolution of 1nm .

The shape and size of silver nanoparticles were determined by SEM and TEM. For SEM and elemental analysis the dried reaction mixtures were subjected to JSM-7610F. SEM operating at 50 kV. For TEM, one drop of sample was loaded on a carbon coated copper grid and it was leaved to dry at room temperature, the micrographs have been obtained using TEM (JEM (1400 plus) operating at 100 kV. The electron diffraction pattern for a selected area was also recorded. The average particle size and size distribution were measured using Image J 1.45s software 1493. Particle size and zeta potential were determined using Zetasizer Nano series, HT Laser, ZEN3600 (Molvern Instrument, UK). The analysis was carried out at a scattering angle of 90 at 25C using samples diluted to different intensity concentration with deionized water. FT-IR measurements were carried out using Nicolet 6700, (Thermo Electron Corporation, USA). FT-IR spectrophotometer by employing KBr pellet technique. All measurements were achieved in the range of 400– 4000 cm^{-1}.

Anti-fungal activity of silver nanoparticles against three different fungi

150 ppm of silver nanoparticle were added to autoclaved potato dextrose agar (PDA), keeping one as Control (PDA without Silver nanoparticle). A disc (6 mm) of mycelia, taken from the edge of 7-day-old fungal cultures, was placed in the centre of each Petri dish containing the PDA culture medium containing silver nanoparticle and incubated at 25±2 °C. The efficiency of silver nanoparticle treatment was evaluated after control competed by measuring the fungi colonies diameters. Each treatment replicated three times. The inhibition rate (%) was calculated by using the following formula:

$$\text{Inhibition \%} = \frac{R - r}{R} \times 100$$

where R is radial growth of fungi in control plate and r is the radial growth of fungi in silver nanoparticle treated plates.

Statistical analysis

All of the data from three independent replicate trials were subjected to analysis using Statistical Package for the Social Sciences (SPSS) 10.0 statistical software (Chicago, USA). The data are reported as the mean ± standard deviations.

Isolation of extracellular proteins

One g of peel plant and 5 ml of sterile deionized water were added. The mixture was agitated overnight at 4°C on a shaker followed 10 min at 4°C by centrifugation at 10,000 rpm . The cell-free filtrate containing the extracellular proteins was analyzed by one-dimensional SDS-PAGE. Filtrate was boiled (95°C for 5 min) with 1% sodium dodecyl sulfate (SDS) solution for 10 min followed by centrifugation at 8,000 rpm for 10 min then supernatant collection for isolation the protein. The protein content of this solution was quantified by Bradford assay. 100 µg of the soluble proteins were heated in 4% β-mercaptoethanol (βme) and 2% SDS at 95°C for 5 min.

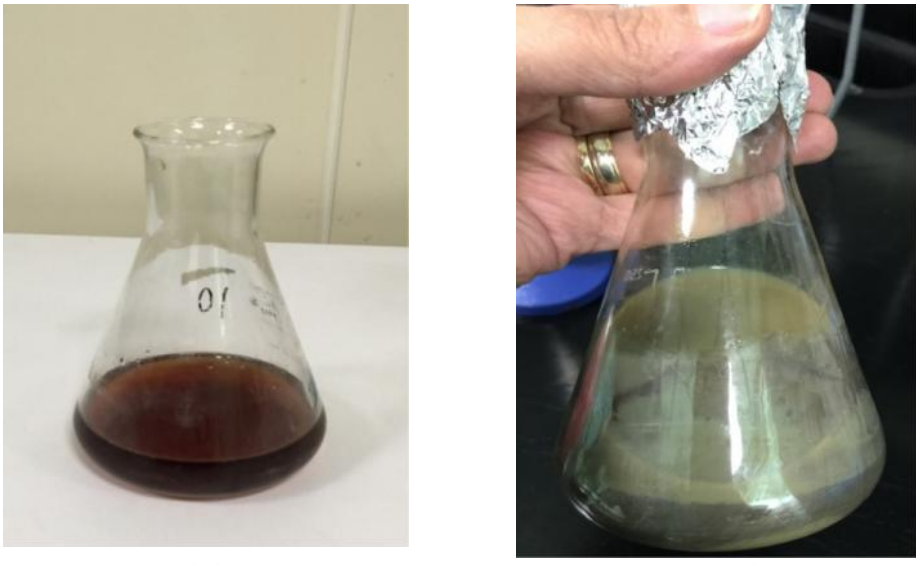
Isolation proteins from silver nanoparticles solution

Silver nanoparticles which synthesis by five tested peels were boiled (95°C for 5 min) with 1% sodium dodecyl sulfate (SDS) solution for 10 min followed by centrifugation at 8,000 rpm for 10 min then 4% βme and 2% SDS were added to supernatant. Super signal molecular weight protein used as (Thermo fisher scientific) molecular marker.

Results and Discussion

Biosynthesis of silver nanoparticles

After addition of AgNO3 into peels extract, the color changed to dark brown with intensity increasing during period of time which confirms the formation of nanoparticles (Ahmad et al., 2012; Nisha et al., 2015). Fig. 1a show the flasks containing the filtrate of the peel extract in aqueous solution of 10^{-3}M AgNO3 after 24 hr. of reaction, check sample (without silver ions) showed no changing in the color under the same conditions. Deposition of silver nanoparticles on the inner surface of the flask after 2week was appeared in Fig. b.



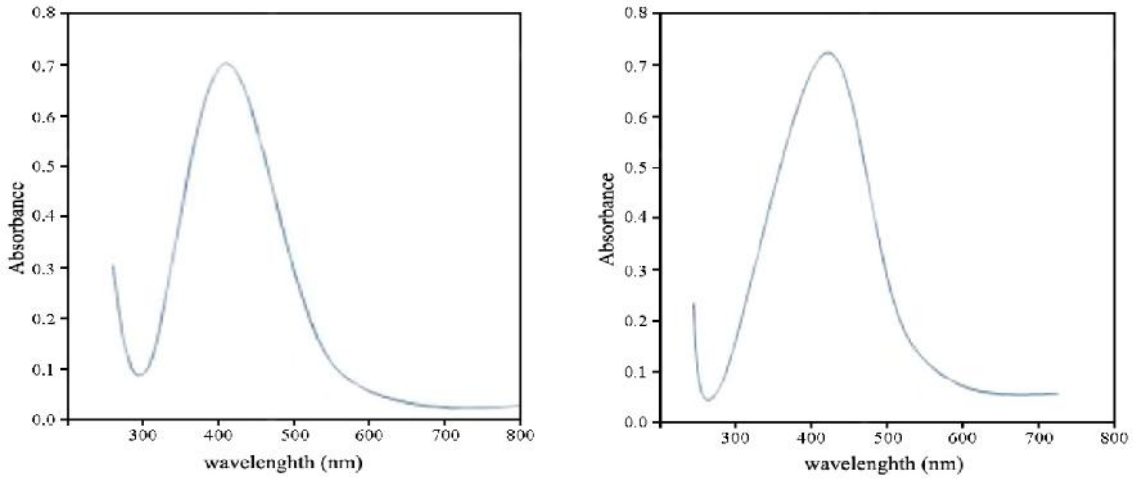
(A) **(B)**

Figure 1. Picture of two flasks containing the pear peel extract in aqueous solution of 10-3 M $AgNO_3$ at the beginning of the reaction (A) and after 24h. of reaction (B).

UV-Vis Spectroscopy

UV–visible spectroscopy is technique to check formation and stability nanoparticles which produced by fruit peel extract. After 24 hours of extract addition, the color of reacting turned into light brown from colorless solution due to formation of colloidal nanoparticles. Figure 2. Showed the UV-Vis spectra recorded for the reaction of pear peel extract (A) and kiwi peel extract (B) with $AgNO_3$ solution.

The synthesis of nanoparticles by pear peel and kiwi peel has been monitored by UV–Visible spectroscopy (UV–Vis) and the characteristic surface plasmon resonance peaks were identified to be 417 and 422 nm. respectively. Which may correspond to the surface plasmon resonance of colloidal Ag nanoparticles (Bankar et al., 2010; Ahmad et al., 2012; Kumar et al., 2015). Absorption bands in the range 400–420 nm and presence in single peak in the UV-Vis spectrum correspond to spherical-shaped metallic nanoparticles **(**Perni et al., 2014**).** Similar kind of results were observed by (Dubey et al., 2010; Roy et al., 2013).



(A) **(B)**

Figure 2. The UV-Vis spectra recorded for the reaction of pear peel extract (A) and kiwi peel extract (B) with AgNO3 solution.

FTIR spectroscopy analysis

FTIR spectroscopy is useful to determine the functional groups on peels extract, FTIR analysis is shown. Fig. (3). The IR spectrum of peel extract showed the various peaks in the range of 3267.85,2207.38,2159.49,2118.90,2015.59,2004.04,1979.69 , 1,635.30 and 437.59 cm-1 . The broad band appearing at 3267.85 cm-1 may assigned the participation of O–H group in the synthesis of nanoparticles. These bonds might be due to the stretching of -OH in enzymes, proteins or polysaccharides present in the extract (Baker and Tatum 1998). The band at 2207.38 might be referred to thiol group (ASH) vibration of L-cysteine amino acid (Solgi and Taghizadeh 2012). The free thiol groups present in the proteins were answerable for the reduction and capping silver nitrate to silver nanoparticle formation Mishra and Sardar 2012; Khatami et al., 2015). The weak bands at 2159.49, 2118.90, 2015.59, 2004.04 indicates carbonyl specific absorption. The peak located at 1,635.30 cm-1 maight be assigned to the C = O stretching in carboxyl or C = N stretch vibrations as well as bending in the amide group. The broad peaks around 437.59 cm-1 are related to AgNPs bonding with oxygen from hydroxyl groups (Bankar et al.,2010; Dubey et al., 2010; Mishra and Sardar 2012) **.** Amino acid and peptides lead to prevent agglomeration by configured a coat which covering the silver nanoparticles as well as may be the possible reason of their stabilization Roy et al.,2013; Abd El-Aziz 2014).

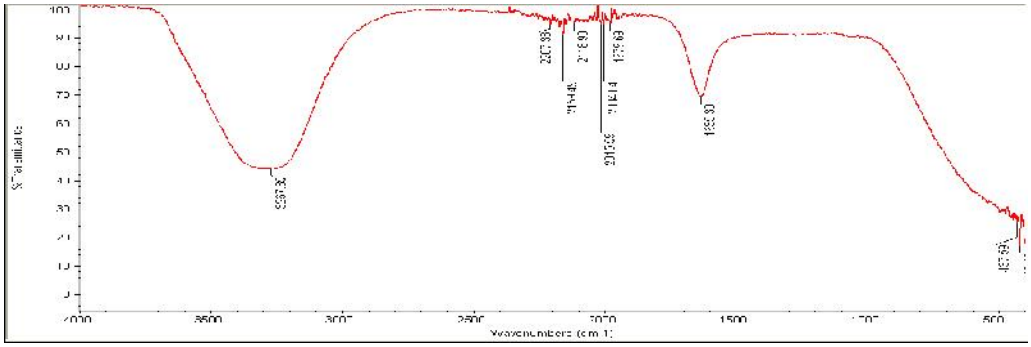
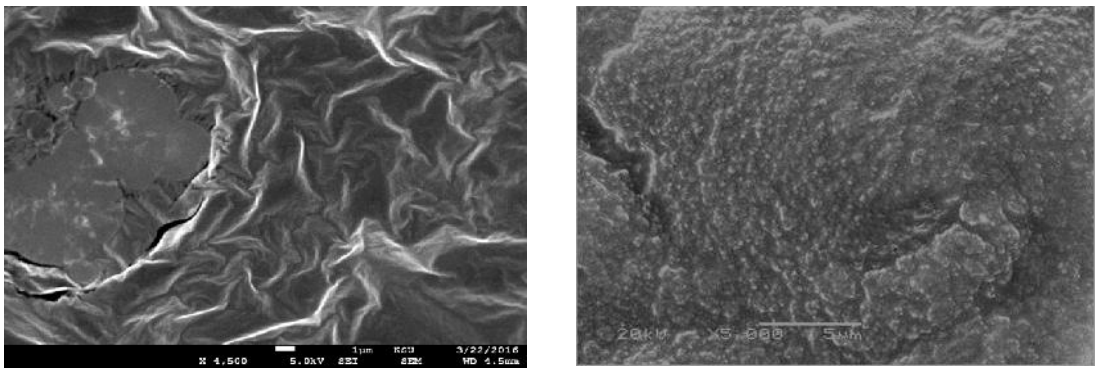


Figure 3: FTIR spectra of silver nanoparticles Synthesis by pear

SEM analysis of SNPs

The surface morphology and topography of the SNPs were examined by scanning electron microscopy (Fig. 4). Well defined spherical SNPs without any agglomeration. The SEM image showing the high density silver nanoparticles synthesized by the different peels extract (Wang et al., 2009; Solg and Taghizadeh 2012).

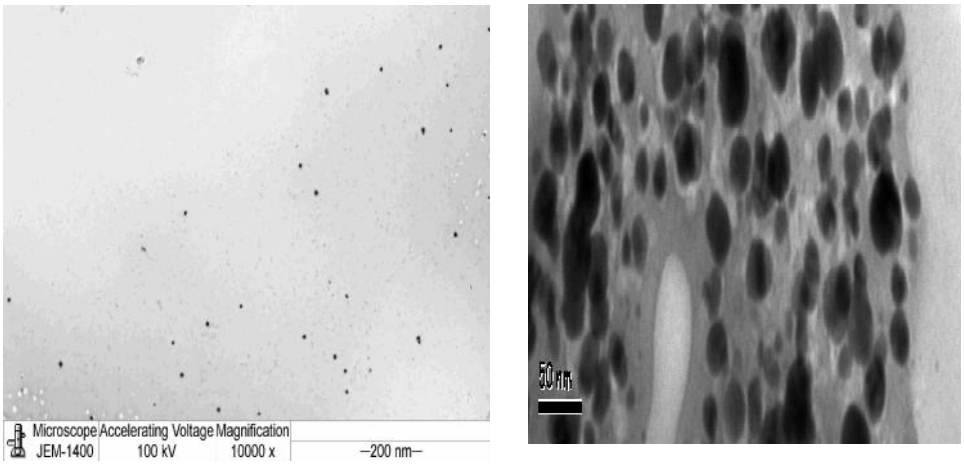


(A) **(B)**

Figure 4: SEM image of silver nanoparticles synthesized *(A)* by *pear peel extract (B)* by *kiwi peel extract*

TEM analysis of SNPs

TEM was utilized to vision the sizes and shapes of Ag nanoparticles. Most of the nanoparticles formed were spherical, and without any agglomerated nanoparticles were observed in Fig. 5. The size and shape of the biosynthesized nanoparticles depend on the plant type (Abd El-Aziz 2014; Kumar et al., 2015; Khatami; 2015).



(A) **(B)**

Figure 5: TEM image of silver nanoparticles synthesized *(A)* by *pear peel extract (B)* by *kiwi peel extract*

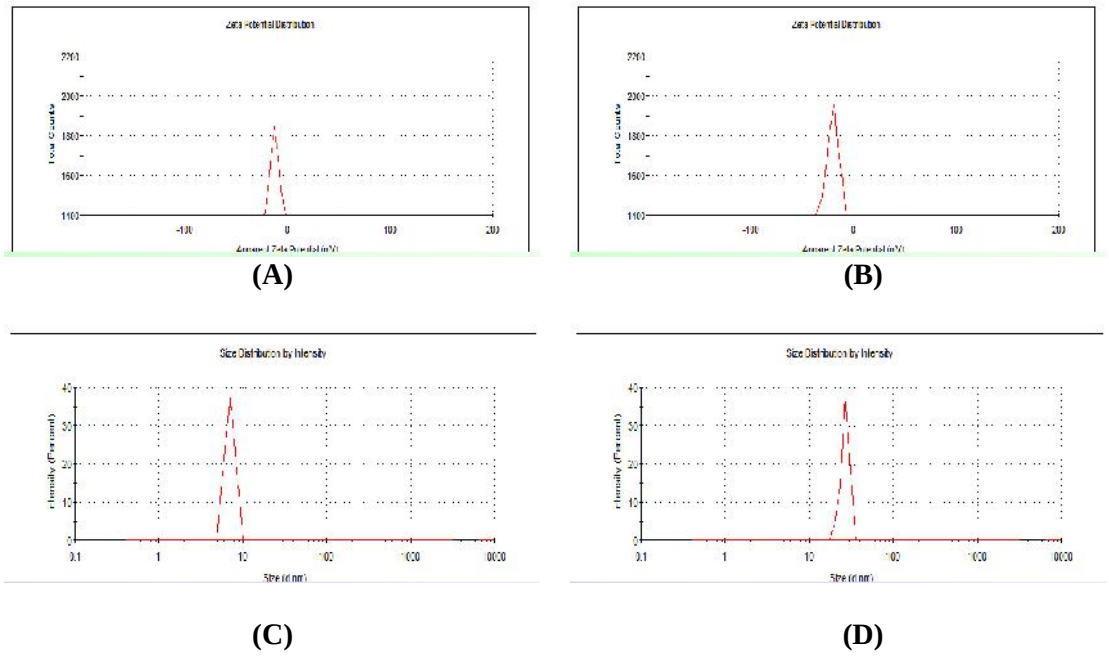
Particle size and zeta potential

The size and zeta potential of the nanoparticle suspension were analyzed using the Zetasizer analysis is depicted in Table 1 and Figure 6a. Which observed the particles size were ranged from 5 to 35 nm. The average size of the synthesized silver nanoparticles using pear, banana, orange, mandarin and kiwi peel extract were 8,21,15,13 and 25 nm. respectively. Sizes and shapes of metal nanoparticles are influenced by a number of factors including pH, temperature as well as method of preparation (Umoren et al., 2015).

Table 1: Average particle size and zeta potential values measured with a Zetasizer Nano

Friuts peel	Size (nm)	Zeta potential (mV)
Banana	21	±11.4
Pear	8	±15.6
Orange	15	±17.3
Mandarins	13	±16.9
Kiwi	25	±20

Zeta potential results are shown in Figure 6b the indicated that the surfaces of silver nanoparticles produced by peel extracts (pear, banana, orange, mandarin, kiwi) had a negative charge of approximately -15.6,-11.4, -17.3 ,-16.9 and -20 mV, The negative value indicated the stability of the nanoparticles and it evaded the agglomeration of nanoparticles (Patil et al., 2014). The result is consistent with the silver nanoparticles synthesized from the leaf extract of *F. religiosa* (Antony et al., 2013). The negative potential value shown by AgNPs could be due to the capping of the bio-organic components present in the extract (Edison and Sethuraman 2012; Parameshwaran et al., 2013). The zeta potential is the electrical superficial charge of particles and is influenced by the particle composition (Singh and Lillard 2009).



(A) **(B)**

(C) **(D)**

Figure 6: (A) Zeta potential of silver nanoparticles Synthesis by pear showed 15.6 mV. (B) Zeta potential of silver nanoparticles Synthesis by kiwi showed 20 mV.(c) Zeta particle size of silver nanoparticles Synthesis by pear showed a range of 5-10nm. (D) Zeta particle size of silver nanoparticles Synthesis by kiwi showed a range of 25-35nm.

Anti-fungal activity of silver nanoparticles against three different fungi

Our results showed that the effect of different size of silver nanoparticles on growth diameter of fungal. It was noticed that the smallest-sized spherical AgNPs demonstrated a better antifungal activity three against tested fungi. In general, inhibition % ranged maximum (87.3%) at silver nanoparticles which produced by pear peel whereas inhibition % was the lowest rate at silver nanoparticles which produced by kiwi peel. The smaller AgNPs showed better inhibitory action because a significantly large surface area was in contact with the microorganisms effluent owing to the larger surface to volume ratio as compared to larger l AgNPs. Thus, smaller particles released more silver ions than larger particles to kill more microorganisms (Xiu et al., 2011; Pal et al., 2007).

Table 2 Effect of silver nanoparticles on inhibition percentage of *A. niger*, *A. flavus* and *F. solani.*

Treatment	Inhibition %		
	Aspergillus niger	*Aspergillus flavus*	*Fusarium solani*
Banana	61.4±0.28	65.1±0.29	67.6±0.14
Pear	78.1±0.39	81.3±0.34	87.3±0.42
Orange	63.6±0.22	67.3±0.24	70.5±0.39
Mandarins	71.2±0.31	73.0±0.18	76.8±0.24
Kiwi	50.3±0.17	53.1±0.21	57.6±0.18
Control	0.0±0.00	0.0±0.00	0.0±0.00

Values in the same column followed by (±) are significantly different (P = 0.05). The presented data are the mean (n = 3) ± standard error of three replicates.

Identification of SNPs proteins were analysis by SDS-PAGE which appearing and the protein bands (Figure 7). Five proteins were identified from SNPs biosynthesis associated proteins were the part of oxido-reductive reactions and showed involvement in ATP synthesis. Histone (H4) (11.4 kDa) was appeared in all plants, Superoxide dismutase (SOD) (23.9 kDa) was appeared in three plant. Table (3) showed summery for major SNPs associated proteins. Our experimental finding of histone (H4) protein association with biosynthesized SNPs indicates the important role of H4 protein in capping SNPs in biological system (Barwal et al., 211). The H4 is well known for the reductant of silver ammonia complex into SNPs and lysine rich-binding domain (Black et al., 1966; Zoroddu et al., 2000). The H4 contribute significantly to redox reaction activity and SNPs synthesis is this domain. The previous reaction played important part to converting Ag^{+} into SNPs (Cassan et al., 2005). Also, superoxide dismutase (SOD) protein binding of with SNPs and reduction of Ag+ to SNPs. Recently, superoxide (O_2^{-}) is able to reduce Ag^{+} to Ag^{0} Further, this step might be sufficient for nucleation step in SNPs synthesis (Jones et al., 2011).

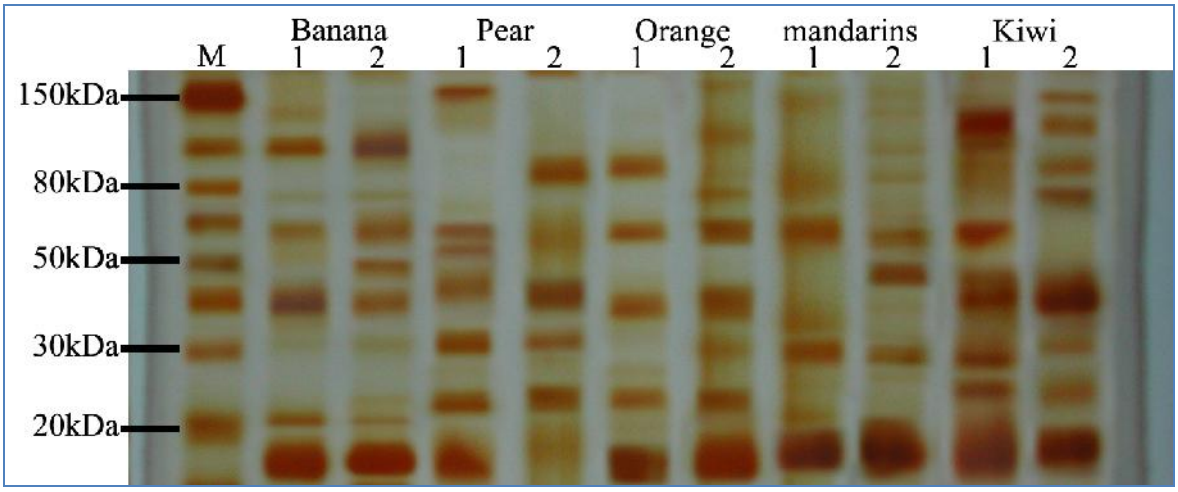


Fig (7) Cellular protein profiling of (1) peel plant extract (2) biosynthesized SNPs from peel plant extract of banana, pear, orange, mandarins and kiwi.

Table 3 Summery for major proteins associated silver nanoparticles

Proteins	MW	Banana		Pear		Orange		Mandarin		Kiwi	
		1	2	1	2	1	2	1	2	1	2
ATP synthase subunit alpha	54	+	+	-	-	+	+	+	+	+	+
Ferredoxin–NADP reductase	39	+	+	-	-	+	+	-	-	+	+
Oxygen evolving enhancer protein (OEE) 1	30	-	-	+	+	-	-	+	+	-	-
Superoxide dismutase	23	-	-	+	+	+	+	-	-	+	+
Histone H4	11	+	+	+	+	+	+	+	+	+	+

Proteins of plant extract (1) and proteins of plant extract nanoparticles (2)

Conclusions

We have carried out production of silver nanoparticles as safe and economically viable by successfully synthesized using five fruits peel extract. The morphology of the silver nanoparticles was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). TEM and SEM were utilized to vision the sizes and shapes of Ag nanoparticles. Most of the nanoparticles formed were spherical, and without any agglomerated nanoparticles were observed. Fourier transform infrared spectroscopy (FTIR) was used to key out the specific functional groups responsible for the reduction of silver nitrate to form silver nanoparticles and the capping agents present in the waste extract. The stability of the silver nanoparticles was analyzed by zeta potential measurements synthesis of silver nanoparticles by pear peel and kiwi peel were negative zeta potential. Silver nanoparticles have a high antimicrobial activity and used for inhibition three fungal growth (*A. niger*, *A. flavus* and *F. solani*). Detection of proteins capping and stability SPNs from five plants as model system.

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