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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICALS USING DATES SEEDS (*PHOENIX DACTYLIFERA* L) EXTRACT FROM SIWA, EGYPT.

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ABSTRACT

In the current study, silver nanoparticles (AgNPs) were biosynthesised using Dates seeds (*Phoenix dactylifera* L) aqueous extract (LAE). HPLC analysis was used to identify the chemical components, flavonoids and phenolics, using both descriptive and quantitative analytics. Transmission Electron Microscopy (TEM), Energy Dispersive X-Ray (EDX), Zeta Potential, and FT-IR analysis were used to characterize the generated powder of NPs. The chemicals that were most frequently identified in the Dates seeds (*Phoenix dactylifera* L) **.**Extract by HPLC analysis were gallic acid, chlorogenic acid, pyrocatechol, and methyl gallate. The AgNPs were spherical in form and ranged in diameter from 8.70 nm to 13.4 nm. AgNPs have charged potentials of -10.1 according to zeta potential. FTIR spectra were used to characterize the hydroxyl functional group found in alcohols and phenolic substances. This demonstrated that using dates seeds (*Phoenix dactylifera* L) extract from the Siwa region of Egypt to create green silver nanoparticles was successful.

Key Words: Dates (*Phoenix dactylifera* L), Date seeds, bioactive compounds. AgNPs green silver nanoparticles·

INTRODUCTION

The potential of nanotechnology is vast, and ongoing research is continually unlocking new possibilities. As the field advances, we can expect to see even more groundbreaking applications in the years to come **(Chehelgerdi *et al.*, 2023 and Huang *et al.*, 2024).**

Nanotechnology, the manipulation of matter at the nanoscale, has indeed revolutionized various fields, including medicine, agriculture, and industry. Here's a breakdown of its significant contributions medicine targeted drug delivery, enhanced diagnostics, regenerative medicine and imaging **(Mariappan, 2019 and Malik *et al.*, 2023).**

In agriculture, improved crop yields, sustainable agriculture and food safety in industry, advanced materials, energy storage, environmental remediation and many more **(Gupta *et al.*, 2023 and Miteu *et al.*, 2023).**

Because of their special physical and chemical characteristics, silver nanoparticles (AgNPs) are being employed more and more in a variety of industries, including consumer, industrial, food, medical, and health care. These include biological qualities, strong electrical conductivity, and optical, electrical, and thermal characteristics. They have been utilized for a variety of purposes because of their unique characteristics. Three techniques are used to synthesize silver nanoparticles: physical, chemical, and biological **(Zhang *et al*., 2016, Hussein & Abdullah, 2022 and Gupta *et al.*, 2024).**

All of the issues with physical and chemical procedures have been resolved by the development of biological approaches. Different biological systems, such as bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids, are used to synthesize silver nanoparticles with a specific size as an alternative to chemical methods **(Iravani *et al.*, 2014 and Zahoor *et al.*, 2021).** This process is used not only for AgNPs but also for the synthesis of several other nanoparticles. Researchers have recently placed a great deal of emphasis on creating effective green chemistry techniques that use natural reducing, capping, and stabilizing agents to create silver nanoparticles with the appropriate size and shape **(Osman *et al.*, 2024 and Akhter *et al.*, 2024).**

It is possible to create silver nanoparticles using biological processes instead than harsh, costly, and poisonous chemicals. Combinations of biomolecules present in some organism extracts (such as enzymes/proteins, amino acids, alkaloids, alcohol, polysaccharides, and vitamins) can bioreduce metal ions in an environmentally beneficial manner **(Garg *et al.*, 2020 and Mikhailova, 2020).**

It has been demonstrated that biologically-mediated nanoparticle synthesis is an easy, reliable, economical, & environmentally benign process, and the high yield production of AgNPs has received a lot of interest **(Habeeb Rahuman *et al.*, 2022 and Burlec *et al.*, 2023).**

The production of dates, which provide numerous seeds that are rich in significant active components that are categorized as compounds with antioxidant activity due to their high content of phenolic and flavonoid compounds, makes Egypt one of the most significant countries in the world **(Siddiqi *et al.*, 2020, Swaidan *et al.*, 2023 and Manai *et al.*, 2024).**

However, because of the active groups they contain, these compounds—like those found in Dates (*Phoenix dactylifera* L) have the capacity to decrease metal ions, resulting in the creation of valuable nanocomposites that have a wide range of medical and agricultural uses **(Abdullah *et al*., 2020).**

Therefore, the goal of these investigations was to synthesize high-value nanocomposites that are useful in a variety of industries using Dates seeds (*Phoenix dactylifera* L), which are found in the Egyptian environment

MATERIALS AND METHODS

All chemicals used in the experiments were purchased from Al-Gomhoria Chemical Company and Sigma Company.

Plant specimen collection and preparation

Dates (*Phoenix dactylifera* L) were obtained from Seiwa, Egypt. After removing the Date seeds from the fruit. Date seeds were cleaned with water. After being cleaned under running water and kept out of direct sunlight for seven days for drying, the seeds were ground in a coffee grinder and filtered to a fine powder.

After the seeds were ground, distilled water was added to the sample at a ratio of 1:10 for the seeds powder and the water, respectively, to create the aqueous extract., The extraction procedure was conducted on a magnetic thermal stirrer at 80°C for 120 minutes, with the temperature gradually rising throughout.

Phytochemical screening examinations were achieved for the Dates seeds extract according to standard methods **(Harborne 1998).**

Test for Phenols (ferric chloride test)

Phenol estimation had been done by adding 3-4 drops of 5% $FeCl_3$ into 0.5 ml of seeds extract gives black colour, indicated the presences of phenols.

Tests for tannins:

Adding few drops of 1% $FeCl_3$ solution, with 0.5ml extract, gives intense green to black colour, confirms the presences of tannins.

Flavonoids*:*

3ml of seeds extract were pipette out and 10 ml of distilled water was added to it . Then it was shaken and 2 ml of 10% sodium hydroxide was also added to the mixture. A yellow colour was observed showing the presence of flavonoid.

HPLC analysis

HPLC analysis of the Dates seeds extract was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 15 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

Synthesis of green silver nanoparticles

According to **Al-Othman *et al.*, (2017)**, Date seeds extract were used to create silver nanoparticles. 100 ml of a 1 mM silver nitrate solution was combined with 10 ml of Dates seeds aqueous extract, and the mixture was heated for 25 minutes at 50 °C on a magnetic vibrating hot plate. The mixture's hue shift was noted as the first sign that silver nanoparticles were forming.

Transmission Electron Microscopy Analysis (TEM)

The samples were prepared for transmission electron microscopy (TEM) analysis by applying a droplet of the green AgNPs solution to a conventional carbon-coated copper connector. The final images were then analyzed using GEOL's Image software with their GEM-1010 TEM running at 70 kV **(Salayová *et al.*, 2021).**

EDX,or energy dispersive X-ray

EDX analysis was used to confirm the presence of iron and silver. An EDX microscopic analysis was carried out using the Oxford 6587 INCA X-ray precision analyzer, and the JEOL JSM-5500 LV electron microscope was scanned at a voltage of 20 kV **(Puchalski *et al.*, 2007).**

Zeta Potential

In order to assess and quantify the electrical charge and demonstrate the stability of the green AgNPs solution, a zeta voltage test was conducted using a Zeta sizer manufactured by Malvern Instruments Inc., UK **(Wypij *et al.*, 2021).**

FT-IR Examination

By using FTIR analysis (Shimadzu equipment), the functional groups of Date seeds extract and the surfaces of the AgNPs that it produces were described. This was achieved through meticulous scanning with a single FTIR type spectrum at a resolution of 4 cm−1 and a range of 4000–400 cm−1 (**Abdellatif *et al.*, 2022).**

RESULTS AND DISCUSSION

Phytochemical Tests:

Screening of the Dates seeds extract for various phytochemical constituents were carried out using standard methods. The data shown in Table 1 shows screening of aqueous extract. These tests reveal the presence of various bioactive secondary metabolites, which might be responsible for their medicinal and others attributes . The observations of the phytochemical tests are presented as follow:

Test for Tannins:

A green black precipitate was observed in dates seeds extract indicating the presence of tannins in the extract.

Test for Flavonoids:

A yellow colouration was also observed in date seeds extract indicating thereby the presence of flavonoid.

Test for Phenols (Ferric Chloride test)

The black colour was detected in Dates seeds extract indicating the presence of phenols in extract.

The obtained results are consistent with **(Sadeq *et al.*, 2021 and Ahmad et *al.*, 2022).**

Plant-based substances called phenolic compounds, flavonoids, and tannins can all function as reducing agents for silver nitrate salt in order to create silver nanoparticles using the environmentally friendly process **(Dhaka *et al.*, 2023 and Tesfaye *et al.*, 2023).** In addition to the fact that these substances essentially function as antioxidants, antibacterials, and antifungals, their versatility in application is advantageous and adds value, particularly if it is enhanced by their usage in the creation of nanocomposites **(Srivastava *et al.*, 2023 and Luna-Guevara *et al.*, 2018).**

Table (1): Phytochemical constituents of Date seeds aqueous extract

No	Secondary Metabolites	The result
1	Flavonoids	+ve
2	Tannins	+ve
3	Phenols	+ve

+ Ve = Present - Ve = absent

HPLC analysis

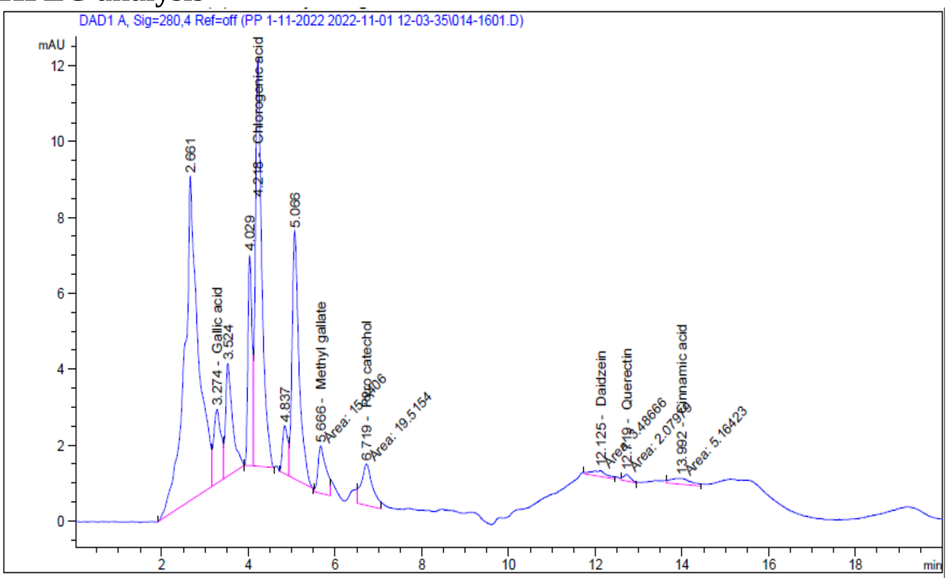

Fig (1): HPLC chromotogram of Dates seeds aqueous extract

In Figure (1) and Table (2) show HPLC analysis of Dates seeds extract . The results indicated the presence of polyphenol compounds such as Gallic acid, Chlorogenic acid, Methyl gallate, Pyro catechol, Daidzein, Querectin, and Cinnamic acid. Results show that Dates seeds extract recorded (995.19, 9338.91, 520.17, 1536.19,122.93 and 146.77 µg/g), respectively. All of these compounds were classified as antioxidant compounds and were used for many medicinal and other purposes (**Bentrad & Gaceb-Terrak, 2020 and Hilary *et al.*, 2020).** It could be concluded that the aqueous extract of Dates seeds is generally appropriate for the synthesis of plant-related nano-compounds due to the existence of these compounds, particularly after their quantitative measurement.

Table (2): HPLC Chromotogram of Dates seeds aqueous **extract**

Polyphenol Compounds	RetTime [min]	Area	Conc. (µg/g)
Gallic acid	3.274	22.78011	995.19
Chlorogenic acid	4.218	112.64	9338.91
Methyl gallate	5.666	15.84	520.17
Pyro catechol	6.719	19.52	1536.19
Daidzein	12.125	3.49	122.93
Querectin	12.719	2.08	146.77
Cinnamic acid	13.992	5.16	57.47

Characterization of AgNPs:

Transmition electron microscopy (TEM)

Transmition Electron Microscopy (TEM) was used to characterize the morphology and size of nanoparticle image of silver nanoparticles synthesized by reduction method and green synthesis process by using Dates seeds extract and 1mM $AgNO_3$ concentration (Figure 2). Where powdered nanoparticles were sent to make dilutions on them with different concentrations. The slides were prepared by adding a small drop of suspension of nanoparticles for biosynthesis on the slides, leaving them to dry and then analyzing them by (TEM) gave a clear image of highly dense silver nanoparticles. The TEM image showing silver nanoparticles synthesized using Dates seeds extract confirmed the development of silver nanostructures. As shown in the microscope image, the particles have a dense spherical shape with distinct sizes of the formed nanoparticles. The nanoparticle sizes ranged from 8.70 nm to 13.4 nm These results are consistent with the results obtained in previous studies **(Bankura *et al.*, 2012 ; Ansari & Alzohairy, 2018 ; Adnan *et al.*, 2023 and Al-Dulaimi *et al.*, 2024).**

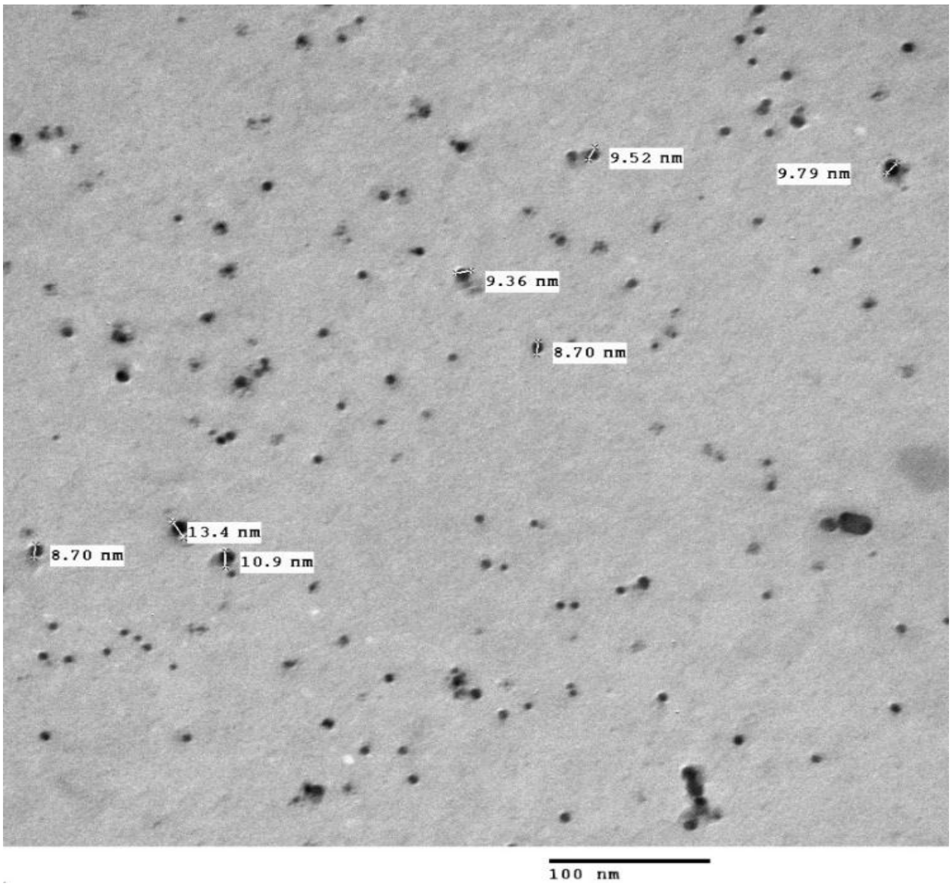

Fig (2). TEM image for AgNPs synthesized by Dates seeds aqueous extract.

Energy Dispersive X-ray (EDX)

Content of the AgNPs that were synthesized utilizing Dates seeds aqueous extract which are mentioned in the Table. The elemental composition of AgNPs was revealed by the EDX (Figure 3), which included sodium (Na) at 3.18%, carbon (C) at 41.41%, oxygen (O) at 48.83%, magnesium (Mg) 1.33%, chloride (Cl) 0.63%, calcium (Ca) 1.46%, and silver (Ag) at 3.15% due to their presence in Dates seeds These ingredients guarantee that the quantity of silver was reduced throughout the nanoparticle creation process **(Rónavári *et al.,* 2021 and Dhaka *et al.,* 2023).**

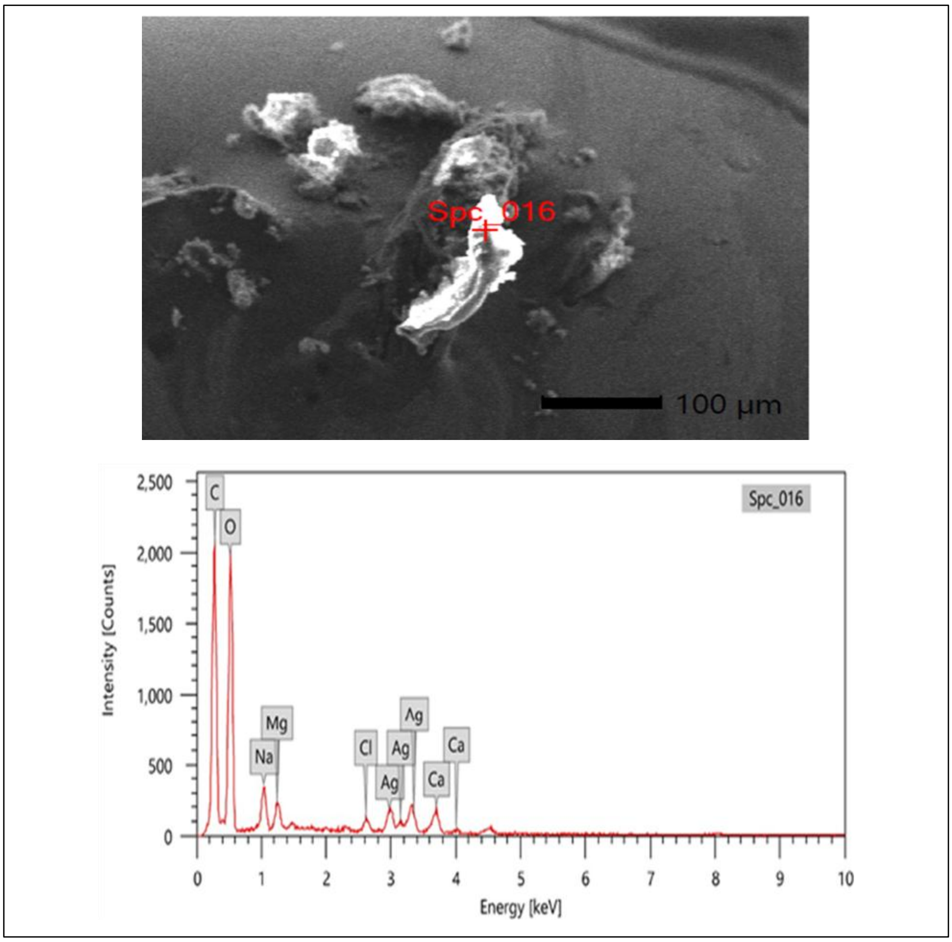

Fig (3) . EDX analysis of biosynthesized AgNPs by Dates aqueous extract.

Zeta potential values

When applied, the zeta potential values of AgNPs produced by the aqueous extract of dates seeds (*Phoenix dactylifera* L) are shown in Figure 4. The charge potentials of the biosynthesized AgNPs were discovered to be -10.1. This result implies a direct relationship between the stability of the green production of nanoparticles utilizing the aqueous extract of dates seeds and the surface potential (zeta potential) **(Dhaka *et al.*, 2023).** So, to improve their stability, reactivity, or environmentally advantageous qualities, AgNPs can be functionalized or coated with natural extracts. This will ultimately impact the zeta potential of NPs **(Akhter *et al.*, 2024).** A zeta potential that indicates adequate physical stability along with possible antifungal and antibacterial properties and other uses.

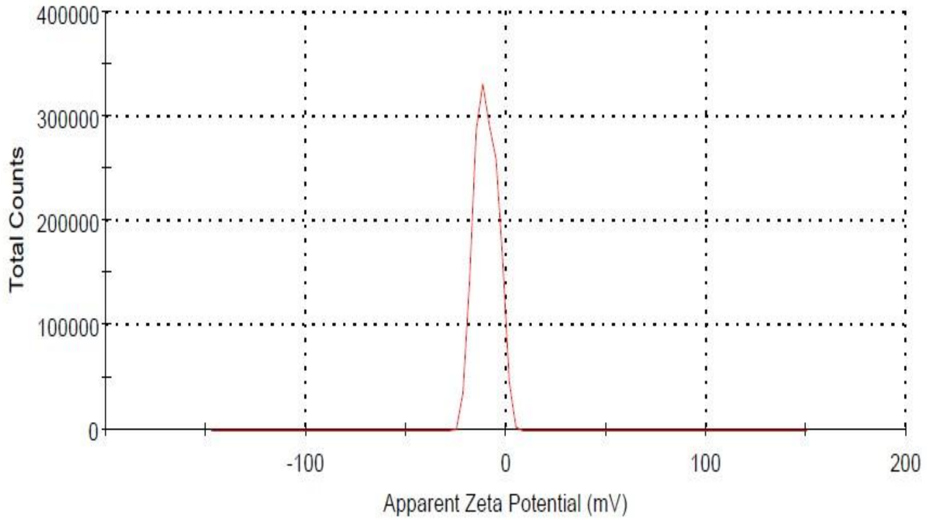

Fig (4) . Zeta potential values analysis of biosynthesized AgNPs by Dates seeds aqueous extract.

FTIR Spectral Analysis

Flavonoids and polyphenols were among the several biomolecules found in Dates seeds that may produce the NPs, according to the FTIR spectra Figure5. Certain specific peaks of the biosynthesized AgNPs were displaced in comparison to Dates seeds. The O-H broadening vibrations of the C-OH or H2O and CH2 stretching vibrations of the phenolics in the extract, as well as the OH-stretching of alcohol compounds, are indicated by the peaks found at about 3438 cm−1. Peaks were found between 1063.43 and 1233.13. Conversely, extracts of alcohols, carboxylic acids, esters, and aromatic ethers The metal's intrinsic stretching vibrations at the tetrahedral A-site Ag–O are responsible for the absorption band at about 560 cm−1. The synthesis of NPs is confirmed by the shifted absorption band at 560 cm−1 for the Dates seeds extract band at 591 cm−1, which corresponds to Ag–O stretches of Ag. The extract's lowering involvement in the formation was confirmed by the observed absorption bands, which were stronger than the extract **(Liu, *et al.*, 2009 and Abdullah *et al.*, 2023).**

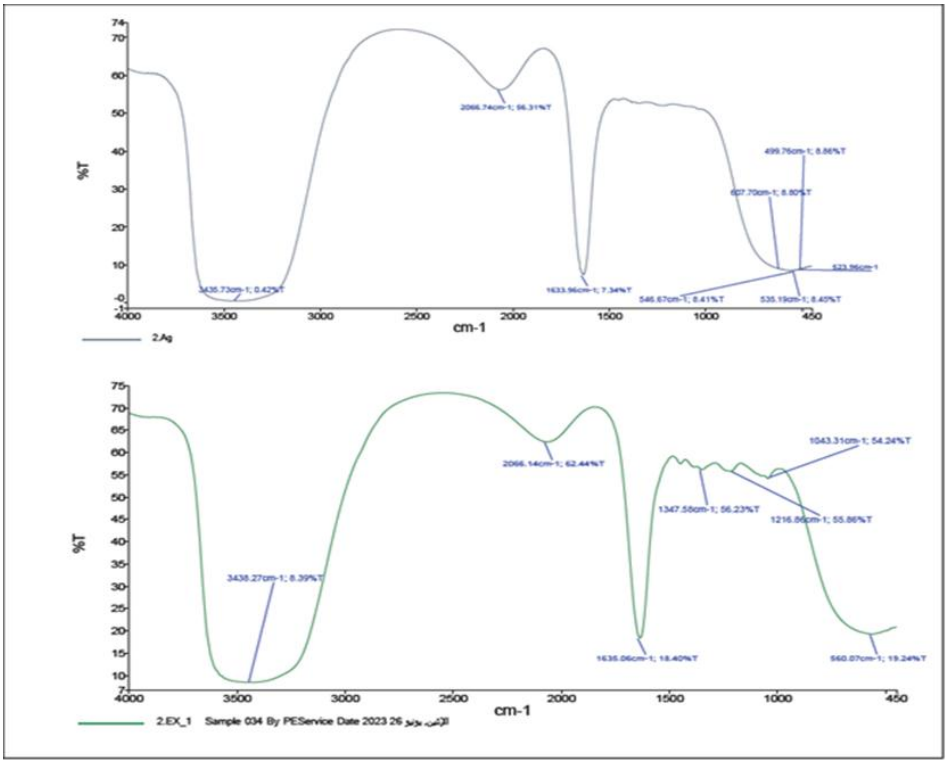

Fig (5). FTIR for AgNPs by the aqueous Dates seeds

CONCLUSION

The biosynthesized AgNPs using Dates seeds aqueous extract were characterized and performed by TEM, EDX, Zeta potential, and FTIR techniques. The valuable phytochemicals presented in Dates seeds extract were the reason for stable AgNPs formation in the solution. The size of the produced nanoparticles by TEM examination was a spherical shape for AgNPs (8.70 – 13.4 nm). This indicates the effective production of green, eco-friendly nanoparticles from the extract of Dates seeds , which were cultivated in Siwa, Egypt, and show promise for a variety of applications, including antifungal and antibacterial ones.

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الجسيمات النانوية باستخدام مستخلص نوي التمر (*Phoenix dactylifera* L) من سيوة، مصر.

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في هذه الدراسة ، تم تخليق جزيئات الفضة النانوية (AgNPs) حيويا باستخدام المستخلص المائي لنوي التمر (LAE) (*Phoenix dactylifera* L). تم استخدام تحليل HPLC لتحديد المكونات الكيميائية والفلافونويدات والفينولات، وذلك باستخدام التحليلات الوصفية والكمية. تم استخدام المجهر الإلكتروني (TEM)، والأشعة السينية المشتتة للطاقة (EDX)، و تحليل زيتا، وتحليل FT-IR لتوصيف NPs الناتجة . المواد الكيميائية التي تم تحديدها بشكل متكرر في مستخلص نوي التمر (*Phoenix dactylifera* L.) عن طريق تحليل HPLC هي حمض الجاليك، وحمض الكلوروجينيك، والبيروكاتيكول، وميثيل جالات. كانت جسيمات AgNPs المخلقة كروية الشكل وتراوح قطرها من 8.70 نانومتر إلى 13.4 نانومتر. حيث كانت جسيمات AgNPs مشحونة ب-10.1 طبقا لقيلس زيتا. حيث يستخدم أطياف FTIR لتوصيف مجموعة الهيدروكسيل الوظيفية الموجودة في الكحوليات والمواد الفينولية. وهذا يوضح أن استخدام مستخلص نوي التمر من منطقة سيوة في مصر يشير إلى الإنتاج الفعال لجزيئات النانو الخضراء والصديقة للبيئة من مستخلص بذور التمر، التي تم زراعتها في سيوة، مصر، وتظهر لمجموعة متنوعة من التطبيقات، بما في ذلك التطبيقات المضادة للفطريات والبكتيريا. لتخليق جزيئات الفضة النانوية الخضراء كان ناجحًا.