



## Original article

# Biosynthesis of silver nanoparticles by using of the marine brown alga *Padina pavonia* and their characterization

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## ABSTRACT

Silver nanoparticles (AgNPs) are gaining considerable importance due to their attractive physicochemical properties for many applications. In the present study, (Ag NPs) were synthesized by the reduction of aqueous solutions of silver nitrate (AgNO<sub>3</sub>) with powder and solvent extracts of *Padina pavonia* (brown algae). The obtained nanoparticles exhibited high stability, rapid formation of the biogenic process (2 min -3 h), small size (49.58–86.37 nm) (the diameter of formed nanoparticles was measured by TEM and DLS) and variable shapes (spherical, triangular, rectangle, polyhedral and hexagonal). Preliminary characterization of nanoparticles was monitored by using UV–visible spectroscopy (UV–vis), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and finally by Fourier Transform Infrared spectroscopy (FTIR). The ratios of converted Ag NPs were recorded as 88.5; 86.2 and 90.5% in case of *P. pavonia* powder, extract and chloroform extract, respectively.

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## 1. Introduction

The novel properties of chemical nanoparticles are highly sought after in a number of new and existing industries. The application of chemical methods in synthesis of nanoparticle have shown limited success and substituted by the use of a biological approach to overcome many of the obstacles (Dhas et al., 2012). So, the bioreduction method as a novel nanoparticle synthesizing technology has attracted increasing attention because it overcame the disadvantages of conventional chemical methods such as formation, monodispersity of the particles and thermodynamic stability (Li et al., 2009). Furthermore, the bioreduction green chemistry

processes led to environmental friendly synthesis and safe process as compared to other methods (Mohanpuria et al., 2008; Ibraheem et al., 2016). Biological sources such as marine algae can catalyze specific reactions as a part of modern and realistic biosynthetic strategies (Govindaraju et al., 2008). These can be alternatives to other non-biological methods which used ultraviolet, irradiation, lithography, laser ablation, ultrasonic fields and photochemical reduction techniques. The use of marine natural products in the synthesis of metallic nanoparticles has the wide range of applications in the field of nanotechnology. Biogenic synthesis of NPs prolongs superiority over the chemical processes, whereas the biogenic synthesis doesn't need to use energy, temperature, high pressure or toxic chemicals (Parashar et al., 2009). The use of biological source like the brown alga *Padina pavonia* for a synthesis of AgNPs submits numerous benefits for pharmaceutical and biomedical applications as they do not use toxicity. Marine macroalgae have various chemicals such as flavonoids, alkaloids, steroids, phenols, polysaccharides, saponins, hydroxyl, carboxyl, and amino functional groups, which can serve as effective metal-reducing and capping agent's to provide a robust coating on the metal nanoparticles in a single step (Abdel-Raouf et al., 2017). The present study aid to the synthesis of AgNPs by using of the marine macro alga *Padina pavonia*.

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## 2. Materials and methods

### 2.1. Collection and preparation of algal material

The brown alga *Padina pavonia* (commonly distributed at Umluj seashore) was collected from marine water seashore, Umluj coast of Saudi Arabia during our previous studies in this region (Ibraheem et al., 2014). Collected sample was immediately brought to the laboratory in new plastic bags containing pond water to prevent evaporation. The algal material was washed thoroughly with tap water and distilled water to remove extraneous materials and shade-dried for 5 days and oven dried at 60 °C until constant weight was obtained, then was grind into a fine powder using the electric mixer and stored at 4 °C for future use.

### 2.2. Biosynthesis of silver nanoparticles by algal powder

Formation of Ag<sup>0</sup> was carried out according to Meena et al. (2007) by mixing 1 g of *P. pavonia* dry matter in a 250 ml Erlenmeyer flask with 100 ml of 10<sup>-3</sup> M AgNO<sub>3</sub> aqueous solution. The bioreduction of AgNO<sub>3</sub> ions occurred within 3hr in light at room temperature with the continuous stirring condition. The change in color was envisaged as the evidence of bioreduction.

### 2.3. Biosynthesis of silver nanoparticles by ethanol and chloroform extracts

The extraction was achieved using two different solvents separately (Ethanol and Chloroform) where; the dry algal biomass was crushed and then fed to a Soxhlet extractor. The extraction was executed on a water bath for 20 h using 200 g of algal powder in a 1 L solvent. After them, all crude extracts were collected and filtered through Whatman No. 1 filter paper and the solvents of the filtrate were evaporated by rotary evaporator under vacuum (50 °C). The resulting dried sample was crushed into powder. Two hundreds mg of the algal extract was added to 100 ml of 10<sup>-3</sup> M aqueous AgNO<sub>3</sub> solution and kept at room temperature for 2 min with stirring. After the reaction, the residual solution was centrifuged at 5000 rpm for 10 min (Meena et al., 2007).

### 2.4. Characterization of silver nanoparticles

Characterization of the purified particles was carried out according to the method described previously (Kalimuthu et al., 2008; Kalishwaralal et al., 2008) as following:

#### 2.4.1. UV-visible spectroscopy analysis

The color change in the reaction mixture of the silver nitrate solution with *Padina pavonia* (powder or extract) was recorded through visual observation and the reduction of Ag<sup>+</sup> ions was recorded by UV-vis spectrum. The analysis was done using UV-vis spectrophotometer (Shimadzu UV-1601PC) in the range of 300–700 nm. NPs solution was centrifuged at 10,000 rpm for 15 min for three times then washed with deionized water for further purification to get the pure form. The sample was completely dried at 60 °C.

#### 2.4.2. TEM analysis

The structural characterization of the silver nanoparticles was carried by Transmission Electron Microcopy using JEOL JEM 1011 high-resolution transmission electron microscope (Japan). The sample was prepared by dispensing a drop of silver nanoparticles solution directly onto a carbon coated copper grids and allowed to dry completely at room temperature and the size and morphology of the AgNPs were characterized.

#### 2.4.3. Dynamic Light Scattering measurements (DLS)

Size average formed algal AgNPs was evaluated by using of Dynamic Light Scattering (DLS) studies (Malvern-Zetasizer, Nano-Z590 instrument, United Kingdom).

#### 2.4.4. FTIR measurement

Identification of the possible biomolecules responsible for the reduction of the Ag ions and capping of the reduced AgNPs synthesized by algal powder or extract was recorded by Fourier Transforms Infrared Spectroscopy (FTIR). Silver nanoparticles powder samples were prepared by centrifuging the synthesized Ag nanoparticles solution at 14,000 rpm for 10 min. The pellet which contains AgNPs was re-dispersed with sterile deionized water three times to get rid of the unattached biological impurities and remove the free proteins/enzymes (to get rid of any impurities) that are not capping ligands for the silver nanoparticles. The samples were dried in an oven overnight at 60 °C and grinded with KBr pellets and analyzed on a NICOLET 6700, Thermo, USA, in the diffuse reflectance mode operating at a resolution of 4 cm<sup>-1</sup>.

## 3. Result and discussion

### 3.1. Synthesis and characterization of AgNPs synthesized by *P. pavonia*

The formation of AgNPs was visually approved by color change (Plate 1) from colorless (A) to yellowish brown (B), or brownish black color (C and D). The formation AgNPs was very fast (2 min and 3 h) for algal extracts and powder, respectively and keep stable in its color for a long time at room temperature. These results are in harmony with many researchers (Gopinath et al., 2013) who advertise that the appearance of brownish black color in solution suggested the formation of silver nanoparticles. But in the current work, the algal nanoparticles recorded high stability and formed within a short time compared with that formed in the other works. In this respect, Abdel-Raouf et al., reported that the colure of *Galaxaura elongate* extracts changed to intense brown after 3hrs of incubation with AgNO<sub>3</sub> (Abdel-Raouf et al., 2017).



**Plate 1.** Tubes containing the aqueous solution of 10<sup>-3</sup> M of AgNO<sub>3</sub> with colorless at the beginning of reaction (A), after adding 1 g of *P. pavonia* powder with color change to yellowish brown color within 3 h (B), after adding 200 mg of *P. pavonia* ethanolic extract with color change to brownish black color within 2 min (C) and after adding 200 mg of *P. pavonia* chloroform extract with color change to dark brown color within 2 min (D).

### 3.2. Characterization of synthesized AgNPs

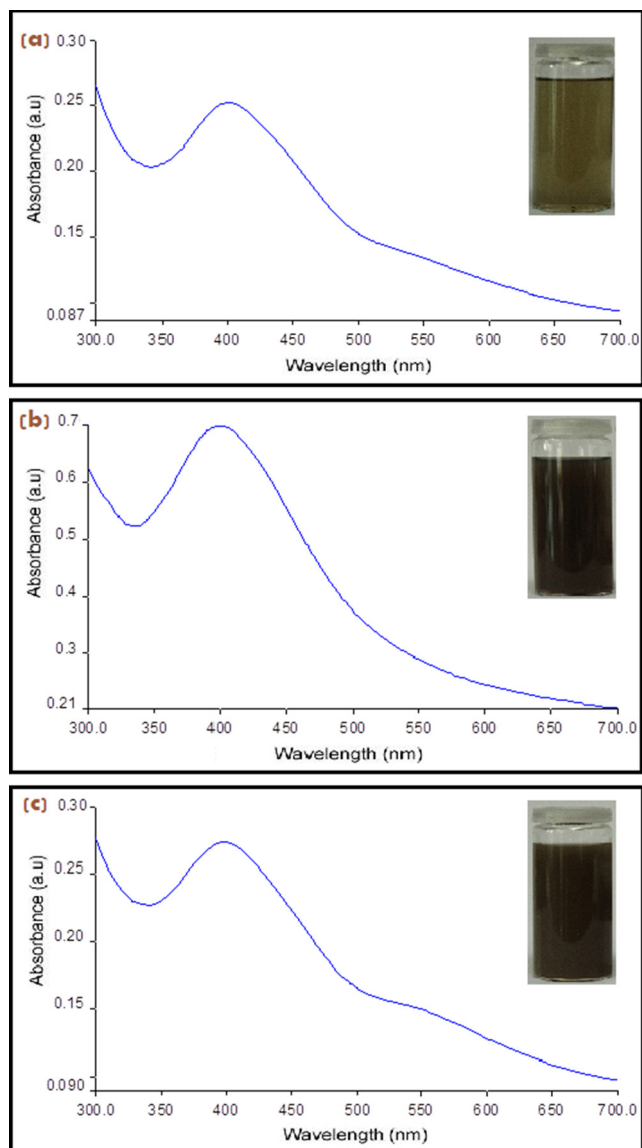
#### 3.2.1. UV-visible spectroscopy analysis of AgNPs synthesized by *P. pavonia*

The UV-visible spectroscopic analysis is an honorable technique to achieve the formation and stability of metal nanoparticles in aqueous solution. UV-visible spectra of the AgNPs synthesized by algal powder indicated that AgNPs surface Plasmon band occurs in the range of 400–500 nm in an aqueous medium (Fig. 1a). It is observed that the silver surface Plasmon resonance band of NPs in alga powder occurs at 401 nm after 3hrs of reaction and steadily increases in intensity as a function of reaction time. Also, UV-visible absorption spectra in Fig. 1b&c indicated a strong plasma resonance bands that are located at 408 and 400 nm for AgNPs formed by both *P. pavonia* ethanol and chloroform extracts, respectively. AgNPs have free electrons which give rise to an SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave (Dubey et al., 2010). Sharma et al. reported that the frequency and width of the surface Plasmon absorption be based on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the

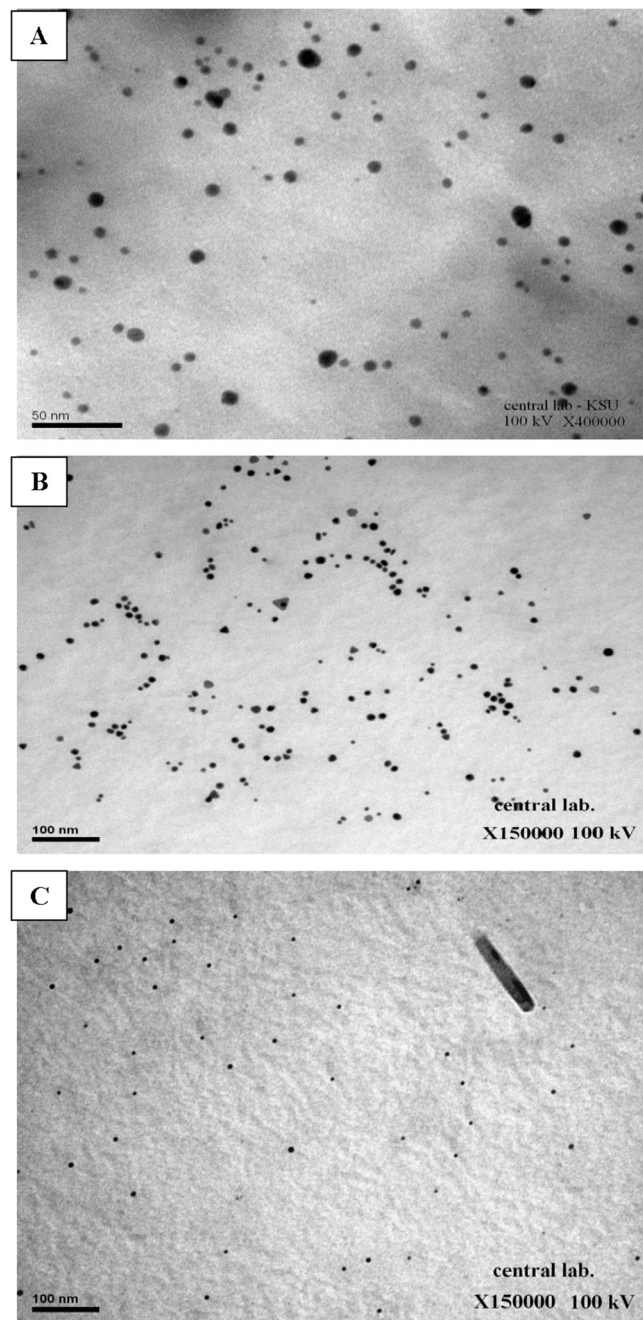
surrounding medium (Sharma et al., 2009). It is interesting; the formed *P. pavonia* nanoparticles were highly stabilized with no complexity of the formed AgNPs (see Fig. 1). The resulting resonances were severe and indicating no evidence for accumulation of the AgNPs in the three studied cases (powder and ethanol or chloroform extracts). It is clear from the previous results that, the alga under investigating contains some reducing agents which releasing into solutions and responsible for the formation of AgNPs.

#### 3.2.2. TEM and DLS analyses of the synthesized AgNPs by *P. pavonia*

TEM and Zetasizer measurements were applied to characterize the nanoparticles size and morphology. TEM micrograph of representative silver nanoparticles synthesized by powder, ethanol and chloroform extracts of *P. pavonia* are shown in Plate 2 A, B, and C.



**Fig. 1.** UV-visible rang spectra of AgNPs synthesized from (a) *P. pavonia* powder, (b) ethanol extract and (c) chloroform extract of *P. pavonia*.



**Plate 2.** TEM micrographs of AgNPs synthesized by (A) *P. pavonia* powder, (B) ethanol extract of *P. pavonia* and (C) chloroform extract of *P. pavonia*.

The images were recorded at different magnifications. These results showed that ethanol extract of *P. pavonia* strongly affected the size and shape of the silver nanoparticles. Plate 2A revealed that silver nanoparticles produced by *P. pavonia* powder were more uniform mostly spherical and polygonal in shape with size

ranging from 0.5 to 50 nm. These results confirmed by the characteristic peak observed in Zetasizer (Fig. 2i) which indicated the average size of silver nanoparticles of *P. pavonia* powder was 86.3 nm. Plate 2B appeared that the silver nanoparticles formed from the ethanol extract of *P. pavonia* were not uniform

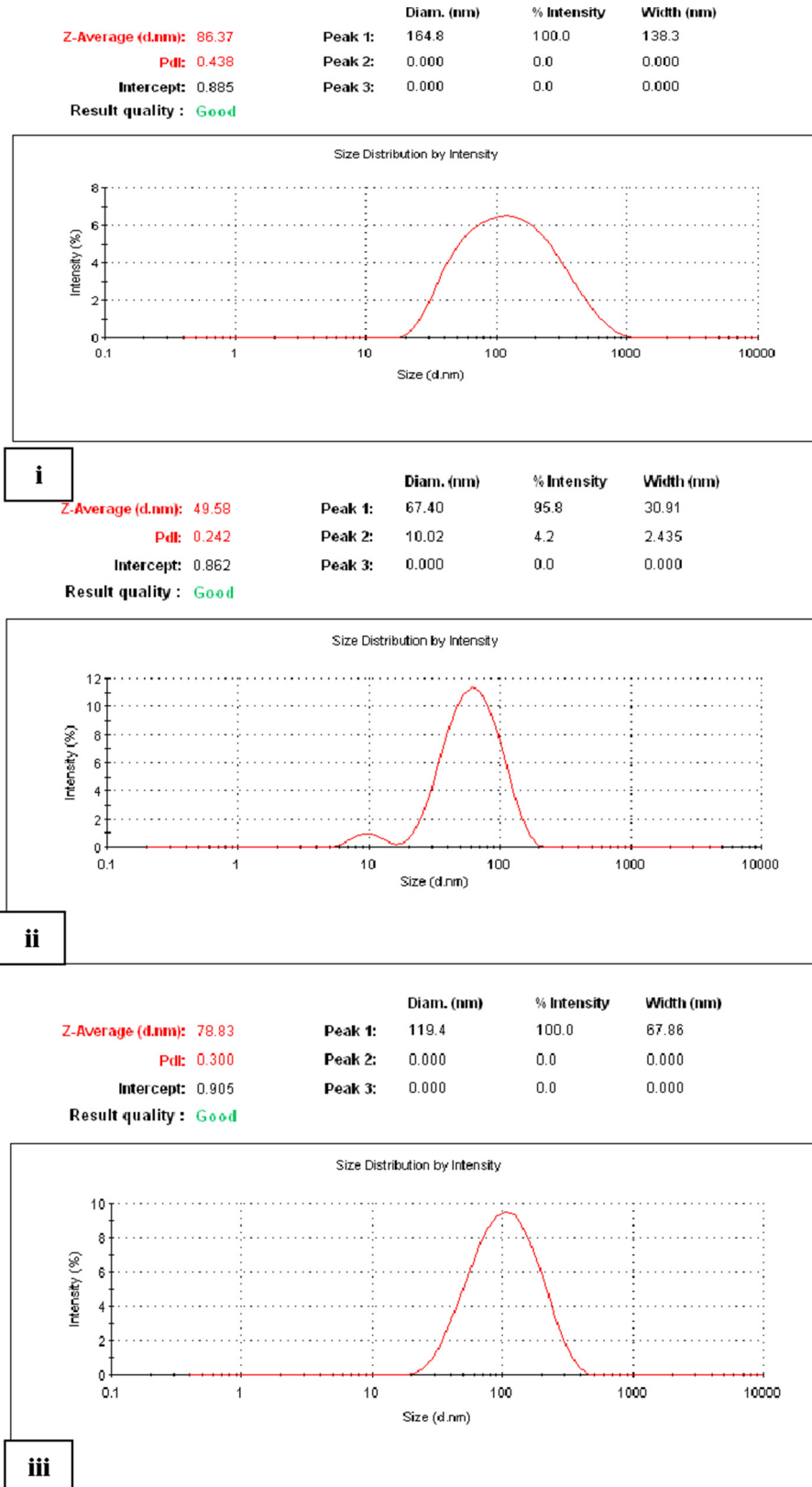
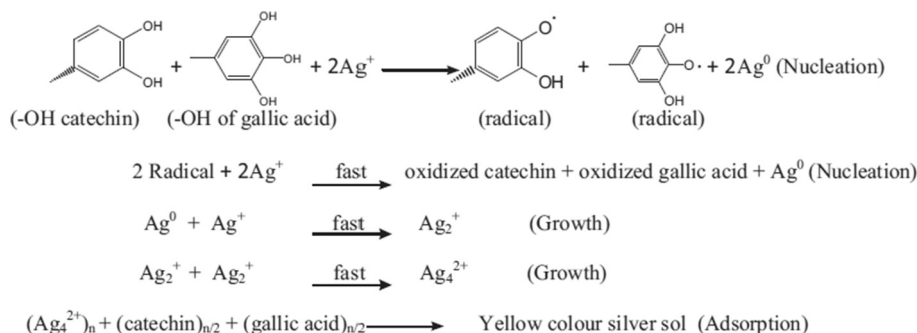


Fig. 2. Zetasizer illustration of AgNPs produced by (i) *P. pavonia* powder, (ii) ethanol extract and (iii) chloroform extract of *P. pavonia*.

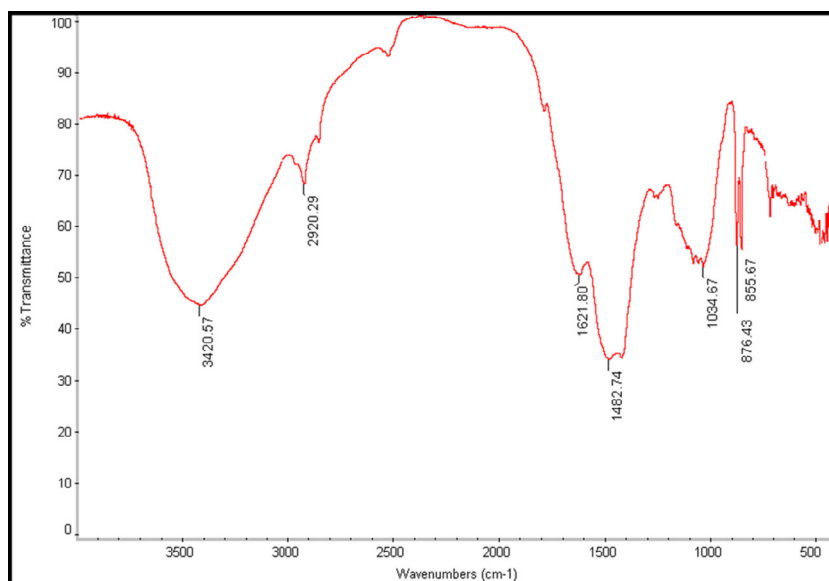


in size and shape. The nanoparticles were polydispersed with many different shapes such as pyramidal, spherical, polygonal, rod and hexagonal with highly smooth edges. The previous image contains SNPs in smaller size ranging from 1 to 49.5 nm according to TEM and Zetasizer estimation, respectively (Fig. 2ii). It is known that polygonal and pyramidal nanoparticles more active than the spherical shape because they have more active sites than the spherical shape. Also, Plate 2C of TEM observed that the smaller size of chloroform AgNPs at 0.8 nm in regular spherical shape with average size 78.8 nm which measured by Zetasizer (Fig. 2iii). These results indicated that the powder, ethanol and chloroform extracts of *P. pavonia* strongly affected the size and shape of the silver nanoparticles we hypothesize that the bioactive constituents of the studied alga play a pivotal reducing and controlling role in the formation of AgNPs in the solution. Ethanol and chloroform extracts of *P. pavonia* contain an amide group, carboxylation of the amino acids aspartate, glutamate and fatty acids. In addition to we conclude that the presence of powder and extracts of the studied alga in solution prevented the formation of aggregates as a result of algal bioactive constituents induced stabilization under the experiment. These results were confirmed and supported by the authors through chemical analyses for the same algal extracts by HPLC and GC-MS (Alenazi, 2013) who showed the presence of high percentages of Neophytadiene (41.82 and 57.8%) as a terpenoids and trans-phytol (21.6 and 16.9%) as diterpenes in ethanol and chloroform extracts, respectively. These results in agreement with Plaza et al., who

reported that macro marine algae especially, brown algae contain high values of natural functional compounds as terpenes, alkaloids amino acids and fatty acids which act as stabilizers and prevent the formation of nanoparticles aggregations (Plaza et al., 2010). *Padina pavonia* as recorded by Alenazi contains high values of bioflavonoids as Epigallocatechin (EGC) 78.2, Epicatechin (EC) 10.69, Catechin (C) 7.9, Gallic acid 2.96 and Epigallocatechin gallate (EGCG) 2.62 ppm (Alenazi, 2013). These bioflavonoids are known as excellent stabilizing and reducing agent to reduce  $\text{Ag}^+$  to corresponding  $\text{Ag}^0$  nanoparticles. These reactions proceed to complete within 2 min and 3 h for algal extracts and powder, respectively. So, it was the novelty of our study that the identification of the compounds which were responsible for the synthesis of silver nanoparticles and act as reducing, stabilizing and capping agent. On the basis of above results, a possible mechanism was proposed to the reduction of  $\text{Ag}^+$  ions by the catechin group and gallic acid of *P. pavonia* extracts. In Scheme 1, first reaction nucleation, represent the reduction of  $\text{Ag}^+$  ions into metallic  $\text{Ag}^0$  by  $-\text{OH}$  of catechin group and gallic acid which were main constituents of extract (one step one electron oxidation – reduction mechanism to  $\text{Ag}^0$  and radical, nucleation, the rate determining step) (Tamuly et al., 2013). After the slow electron transfer step, reaction growth to adsorption may follow. The complexation of the formation of  $\text{Ag}^0$  atoms with  $\text{Ag}^+$  ions yield  $\text{Ag}_2^+$  ions and  $\text{Ag}_2^+$  dimerize to give yellow solution  $\text{Ag}_4^+$ . The adsorption step was further supported by the presence of a faint layer of organic molecules onto the surface of the Ag nanoparticles.



**Scheme 1.** Mechanism to the reduction of  $\text{Ag}^+$  ions by the  $-\text{OH}$  groups of Catechin group (Epigallocatechin, Epicatechin, Catechin and Epigallocatechin gallate) and Gallic acid of *P. pavonia* extracts.



**Fig. 3.** FT-IR spectrum of *P. pavonia* powder.

### 3.2.3. FT-IR analysis of AgNPs synthesized by *P. pavonia*

3.2.3.1. FT-IR analysis of AgNPs synthesized by *P. pavonia* powder. The control spectra (Fig. 3) showed a number of peaks thus reflecting a complex nature of the algal powder. On the other hand, the band intensities in different regions of the spectrum were analyzed and are shown in Figs. 3 and 4. There was a shift in the following peaks: 3420–3274 and 1621–1635  $\text{cm}^{-1}$ . The peak located at around 3420  $\text{cm}^{-1}$  was attributed to N–H formation of strong hydrogen bonding between two molecules. It is worthily mentioned that the U-shape peak around 3200–3500  $\text{cm}^{-1}$  contains both N–H amine and O–H hydroxyl groups. So, this peak more observed on silver nanoparticles which synthesized from algal powder comparing to its control. Accordingly, the peak shift from 3420 to 3274  $\text{cm}^{-1}$  implicated that these groups may be involved in the process of nanoparticles synthesis (Dubey et al., 2010). The untreated algal powder sample shows strong absorption bands about at 2920  $\text{cm}^{-1}$  suggesting aliphatic C–H stretching (Kaswar et al., 2009). A clear shift in this peak (from 2920 to 2159  $\text{cm}^{-1}$ ) indicated the possible involvement of amino or amide groups of

algal powder in nanoparticles synthesis. The peak located at 1621  $\text{cm}^{-1}$  could be assigned to the stretch vibration of C=C [(in-ring) aromatic]. A broad peak shift from 1621 to 1635  $\text{cm}^{-1}$  indicated the presence of amide I band in the AgNPs synthesized by algal powder.

The vibration shift around 1482  $\text{cm}^{-1}$  of untreated *P. pavonia* powder (Fig. 3) was suggestive of the involvement of aliphatic –CH and aromatic –OH groups such as hydroxy flavones and hydroxy xanthenes. Also, the control spectra (Fig. 3) showed that the peak located at around 1034  $\text{cm}^{-1}$  was attributed to the primary amine C–N stretching vibrations of aliphatic amines. The total disappearance of these two bands (1482 and 1034  $\text{cm}^{-1}$ ) in Fig. 4 after the bio-reduction may be due to the fact that the polyols are mainly responsible for the reduction of Ag ions. As stated earlier, cell walls of macro marine algae are mainly composed of pectin, cellulose, and hemicellulose, especially *P. pavonia* contains large quantities of fucoidan (it is a reducing and stabilizing agent) (polysaccharides) which used as reducing and stabilizing agent (Narayanan and Sakthivel, 2011). The functional groups associated

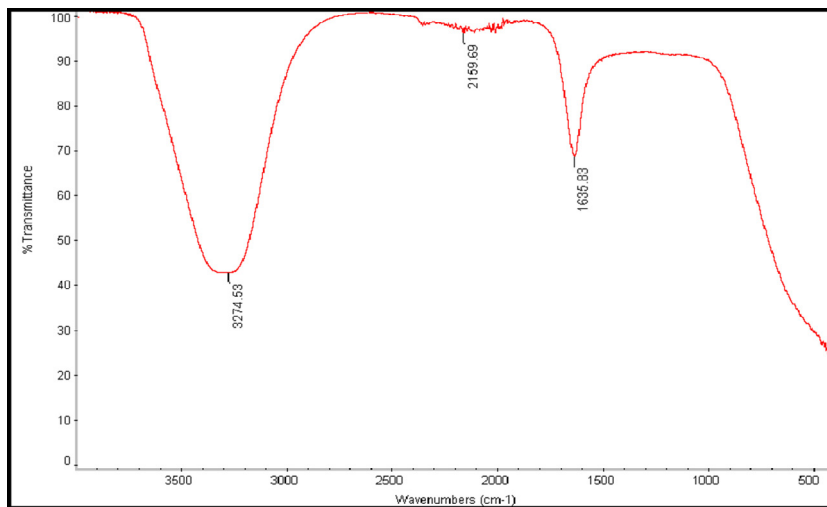


Fig. 4. FT-IR spectrum of AgNPs synthesized by *P. pavonia* powder.

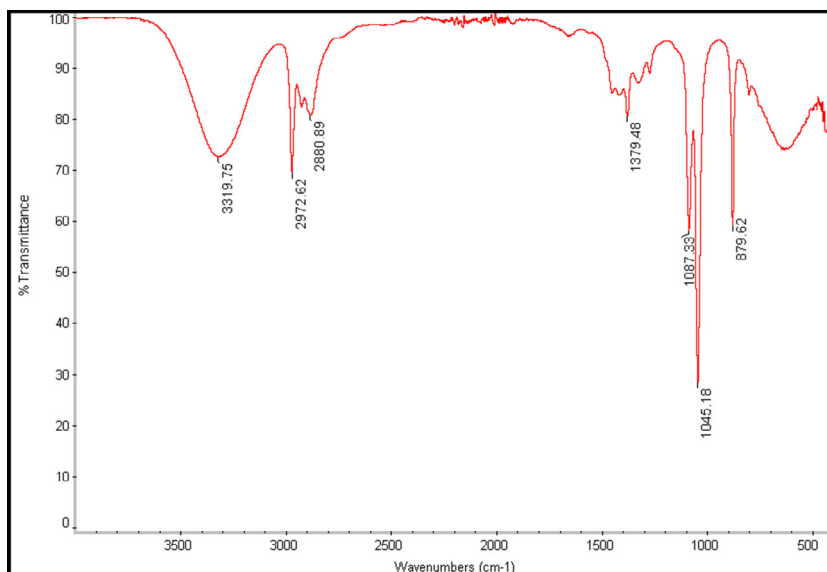


Fig. 5. FT-IR spectrum of *P. pavonia* ethanol extract.

with these polymers as well as the proteinaceous and polysaccharides matters may be involved in reducing the silver salt to  $\text{Ag}^0$ . Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles (Bar et al., 2009).

**3.2.3.2. FT-IR analysis of AgNPs synthesized by *P. pavonia* ethanol extract.** Fourier Transforms Infrared Spectroscopy analysis was performed in order to detect the functional groups on *P. pavonia* ethanol extract and predict their role in the synthesis of AgNPs. Data in Figs. 5 and 6 revealed the band intensities in different regions of the spectrum. It was showing that there was a shift in the following peaks: 3319–3426, 2972–2919, and 2880–2850 and 1379–1384  $\text{cm}^{-1}$ . Also, it was observed that less intense peaks with slight shifts around 3319–3426  $\text{cm}^{-1}$  due to a presence of O–H stretching. Further, supports by Mata et al. who described that hydroxyl groups (OH) are very abundant in polysaccharides

which are majorly responsible for reduction process (Mata et al., 2009).

The IR bands at 2972 and 2880  $\text{cm}^{-1}$  are characteristic of the C–H stretching symmetric and antisymmetric aliphatic and aromatic modes, respectively (Fig. 5). Also, these bands appeared as very strong bands in AgNPs of *P. pavonia* ethanol extract with a low shift to 2919 and 2850  $\text{cm}^{-1}$ , respectively (see Fig. 6). In addition, there are significant peaks at 2850, 1384 and 1467  $\text{cm}^{-1}$  were indicative of the role of aromatic groups in reduction of silver ions. It is notable that a new band at about 1736  $\text{cm}^{-1}$  corresponding to carbonyl stretch vibrations in ketones, aldehydes, and carboxylic acids indicating that the reduction of the silver ions is coupled to the oxidation of the hydroxyl groups in ethanol algal extract. These results were in agreement with Sanghi and Verma (2009).

Fig. 6 showed that the most useful IR band for the direct measurement of the secondary structure of the protein is a wide band found between 1736 and 1236  $\text{cm}^{-1}$ . The maximum absorbances

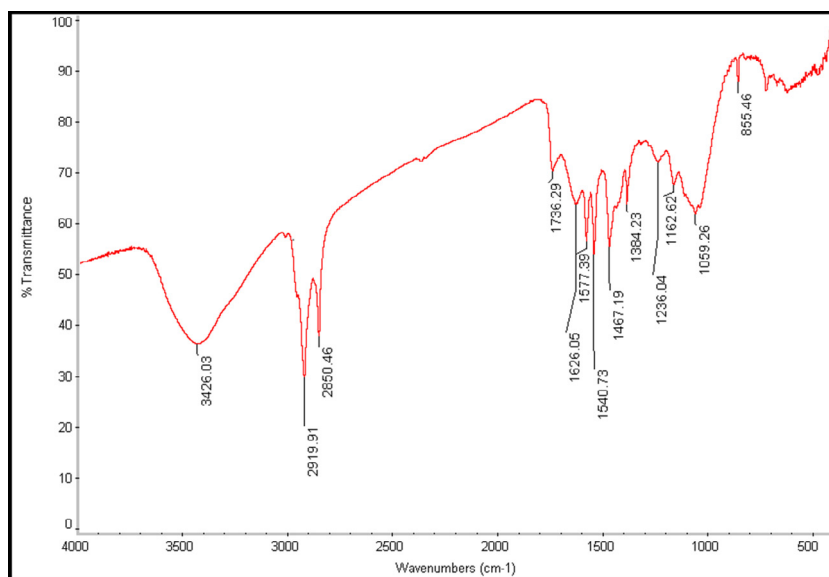


Fig. 6. FT-IR spectrum of AgNPs synthesized by *P. pavonia* ethanol extract.

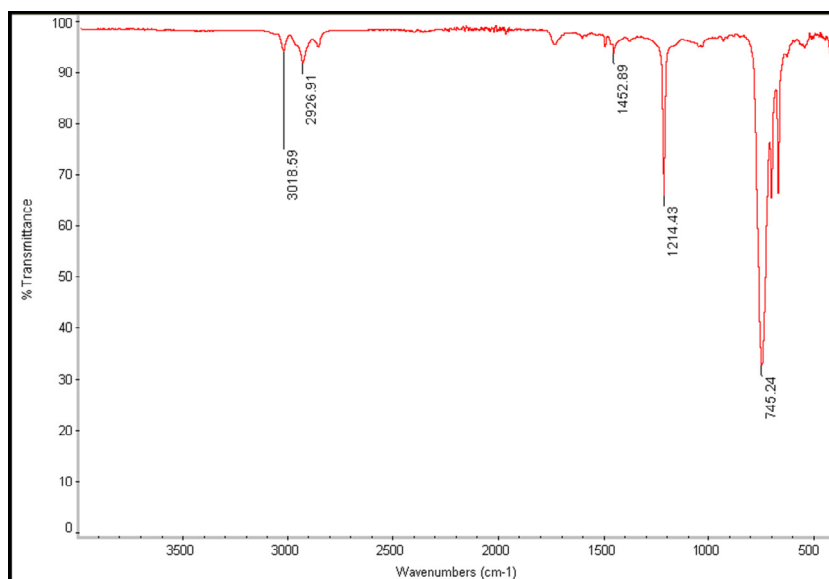


Fig. 7. FT-IR spectrum of *P. pavonia* chloroform extract.

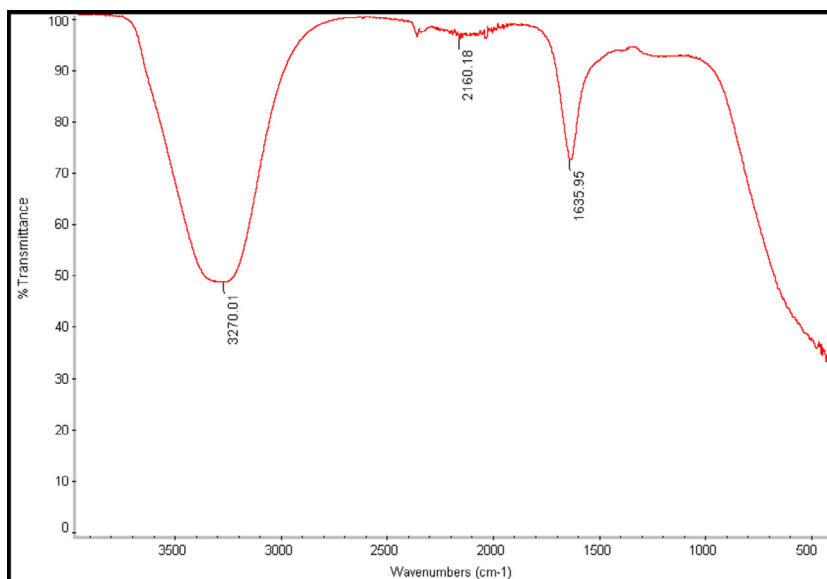


Fig. 8. FT-IR spectrum of AgNPs synthesized by *P. pavonia* chloroform extract.

of the amide I band of proteins were found in SNPs of ethanol *P. pavonia* extract samples near at 1736 and 1626  $\text{cm}^{-1}$ . Also, the amides II and III appeared at 1540 and 1236  $\text{cm}^{-1}$  respectively. The amide II band is a combination of N–H in-plane bending and C–N stretching and is centered on a small peak at 1540  $\text{cm}^{-1}$ . This indicated that the secondary structure of the proteins is affected as a consequence of reaction with the Ag ions or binding with the algal silver NPs (Binupriya et al., 2010).

**3.2.3.3. FT-IR analysis of AgNPs synthesized by *P. pavonia* chloroform extract.** The FTIR of untreated and treated chloroform *P. pavonia* extract samples containing AgNPs are depicted in Figs. 7 and 8. The untreated chloroform extract sample shows absorption bands at 3018, 2926, 1452 and 1214  $\text{cm}^{-1}$ . For untreated sample (Fig. 7) the weaker absorption band at 3018  $\text{cm}^{-1}$  corresponding to H group. Also, weak aldehydic CH stretching was recorded at 2926  $\text{cm}^{-1}$ . Furthermore, a thinner strong band of Amide III shown at 1214  $\text{cm}^{-1}$  while weak symmetric stretching vibrations of –COO– absorbed at 1452  $\text{cm}^{-1}$ . Fig. 8 showed that formation of strong U-shape peak occurred at 3270  $\text{cm}^{-1}$  which included amide bands and O–H Stretching comparing with its control (Fig. 7). Additionally, slight amide bands of proteins recorded at 2160  $\text{cm}^{-1}$  and moderate amide I bands absorbed at 1635  $\text{cm}^{-1}$ . Comparison between spectra of untreated to the treated samples, AgNPs (Figs. 7 and 8) indicated that only a major shift between untreated *P. pavonia* extract at 3018 to treated sample at 3270  $\text{cm}^{-1}$  but a minor shift in amine groups of proteins. These results revealed that the studied alga should be the origin of the bioactive groups which may be responsible for the reduction of metal ion to metal nanoparticles. Also, the used solvent may play an important role in the extraction of these compounds (Abdel-Raouf et al., 2017; Al-Saif et al., 2014).

#### 4. Conclusions

Synthesis of AgNPs using biological resources like marine algae is a challenging alternative to chemical synthesis since this novel biogenic method were eco-friendly methods. The obtained data clearly indicate the algal extracts can be used as an effective capping as well as the reducing agent for the synthesis of AgNPs. Silver nanoparticles synthesized by *P. pavonia* are quite stable and no visible changes in a long time. This can be supportive for medical

uses. All used analysis showed that there is a major distribution of particle size with many different shapes such as pyramidal, spherical, polygonal, rod and hexagonal with highly smooth edges. Their size ranged from 49.58 to 86.37 nm.

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