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Evaluation of Aflatoxin B1 detoxification by using green Ag nanoparticles synthesis from some plant extracts

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ABSTRACT— A four medicinal leaves plants were gainted from local market and the alcohol extraction were doing using 75% methanol. Green silver nanoparticles synthesis from foure medicinal plant extracts. Scanning electron microscopy (SEM), UV visible spectroscopy, Fourier Transform Infrared Spectroscopy(FTIR), and Energy Dispersive Spectroscopy (EDS). The results showed nanoparticles, smooth spherical nanoparticles ranging in size from 29.03 to 88.00 nm. The standard concentration of aflatoxin B1 is (29) ppb, according to the findings of a study on the effectiveness of green nanoparticles against aflatoxin B1 using high-performance liquid chromatography technology to assess its concentration. The extracts were examined by HPLC, and found that the concentration of aflatoxin B1 is The present in the alcoholic extracts. (*Thymus vulgaris*: 0.065ppb, *Rosmarinus officinalis*:0.580ppb, *Mentha spicata*:0.377 ppb, and *Eucalyptus camaldulensis*:1.067ppb). But Ag nanoparticles alcohol extracts (*T. vulgaris*:rare, *R. officinalis*:0.065ppb, *M. spicata*:1.083 ppb, and *E. camaldulensis*:0.615ppb). The remove rate in green Ag nanoparticles of alcohol extract showed aflatoxin B1 detoxification ratios by using rosemary: 99.67 % eucalyptus: 96.92 %, mint: 94.58%, and thyme: 92.07%. However, that alcoholic extracts of thyme: 99.67%, mint: 98.15%, rosemary: 98.15% and eucalyptus: 96.49%. From the results appeared the superiority of the thyme plant detoxification over the rest of the plant extracts while rosemary Ag nanoparticles have higher in aflatoxin B1 detoxification activity than other nanoparticles. The mint plant showed less effectiveness against aflatoxin B1.

KEYWORDS: mint, eucalyptus, thyme, rosemary, alcohol extract, nanotechnology, aflatoxin B1.

1. INTRODUCTION

Aflatoxin B1 (AFB1) is a big concern all over the world [1]. As a result, mycotoxins can induce toxicity in the liver and kidneys, as well as bleeding, cancer, and even death. In *Aspergillus* sp., there is a benefit. It's on the rise all around the world, thanks to the discovery of a growing number of natural *Aspergillus* toxins that have been demonstrated to endanger human and animal health [2], [3]. The threats to the environment posed by pesticide overuse have been widely explored in recent years. As a result, scientists working in the agriculture industry are looking into alternatives to pesticides [4]. In modern agriculture, innovative strategies are being employed to combat pathogen-related losses. Nanotechnology stands out among these technical advancements because of its wide range of applications, including agriculture, and its unique physical, chemical, and biological features [5]. Medicinal plants have been utilized for medicinal purposes since antiquity and are even thought to be the source of modern medicine. Plant-derived chemicals have long been and continue to be a valuable source of therapeutic molecules [6]. Medicinal plant analysis has a lengthy history, especially in terms of determining plant quality. The physiological sensations of taste, smell, and appearance were used as the first sensory procedures. This eventually led to the development of more complex automated techniques. Even though each country's traditional remedies are unique. Many areas of analytical analysis have advanced, including the development of chromatography and spectroscopic processes, as well as the integration of different techniques. The ability to manipulate data with multivariate analysis software has opened the door to metabolic processes, allowing us to better understand the many variations of chemical compounds that occur within medicinal plants, giving us more confidence in the quality of plants and medicines, as well as their suitability for research patients. Technology advancements have allowed for the effective analysis and classification of plants, as well as the detection of pollutants and fraud at extremely low levels. However, technology advancements alone cannot supply all of the information we require to deliver high-quality herbal medicines, and conventional methods of quality assessment remain relevant today [7]. Nanotechnology is a comprehensive discipline that involves manipulating atoms, electrons, protons, and neutrons in a variety of ways to get a new understanding of how materials might be made to solve a range of issues in a variety of fields. For disease prevention and management, several plant nano-devices/ nanomaterials/ nanoparticles have been produced [8].

The aim of the research was including the synthesis and characterization of green Ag nanoparticles by using alcoholic extracts of some plants (mint, thyme, rosemary, and eucalyptus), as well as the assessment of green Ag nanoparticles activity to aflatoxin B1 detoxification.

2. Materials and Methods

In this research, aflatoxin produced from fungal isolates of A.flavus from the Food Contamination Research Center/Environment and Water Department of the Ministry of Science and Technology was taken. These samples were taken during the period from July 2020 to July 2021.Preparation of Medical plants.

The medicinal plants leaves were using in this study are (thyme, mint, rosemary and eucalyptus), and gaint from the local market in Baghdad, and identified and approved from the herb laboratory/ College of Science/ University of Baghdad. Iraq. Dried by air-warmed and powdered, then kept at 4°C until use.

2.1 Extraction of leaves medical plants

To prepare the alcohol extract from mint, thyme, rosemary, and eucalyptus leaves plants, were worked according the method described by [9] with some modifications, 40 grams from mint, thyme, rosemary and eucalyptus leaves at alone were input in a Soxhlet device continuous extractor thimble, and using (500) milliliters of 75% methanol as solvent under 75°C temperature, the extraction process continued for 8 hours and the solvent was evaporated using evaporator rotary device under vacuum pressure under a temperature of 45°C, and the sample was placed in oven at a temperature of 45 °C to obtain a dry powder, which was kept in the refrigerator until use.

2.2 Preparation of Green Silver nanoparticles

Silver nanoparticles were synthesis from thyme, mint, rosemary, and eucalyptus leaves separately using silver nitrate, as described by [10], [11]. 1gm from any mint, thyme, rosemary, and eucalyptus leaves extractes and 10 ml of methanol was dissolved separately, and droppled to 200 ml of 0.1 mM silver AgNo3 under ultrasonic waterbath was for 30 minutes with a power of 100 watts and a frequency 42 kHz and then put on a magnetic sterrer for 8 hours, when the the colour solution was changes. The green Ag nanoparticles were purified by centrifugation at 10,000 rpm for 20 minutes after that worked serial dilution and Centrifugation before filteration by 0.22µ millipoure filter [12], [13].

2.3 Characterization of the prepared nanoparticles

A. Scanning Electron Microscopy (SEM)

SEM was utilized to characterize the size, shape and dispersion of nanoparticles in this work. To do an SEM analysis according to [14] technique.

C. UV–vis spectral analysis

Beckman Coulter (DU739, Hawaii, Germany) was used to study the synthesis of EAgNPs, TAgNPs, MAgNPs and RAgNPs and UV-vis spectroscopy of the elegant complexes of EAgNPs, TAgNPs, MAgNPs and RAgNPs were scanned by collecting transmission spectra via a 2-mm quartz cuvette. Surface plasmon resonance (SPR) is discovered to have a peak in the visible range between 400 and 450 nm. The sample was characterized using water as a reference sample [15].

D. Fourier transform infrared (FT-IR)

To determine the main functional groups in alcoholic plant extracts of mint, thyme, rosemary and eucalyptus leaves. The FTIR analysis was carried out exactly as specified. Control samples (mint, thyme, rosemary and eucalyptus leaf extracts) were converted and examined. Using an FTIR spectrophotometer Nicolet 6700, Thermo Scienti c), collect FTIR spectra with a resolution of 4 cm^{-1} in transmission mode (4000-400 cm^{-1}) [16].

E. Energy-dispersive spectroscopic (EDS) analysis

A SEM apparatus (model JEM 2011) outfitted with the OXFORD EDS 6498 accessory was used to create droplet measurements of reduced silver nanoparticles deposited on carbon-coated copper grids [16].

2.4 Testing the Aflatoxin B1 detoxification activity of green Ag nanoparticles from some plant extracts.

Aflatoxin B1 was produced from *A. flavus* through the culture medium (potato dextrose agar) and it was incubated for a week. Aflatoxin B1was measured in the medium and the aflatoxin B1 29ppb concentration was shown using HPLC. Ag nanoparticles and the extract were added at a concentration of 10 percent, where each Ag nanoparticle was 10 ml with 100 ml of the medium. The extract was separately on the culture medium and then it was incubated for one day and aflatoxin B1 was detected using HPLC. The extracts were examined by HPLC, and found that the concentration of aflatoxin B1 is present in the alcoholic extracts. (*Thymus vulgaris*:0.065ppb, *Rosmarinus officinalis*:0.580ppb, *Mentha spicata*:0.377 ppb, and *Eucalyptus camaldulensis*:1.067ppb). But Ag nanoparticles alcohol extracts (*T. vulgaris*:rare, *R. officinalis*:0.065ppb, *M. spicata*:1.083 ppb, and *E. camaldulensis*:0.615ppb) [17].

2.5 Statistical analysis

An experimental design file should be a complete randomized design (CRD). Also, [18] statistical analysis system program was used to determine the impact of various factors on the study's parameters. Notable distinction For a large comparison in this study, the LSD test was used.

3. Results and discussion

A. Scanning Electron Microscope (SEM)

SEM examination revealed very small spherical particles of RAgNPs, EAgNPs, TAgNPs, and MAgNPs with minimum nm sizes of 29.03 nm, 29.03 nm, 69.33 nm and 48.31 nm for RAgNPs, EAgNPs, TAgNPs, and MAgNPs, respectively (Figure 2). The presence of active compounds on the surface of the nanoparticles, which serve as a covering agent, when the structure of silver nanocomposites (AgNPs) in the presence of aqueous leaf extracts were [19] results agree with our findings (SEM analysis of Tridax procumbens, Euphorbia hirta, and Azadirachta India [19]. The AgNPs were found to be spherical and agglomerated.

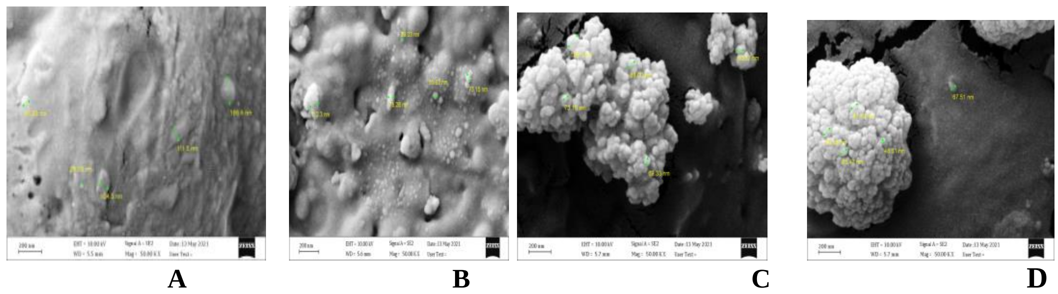

Figure 2. SEM image showed the differences in shapes & Size between RAgNPs (A) EAgNPs (B) TAgNPs (C) MAgNPs (B).

C. Fourier transform infra-red (FT-IR)

From FTIR results of alcohol plant extracts (thyme, mint, rosemary and eucalyptus) show bands at 2,916 and 2,850 cm^{-1} of the fragrant compound's present C–H bond. The band at 1730 cm^{-1} indicates the presence of amide, esters and acids from carbonyl group stretching vibrations in a mint extract and it represents 1590 cm^{-1} (-C-C-) in the aromatic ring in mint and eucalyptus extracts were refer to the hydrophobic band area(figure 3).

The C–O stretch of phenolic compounds in mint is depicted by the high peak at 1019 cm^{-1}, lower peaks at 600 cm^{-1} were detected, indicating the presence of polyphenols in thyme, rosemary, and eucalyptus. As a result, the majority of phenolic and carbonic chemicals are found on the surface of silver nanoparticles. These chemicals were derived from water-soluble components present in the leaves, including as flavonoids, alkaloids, and polyphenols [20], [21].

FTIR spectrometry was used to identify the metabolites responsible for Ag ion reduction. In the presence of phenols and alcohols with a free OH group, instinctive absorption bands emerge at 3423.15 and 3420.77 cm^{-1} in extracts of thyme, rosemary, and eucalyptus [21]. The NH stretching peak [22] stabilizes this band. The presence of alkanes in the lipids of thyme, mint, rosemary, and eucalyptus extracts is shown by the bands at 2,928.45 and 2,928.44 cm^{-1} [21]. Only in the biosynthesized solution does the area of 2359.03 cm^{-1} suggest the presence of asymmetric expansion of COO. The bands at 2240.59 and 2241.03 cm^{-1} are allocated to the CC group. The bands at 2,138.93 and 2,138.90 cm^{-1} show the presence of alkenes, NC, and N = C groups in the R-N = C = S structure. The absorbance bands at 1630.32 and 1631.43 cm^{-1} correspond to C=C in aromatic compounds and amide (NH), out of level. The bands at 1518.75 and 1518.92 cm^{-1} are caused by amide II in proteins. Bands at 1123 and 1150.67 cm^{-1} confirmed the existence of a carboxylic acid group in thyme, rosemary, and eucalyptus. The bands at 821.69cm-^{1}, as well as 674.63 and 675.51 cm^{-1}, show the presence of =CH in aromatic compounds in plant extracts of thyme, rosemary, and mint, as well as biosynthesized Ag NPs. The bands at 603.71 and 602.71 cm^{-1} correspond to C-Cl (alkyl halides) in thyme, rosemary, and eucalyptus. Proteins with free carboxylic groups can bind to metal nanoparticles and stabilize them [23], [24].

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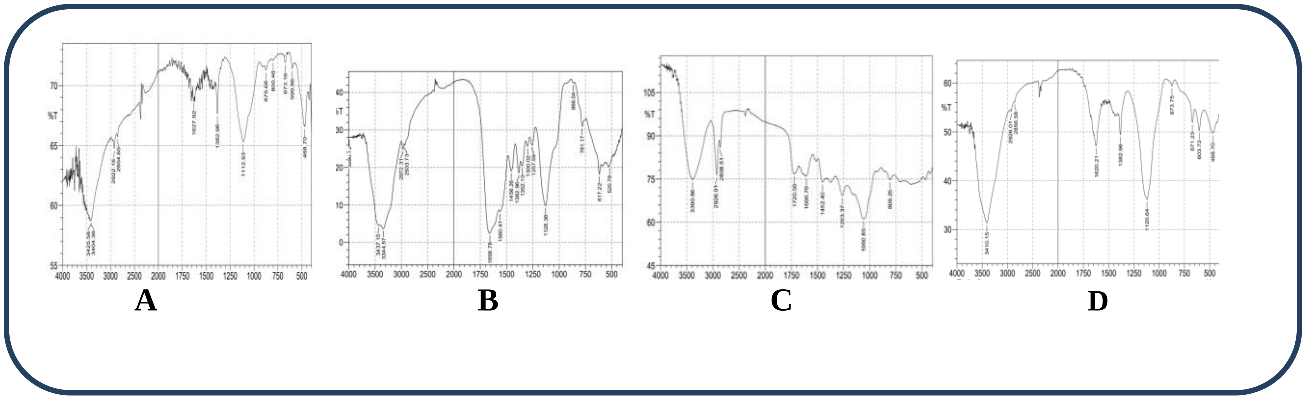

Figure 3. FTIR image showed defferences results of (A) RAgNPs (B) EAgNPs (C) TAgNPs and (D) MAgNPs.

D. UV-Vis spectral analysis

UV-vis spectroscopy was used to study the formation and stability of silver nanoparticles in water. However in the green Ag nanoparticles from alcoholic extract were higher absorbances reached in wavelength of (thyme in 210 nm, rosemary in 210 nm, eucalyptus in 224 nm and mint 282 nm) (figure4). While the alcohol extract were higher absorbances reached in wavelength of (mint in 230 nm, rosemary 215 nm, thyme 233 nm and eucalyptus 280 nm). the peak pattern matches the findings of [25]. The extract's UV spectra the addition of AgNO3 exhibited maximal absorption at around 250 to 400 nm, indicating the production of AgNPs. We successfully produced silver nanoparticles utilizing plant leaf extract in this research [26] Green synthesis advances more than chemical and physical methods because it is less expensive, more environmentally friendly, and more readily scaled up for large-scale synthesis. This technique does not need the use of high pressure, energy, temperature, or hazardous chemicals [25].

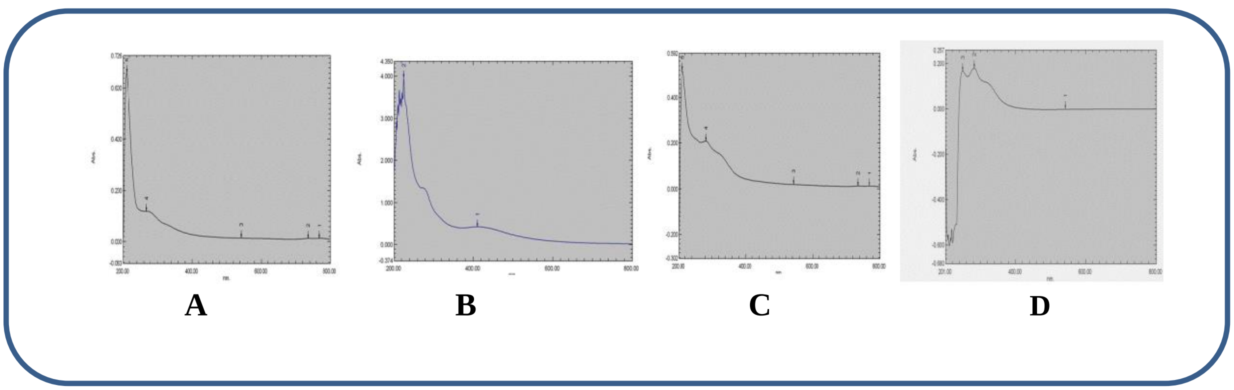

Figure 4. UV-VIS image showed defferences results of (A) RAgNPs (B) EAgNPs (C) TAgNPs and (D) MAgNPs.

E. Energy-dispersive spectroscopic (EDS) analysis

The Ag nanoparticles alcoholic extract of eucalyptus (Ag: 40.2, O: 39.6, C: 20.2), thyme (Ag: 3.0, O: 69.5, C: 25, N: 2.5) and rosemary (Ag:3, O:55.5, C: 41.5) and mint (Ag:0.1, O:39.9, C:40.4, N:19.6). Through the results in the (figure 5) of the examination, we note that the Ag nanoparticle eucalyptus extract is better than the rest of the Ag nanoparticle plant extracts due to the presence of silver ion higher and the presence of other elements for effective compounds. The EDX spectrum displays a significant signal in the silver area, confirming AgNP production. Because of plasmon surface resonance, metallic silver nanocrystals often display an optical absorption peak at 3KeV. Silver is present in eucalyptus extract (Ag 40.20 %) but not in the other plants. Data from energy dispersive spectroscopy (EDX) reveal highly significant silver. This suggests that the reduction of silver ions to the element silver was most likely caused by molecules linked to the surface AgNPs.

The presence of a dense silver peak verified the reduction of silver nitrate to silver nanoparticles. The silver peak has a greater thickness than the other peak. This verifies the spectrum's depiction of the full reduction of silver compounds to AgNPs. The EDX measurement confirmed the presence of the necessary phase of silver (Ag) in the sample [27].

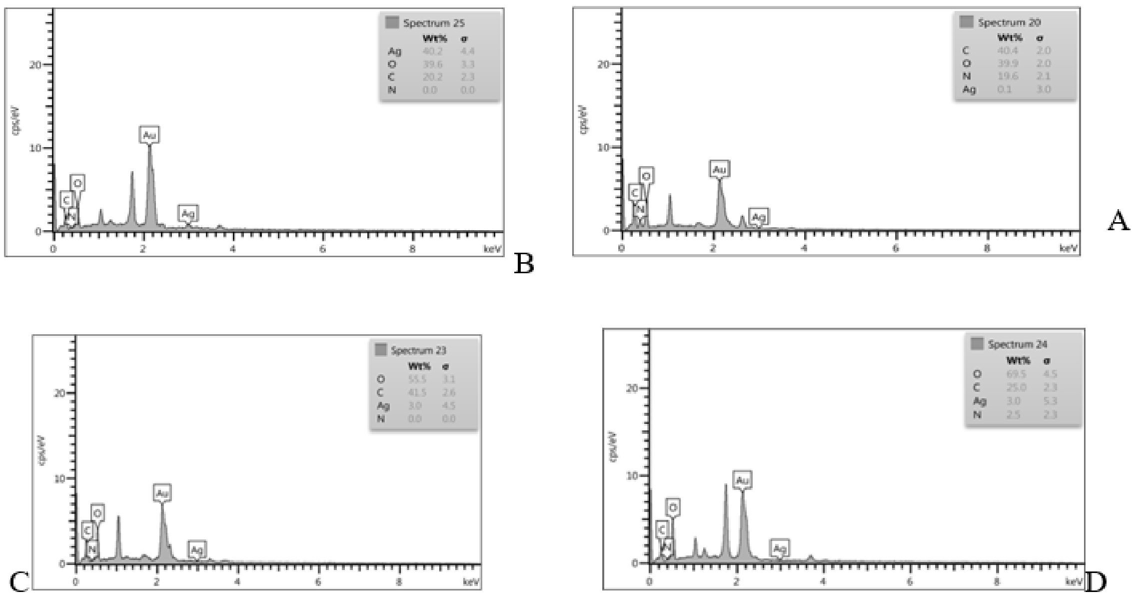

Figure 5. EDS pattern for A (EAgNPs),B (MAgNPs), C (RAgNPs) and D (TAgNPs) with the extracts (alcohol method).

Testing the Aflatoxin B1 detoxification activity of green Ag nanoparticles of some plant extracts.
AFB1 was detected in culture media using high-performance liquid chromatography (HPLC). Alcoholic extracts of mint leaf extract, thyme, rosemary, and eucalyptus were added to medium samples. The remove rate in Ag nanoparticles alcohol samples showed aflatoxin B1 ratios rosemary: 99.67 % eucalyptus: 96.92%, mint: 94.58% and thyme: 92.07%. However, that alcoholic extracts of thyme: 99.67%, mint: 98.15%, rosemary: 98.15%, 92.07 % and eucalyptus: 96.49%. The results showed the superiority of the thyme plant over the rest of the plants, while the mint plant showed less effectiveness. This finding is consistent with the findings of [28], [17].

4. Conclusion

The participation of alcoholic extracts of plants in the synthesis green silver nanoparticles can be considered as one of the techniques in the biotechnology fields, and testing the efficiency of the effectiveness of these nanoparticles in removing or preventing the production of mycotoxins, especially aflatoxin B1, which can be considered one of the modern and promising techniques in the treatment of mycotoxins, which is very difficult removing them and very high costs and because of the danger of these toxins, especially aflatoxin B1 from a healthy sids, which may lead to tumors or may be cancers.

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