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STABILITY OF GREEN SYNTHESIS OF SILVER NANOPARTICLES BY USING *Euphorbia milii* (EUPHORBIACEAE) LEAVES EXTRACT WITH DIFFERENT SOLVENTS AND POLARITIES

(Kestabilan Sintesis Hijau Nanopartikel Perak dari Ekstrak Daun *Euphorbia milii* (Euphorbiaceae) menggunakan Pelarut dan Kekutuban yang Berbeza)

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Abstract

Silver nanoparticle (AgNP) synthesis can be produced by chemical, physical, and green synthesis methods. In this study, green synthesis of AgNPs was conducted by using *Euphorbia milii (E. milii)* leaf extract as the reducing agent. *E. milii*, also called crown-of-thorn or in Malay *'Mahkota duri'* was used as the targeted plant in this study due to its rich phytochemical contents. The leaves were extracted into six different solvents (hexane, chloroform, ethyl acetate, acetone, methanol and distilled water) by increasing polarities to extract different varieties of compounds that were applied as reducing agents in the synthesis of AgNPs. The crude extract was added to 1 mM $AgNO_3$ solution and kept in the dark at room temperature for 24 hours. The process was repeated for all crude extracts with different solvent polarities. The AgNPs solution obtained was analysed by using a UV-Vis spectrophotometer, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscope (SEM). The stability of the AgNPs was observed and compared between different extracts from different polarities. The wavelength of UV-Vis obtained for the 6 sets was between 415 nm and 485.5 nm. The formation of AgNPs based on the surface plasmon resonance band is proved by the peak that appeared around $\lambda = \sim 450$ nm. The FTIR spectroscopy of the various extracts indicated the presence of OH, C-H stretching, C=O stretching, C=C stretching and C-O stretching, respectively, as compared with the FTIR spectrum for AgNPs for the synthesis and stabilization of the AgNPs. SEM images showed the different sizes of AgNPs for different extracts, with a range of about 67 to 843 nm.

Keywords: green synthesis, reducing agent, green AgNPs, euphorbiaceae, *Euphorbia milii*

Abstrak

Sintesis nanopartikel perak (AgNPs) boleh dihasilkan melalui kaedah sintesis kimia, fizikal dan hijau. Dalam kajian ini, sintesis hijau AgNPs telah dijalankan menggunakan ekstrak daun *Euphorbia milii* (*E. milii*) yang bertindak sebagai agen penurunan. *E. milii* atau juga dipanggil mahkota duri atau dalam bahasa Melayunya 'Mahkota duri' telah digunakan sebagai tumbuhan sasaran dalam kajian ini kerana kandungan fitokimia yang kaya. Daun diekstrak ke dalam enam pelarut berbeza (heksana, kloroform, etil asetat, aseton, metanol, dan air suling) dengan meningkatkan kekutuban untuk mengekstrak pelbagai sebatian yang berbeza yang digunakan sebagai agen pengurangan dalam sintesis AgNPs. Ekstrak mentah telah ditambah ke dalam larutan 1 mM $AgNO_3$ dan disimpan dalam gelap pada suhu bilik selama 24 jam. Proses ini diulang untuk semua ekstrak mentah dengan kekutuban pelarut yang berbeza. Penyelesaian AgNP yang diperolehi dianalisis dengan menggunakan spektrofotometer UV-Vis, spektroskopi inframerah transformasi Fourier (FTIR), dan mikroskop elektron pengimbasan (SEM). Kestabilan AgNP diperhatikan dan dibandingkan dari ekstrak yang berbeza kekutubannya. Panjang gelombang UV-Vis yang diperoleh untuk 6 set adalah antara julat 415-485.5 nm. Pembentukan AgNP berdasarkan jalur resonans plasmon permukaan dibuktikan oleh puncak yang muncul sekitar $\lambda = \sim450$ nm. Spektroskopi FTIR bagi pelbagai ekstrak menunjukkan kehadiran OH, regangan CH, regangan C=O, regangan C=C dan regangan CO, masing-masing berbanding dengan spektrum FTIR untuk AgNP untuk sintesis dan penstabilan AgNPs. Imej SEM menunjukkan saiz AgNP yang berbeza untuk ekstrak berbeza dengan julat kira-kira 67 hingga 843 nm.

Kata kunci: sintesis hijau, agen penurun, AgNPs hijau, euphorbiaceae, *Euphorbia milii*

Introduction

Silver nanoparticles (AgNPs) are useful in various applications that have attracted much interest among researchers [1]. The production of AgNPs can involve chemical, physical, biological, and photochemical methods [2]. For the production of AgNPs, chemical and physical methods are usually used at the industrial level [3]. However, this method was a concern in biomedical applications because of the toxicity of reducing and capping agents, high temperature, and pressure in the protocol [4]. Capping agents such as alkylamines and alkanethiols had the function to stabilise the AgNPs from aggregation. The use of this capping agent and the reduction from this method can result in hazardous by-product formation that can affect the use of AgNPs in biomedical fields and chemical applications. To overcome this problem, green synthesis methods are used as a substitute for chemical and physical procedures [3] by using biological extracts such as plants, bacteria, or other potential natural reducing agents [5].

Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals and natural capping agents [6]. In this study, *Euphorbia milii* (*E. milii*) was chosen as a targeted plant because it is rich in bioactive compounds and has high total flavonoid content [7]. Euphorbia is the largest genus, widely distributed primarily in tropical countries that acts as medicinal plants. *E. milii* is a flowering plant commonly known as the "Christ plant" or "Christ thrown" [8] and is widely used in folk medicine for the treatment of warts (South Brazil), cancer, and hepatitis (China). A previous study on *E. milii* was carried out on polar crude extract to isolate the active chemical components [7]. *E. milii* leaves act as reducing biological agents and bind together with silver metal ion solutions to form AgNPs. The reducing agents are widely distributed in the biological system [4]. The study presented here describes the effect of different polarities in the crude extract on the stability of the AgNPs.

Materials and Methods

Materials and instrumentation

Silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich. The absorption spectra of synthesized AgNPs were recorded by using UV-visible spectroscopy from 300-800 nm (Shimadzu UV-1800). The Fourier Transform Infrared (FTIR) extracts and AgNPs were taken by Perkin Elmer Spectrum 100 by using the ATR method and recorded between 4000 and 400 cm^{-1}. The Scanning Electron Microscope (SEM) images were taken by JEOL 6360 LA with working distance and accelerating voltage, 15 mm and 15 kV, respectively. The sample for SEM was sonicated 3 times (15 minutes each) before it was mounted on an Indium tin oxide (ITO) substrate. Then, the sample was coated with metal gold.

Collection of the plant sample

The sample of *Euphorbia milii* leaves was collected from Kota Sarang Semut, Kedah (November 2020). The botanical identity of the plant specimen of *E. milii* leaves was confirmed by the Biological Laboratory, Universiti Malaysia Terengganu.

Preparation and extraction of *E. milii* leaves

E. milii leaves (70 g) were cleaned under running tap water and dried at room temperature for seven days before grounded into powder form by using a grinder. Then, the powders were soaked with the first non-polar solvent, which is hexane and filtered to collect the hexane extract. The residue from hexane was repeated with different solvents by increasing the polarity (chloroform<ethyl acetate<acetone<methanol <distilled water) using the same method continuously at room temperature for 72 hours. Each extract was concentrated by using a rotary evaporator (Büchi Rotavap R-200 CH-9230, Switzerland) under reduced pressure (337-218 mbar) at 35-40 °C for 15-30 min. The six crude extracts obtained were weighed and stored in a chiller (4 °C) for further analysis.

Green synthesis of AgNPs

The green synthesis followed the method developed by Alsalhi, et al. with some modifications. To prepare AgNPs, 10 mg crude extract was added to 10 ml Mili-Q water. Then, it was filtered by using a syringe filter (0.2 µm). The mixture (5 ml) was added to 1 mM AgNPs solution (50 mL) [9]. The mixture was kept in the dark at room temperature for one day (until the color changed from colorless to brown). The process was repeated with different crude extracts, as shown in Figure 1.

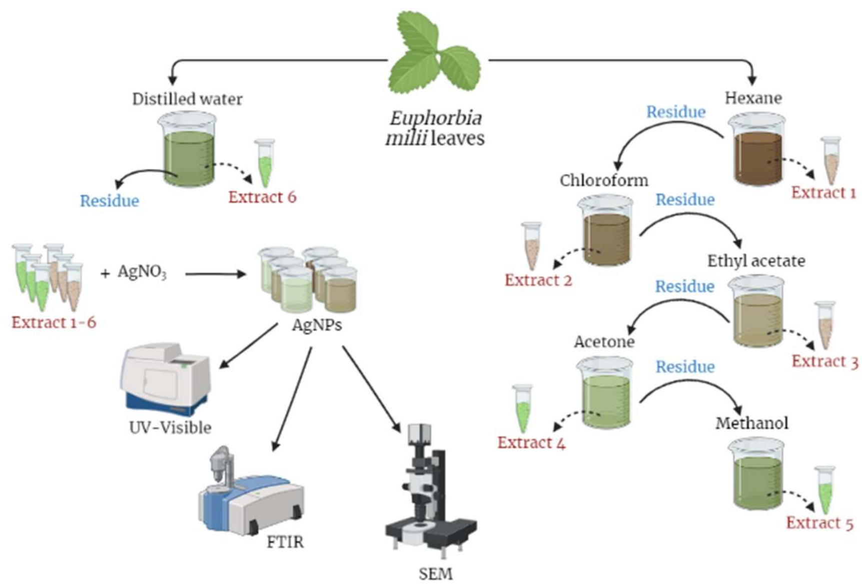

Figure 1. Overall experimental process

Results and Discussion

Green synthesis of AgNPs

The present study explored green synthesis of AgNPs was by using *E. milii* leaf extracts as a reducing agent. The reaction mixture turned from colorless to yellowish brown, which is the preliminary indication of the synthesis of AgNPs. Six conjugated AgNPs were made from $AgNO_3$ with different crude extracts. These conjugates were characterised as AgNPs-hexane extract (SH), AgNPs-chloroform extract (SC), AgNPs-ethyl acetate extract (SE), AgNPs-acetone extract (SA), AgNPs-methanol extract (SM) and AgNPs-distilled water extract (SDW-reference). AgNPs synthesis was confirmed with SEM analysis and discussed in a later section.

Characterization of AgNPs: Fourier-transform infrared spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) analysis of the obtained crude extracts (Figure 2) revealed different characteristic peak values with various functional compounds. Table 1 shows the peak assignment for six extracts. The major bands at 3341 cm^{-1}, 3248 cm^{-1}, and 3333 cm^{-1} represented the O-H group of carboxylic acid. The absorption band observed at ~2926 cm^{-1} corresponded to the stretching vibration of the C-H bond in the aliphatic hydrocarbon chains. The band at ~1636 cm^{-1} was attributed to C=C stretching vibrations of phenolic compounds such as polyphenols and flavonoids [10]. The C-H aliphatic band at approximately 1358-1396 cm^{-1} showed the presence of alkyl halides. The C-N bands at 1211 cm^{-1}, 1219 cm^{-1}, 1242 cm^{-1}, and 1258 cm^{-1} showed the presence of aliphatic amines. Lastly, the C-O bands at 1026 cm^{-1}, 1042 cm^{-1} and 1072 cm^{-1} showed the presence of alcohol, carboxylic acids, esters and ethers. The carboxyl and hydroxyl ions in the molecules develop a protective layer on the surface of AgNPs [9].

The FTIR spectra were carried out to recognize the existence of different functional groups. As shown in Figure 3, all conjugated samples compared to reference samples showed the formation of the O-H group (3333 cm^{-1}), C=C carbonyl-carbonyl group (1636 cm^{-1}), and C-H oop aromatic band (664 cm^{-1}).

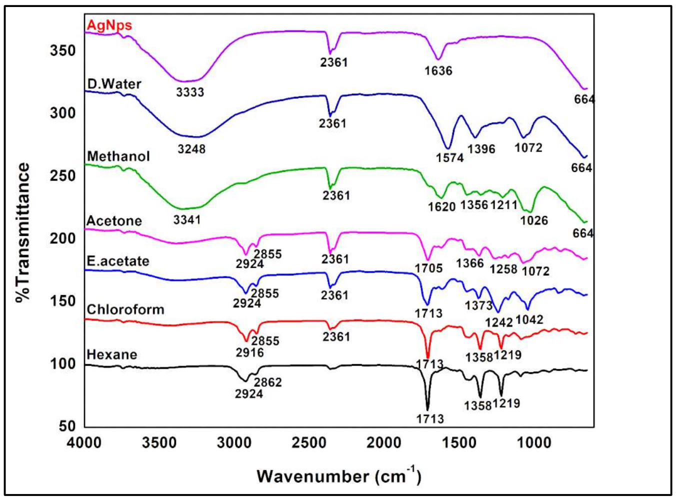

Figure 2. FTIR analysis for crude extracts

Table 1. Peak assignment for crude extracts

Peak Assignment	Wavenumber (cm^{-1})						
	Hexane	Chloroform	Ethyl Acetate	Acetone	Methanol	D. Water	AgNPs
O-H stretching	-	-	-	-	3341	3248	3333
C-H stretching	2926	2916	2924	2924	-	-	-
C=O stretching	1713	1713	1713	1705	-	-	-
C=C stretching	-	-	-	-	1620	1574	1636
C-H aliphatic	1358	1358	1373	1366	1356	1396	-
C-N	1219	1219	1242	1258	1211	-	-
C-O	-	-	1042	1072	1026	1072	-
C-H oop aromatic band	-	-	-	-	664	664	664

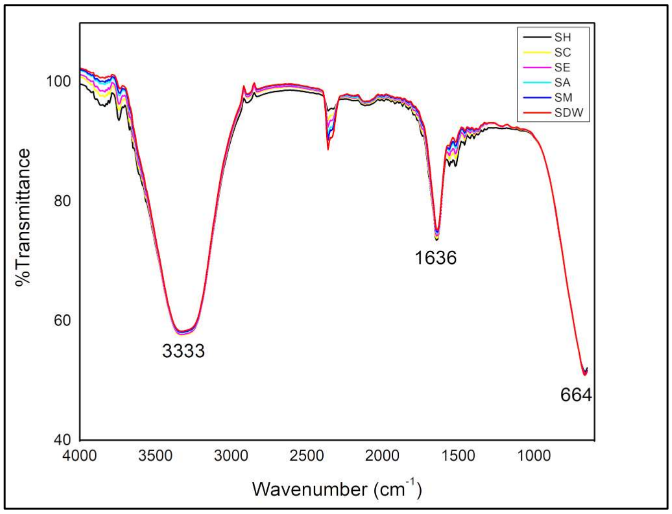

Figure 3. FTIR analysis for conjugated AgNPs

Ultraviolet-visible spectroscopy

The common phenomenon of surface plasmon resonance (SPR) in AgNPs has been studied in metal nanoparticles where electrons are excited by photons of incident light [2]. When excited by light, the induced charges characteristic of surface plasmons can propagate as an electromagnetic wave parallel to the metal surface. The most important factor in determining the frequency and intensity of plasmon resonance is their surface polarization. This is because it provides the main restoring force for electron oscillation [11]. All six conjugates were characterised by using UV-Vis spectroscopy to confirm the formation of the AgNPs. Based on all solvent systems, SH was the most unstable because the peak occurred only in 3 days. There was also no color change after the synthesis. While SC, SE, SA, SM, and SDW were more stable than SH because their peak occurred in 7, 42, 63, 63, and 64 days respectively (Figure 4 - Figure 8). As can be seen in UV-Vis spectra, the range of wavelength is from 401 to 456 nm. Table 2 shows the wavelength of AgNPs for each extract. The surface plasmon resonance band for spherical AgNPs was usually in the range of 390-450 nm [2] and 410-455 [12]. The optical properties of spherical AgNPs were highly dependent on the nanoparticle diameter. Smaller nanospheres primarily absorbed light and had peaks near 400 nm, while larger spheres had peaks that broadened and shifted towards longer wavelengths (known as red-shifting) [13]. Table 3 shows the references from other studies.

Table 2. Maximum peak for each AgNP

Conjugated AgNPs	Wavelength (nm)	Stability (Day)
AgNPs-hexane extract (SH)	427	3
AgNPs-chloroform extract (SC)	416	63
AgNPs-ethyl acetate extract (SE)	436	63
AgNPs-acetone extract (SA)	415	7
AgNPs-methanol extract (SM)	In-plane dipole: 401 In-plane quadrupole: 485	64
AgNPs-distilled water extract (SDW)	456	42

Table 3. References for the maximum peak from other studies

Plant	Solvent Extract	Wavelength (nm)	Stability (Day)	Reference
Ipomoea carnea Jacq.	Methanol	300-550	1	[14]
Terminalia catappa	n-Hexane	419	1	[15]
Terminalia catappa	Chloroform	418	7	[15]
Terminalia catappa	Ethyl acetate	264	1	[15]

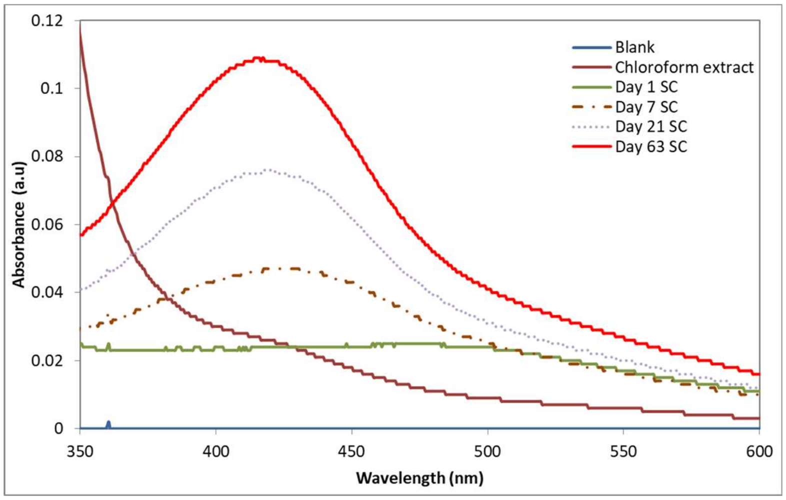

Figure 4. UV-Visible of SC

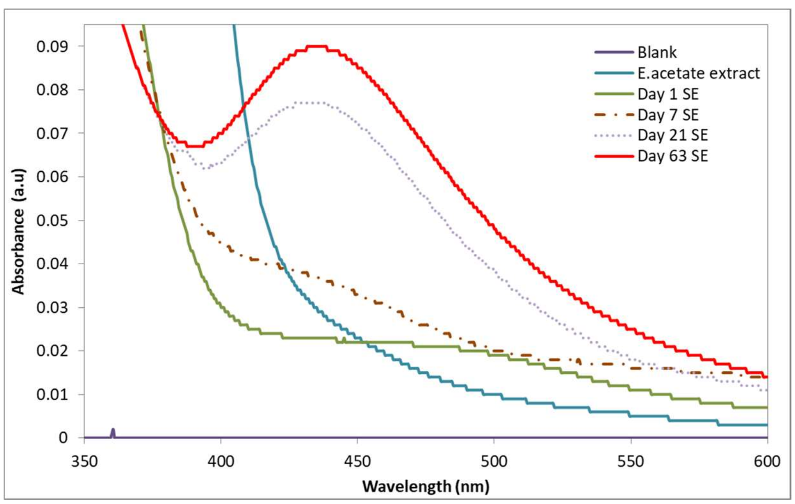

Figure 5. UV-Visible of SE

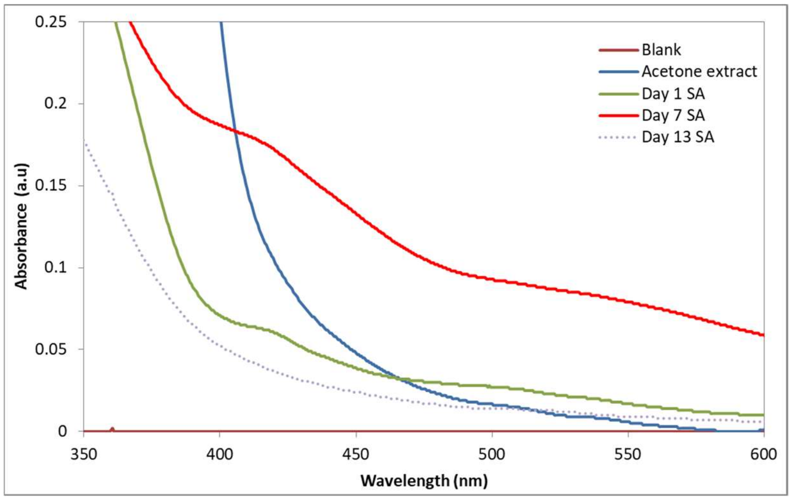

Figure 6. UV-Visible SA

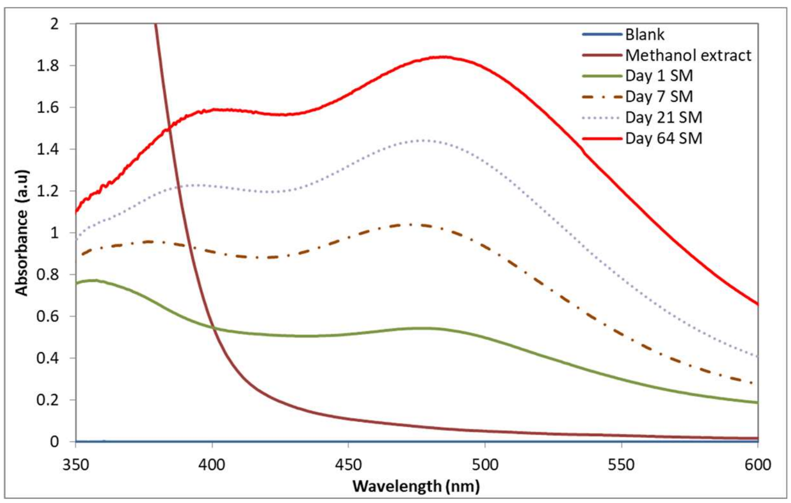

Figure 7. UV-Visible of SM

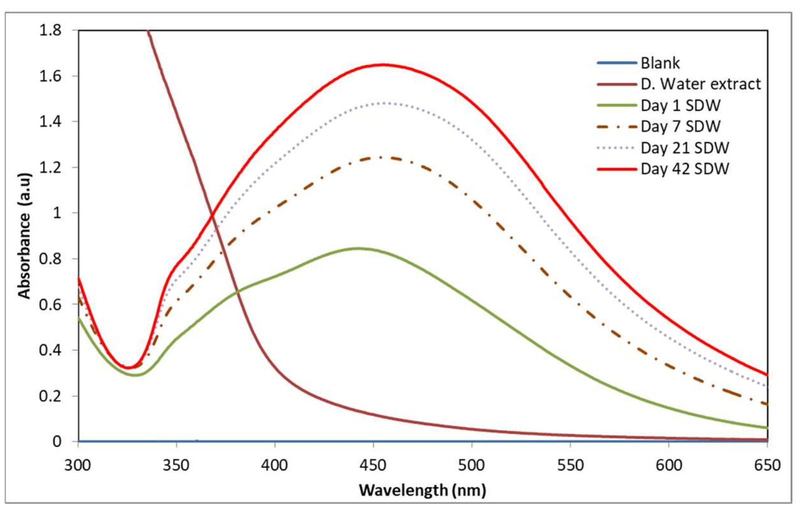

Figure 8. UV-Visible of SDW

Scanning electron microscope (SEM)

SEM technique was employed to visualise the size and shape of AgNPs. The SEM images of the AgNPs for each crude are shown in Figure 9. The smallest was from SE, which is 67-441 nm and the largest was from SH which is 771-843 nm (Table 4). The shape of all crude was mostly spherical. Compared to other studies using aqueous extract, *Euphorbia serpens* leaf had a spherical shape with particle sizes from 30 to80 nm [16], the spherical shape of *Euphorbia hirta* had a range of sizes between 50 and 80 nm [17], range sizes of *Boerhavia procumbens* was 20-80 nm with spherical shape [18], and Shireen, et al. stated that *A. americana, M. spicata, and M. indica* had mostly spherical and few triangular, rods and cubic shape [19].

Table 4. Range of size for AgNPs from various crude extract

AgNPs from Various Crude Extract	Size Range (nm)	Shape	References
(a) AgNps-hexane extract (SH)	771-843	Spherical	-
(b) AgNPs-chloroform extract (SC)	100-186	Spherical	-
(c) AgNPs-ethyl acetate extract (SE)	67-441	Spherical	-
(d) AgNPs-acetone extract (SA)	86-174	Spherical	-
(e) AgNPs-methanol extract (SM)	92-107	Spherical	-
(f) AgNPs-distilled water extract (SDW)	356	Spherical	-
Aqueous extract of *Euphorbia serpens* leaf	30-80	Spherical	[16]
Aqueous extract of *Euphorbia hirta* leaf	50-80	Spherical	[17]
Aqueous extract of *Boerhavia procumbens*	20-80	Spherical	[18]
Aqueous extract of *A. americana, M. spicata, and M. indica*	30-100	Mostly spherical Few triangular, rods and cubic	[19]

Conclusion

Six crude extracts of *E. milii* leaves were successfully utilized as capping and reducing agents for AgNP synthesis. The reaction conditions were optimized for approximate uniform dispersion of green synthesized AgNPs. The FTIR analysis suggested the presence of carboxylic acid, polyphenols, flavonoids, alkyl halides, aliphatic amines, alcohol, esters and ethers, responsible for the capping and stabilizing of AgNPs. UV-Vis spectroscopy confirmed the formation of AgNPs in all crude extracts by exhibiting the SPR band between 401 and 456 nm. SH was the most unstable AgNPs compared to other conjugated AgNPs. The shape for all conjugated AgNPs was spherical, with the sizes SH >SDW >SC >SM >SA >SE. Therefore, these studies concluded that six leaf extracts could be utilised for the green synthesis of AgNPs.

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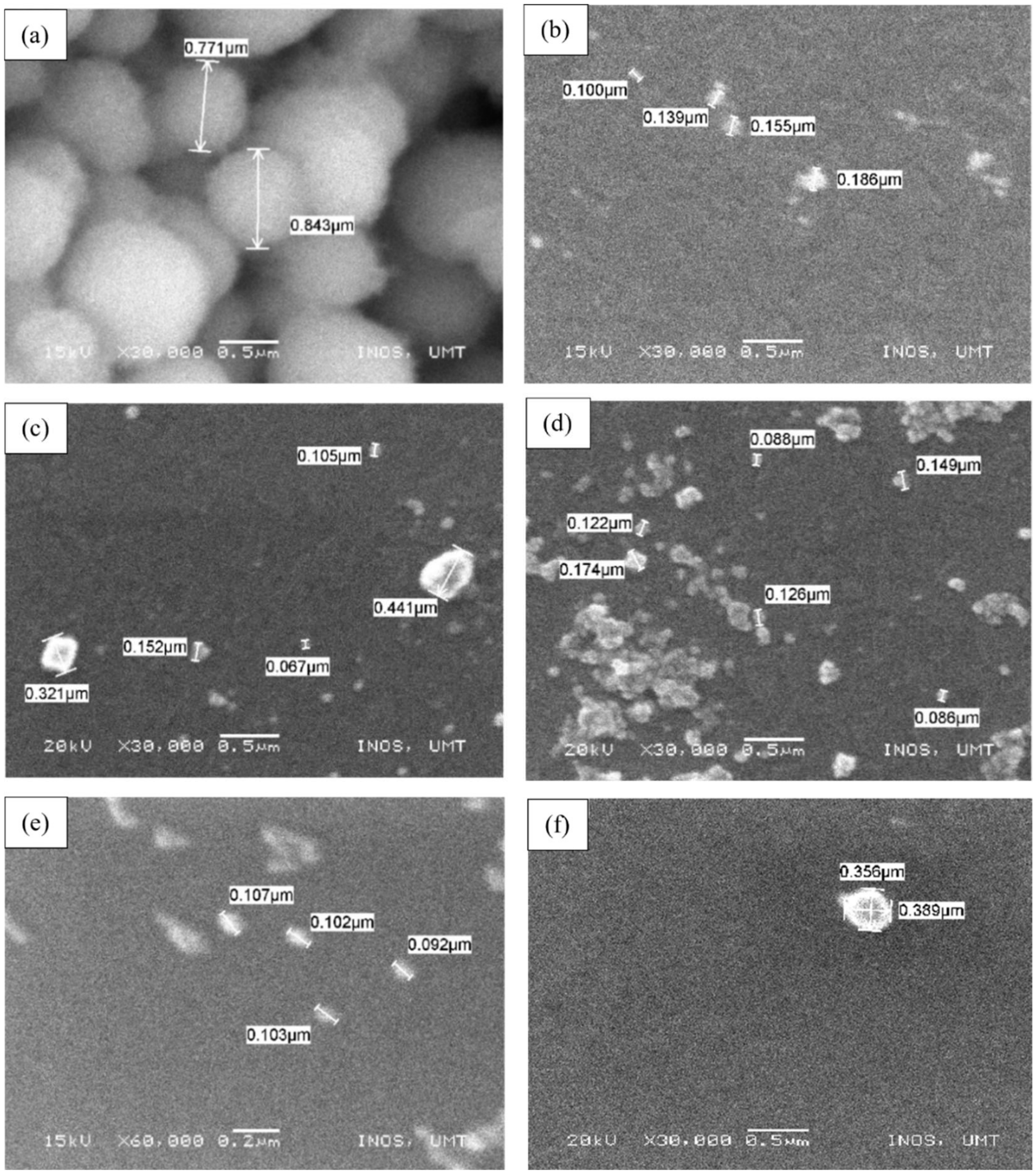

Figure 9. SEM result for AgNPs mediated with a) SH b) SC c) SE d) SA e) SM and f) SDW

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