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Research Article

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Green Synthesis of Silver Nanoparticles and its Antimicrobial, Antioxidant Activity of *Drynaria Quercifolia*

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Abstract: Eco Friendly or green synthesis of metal nanoparticles has become an important branch of nanotechnology and due to their large applications, there is a growing commercial demand for nanoparticles. The plant named' Oak leaf fern' belongs to the Polypodiaceae family and is native to India, Southeast Asia., etc. The main objective of the study is to synthesize silver nanoparticles from *Drynaria quercifolia* and to check its antioxidant and antibacterial activity from synthesized nanoparticles. The phytochemical analysis was carried out for different extract such as methanol extract, ethyl acetate extract and petroleum ether extract. While comparing the three extracts, methanol extract showed the presence of alkaloids, flavonoids, Terpenoids, Saponin and carbohydrates. The Reducing power assay was conducted for methanol extract. The percentage inhibition of free radicals is found to be 632.06µg/ml. In this study, the synthesis of silver nanoparticles using methanol leaf extract of *D.quercifolia* in an eco-friendly and cost effective way is reported. The use of *D.quercifolia* leaf extract as a reduction agent from 1 mM silver nitrate has been examined for the synthesis of silver nanoparticles (SNPs) ($AgNO_3$). The synthesized nanoparticles were characterized by UV–Vis (422 nm), FTIR, SEM, and XRD studies. Within 24 hours of the incubation time, silver nanoparticles were synthesized and generated SNPs had an absorption peak of roughly 400 nm in the UV-visible spectrum. The synthesized AgNPs shows good antibacterial activity against Gram negative bacteria as well as Gram-positive. This technique is quick, easy, without any hazardous chemicals such as reducing or stabilizing agents and economical to synthesize SNPs.

Keywords: *D.quercifolia*, Green synthesis, Silver nanoparticles, UV-Vis, FTIR, SEM, XRD, Antioxidant activity.

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1. INTRODUCTION

Nanotechnology is the perception and control of matter at dimensions ranging from about 1 to 100 nanometres, where unusual phenomena allow for new applications. Nanotechnology includes nanoscale research, engineering, and technology, including the imaging, calculation, simulation, and manipulation of matter at this length scale. The nanotechnology industry has proven to be one of the most active research areas[1]. Nanoparticle synthesis is exponentially increasing due to its wide range of applications in the fields of optoelectronics, biosensors, bio-nanotechnology, biomedicine and so on[2]. The' natural' environmentally friendly processes in chemistry and chemical technology are becoming increasingly popular and are much needed as a result of environmental concerns linked worldwide problems[3]. Silver is one of the most commercialized nano-materials with an annual production of five hundred tons of silver nanoparticles[4], and is expected to grow in the coming years. Including its profound function in the field of high sensitivity biomolecular detection, catalysis, biosensors and medicine; significant inhibitory and bactericidal effects, anti-fungal, anti-inflammatory and anti-angiogenic activities have also been recognized[5]. Medicinal plants have been used for thousands of years in traditional treatments for numerous human diseases, and continue to be an important therapeutic aid to alleviate human diseases[6]. According to the World Health Organization[7], "a medicinal plant" is any plant that contains substances in one or more of its organs that can be used for therapeutic purposes and are precursors for useful drug synthesis[8]. Screening for antimicrobial activity, plant products has shown that the higher plants represent a potential source of novel antibiotic prototypes[9]. The occurrence of multiple resistances in human pathogenic microorganisms has been growing in recent years, largely due to the indiscriminate use of synthetic antimicrobial drugs widely used in the treatment of infectious diseases. This has prompted scientists to search for new antimicrobials from diverse sources such as medicinal plants[10]. *D. quercifolia J*. Smith is a genus of fern in the family Polypodiaceae, commonly known as the Oak leaf fern. It is a big, densely scaly, woody rhizome herb. Leaves in adult plants are two types: 1) sessile, cordate-oblong, permanent bract leaves with a pinky lobed margin clasping the rhizome to serve as human collectors, and 2) oblong, pinnate-like, regular leaves with a rigid, brownish, winged petiole, about a foot long. Bract leaves are up to 9 inches long and up to 6 inches wide, persistent, sticking like brittle, dark, hard structures with a prominent midrib and main lateral veins to the rhizome. Normal lamina leaves 2-3 feet long and about one foot wide pinnatisect each segment being linear with a wide base, tapering apex, and whole margin. Venation is rhetorical. Young plant leaves (up to 2 or 3 years of age) are all sessile, simple lanceolate, with attenuated base and reticulated vein. The Aim of the study is to synthesize Silver Nanoparticles from *D. quercifolia,* and to find its biological activity such as Antimicrobial and Antioxidant activity of synthesized nanoparticles.

2. MATERIAL AND METHODS

2.1 Collection And Extract Preparation

The fresh samples of *D. quercifolia* (AORF00139) obtained from Yercaud hills were authenticated and submitted in Alpha Omega Research Foundation, Salem -636008, Tamil Nadu. The sample materials were washed under running tap water, dried in air and then homogenized to fine powder and placed in refrigerated bottles with airtight effect. Extract of the raw sample was prepared using the Soxhlet apparatus. Around 20 gm of powdered sample content was uniformly packed into a thimble and extracted successively with 250ml of petroleum ether, ethyl acetate and methanol respectively. Extraction were carried out for 24 hours, until the solvent from the siphon tube became colourless. The extract was then taken in a beaker and placed on a hot plate, heated at 30-40°C until all the solvent had been evaporated. Dried extract was kept at 4°C in the refrigerator for future use [41].

2.2 Phytochemical Screening

For all the *D. quercifolia* extracts, preliminary phytochemical analysis was performed according to the standard methods defined by Brain and Turner[11] and Evans[12].

2.2.1 Detection Of Alkaloids

Extracts of *D. quercifolia* were dissolved and washed individually in dilute hydrochloric acid. The filtrates were used to test for alkaloid presence. Filtrates have been treated with reagent Mayer. Formation of a precipitated yellow cream indicates the presence of alkaloids.

2.2.2 Detection Of Flavonoids

Sulphuric acid test: Few drops of H_2SO_4 were treated with extracts. Orange colour formation indicates the presence of the flavonoids.

2.2.3 Detection Of Steroids

0.5 g of the extracts were combined with 2ml of acetic anhydride, each with 2ml of H_2SO_4. In some samples the colour change from violet to blue or green suggests the presence of steroids.

2.3 Detection Of Terpenoids

2.3.1 Salkowski's test

0.2 g of the whole plant sample extract was carefully applied and mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml).

2.4 Detection Of Anthraquinones

2.4.1 Borntrager's test

Approximately 0.2 g of the extract was boiled for few minutes in a water bath with 10 percent HCl. It was filtered, and allowed to cool. The filtrate was replaced with equal volume of chloroform. A few drops of 10 per cent NH_3 have been added and heated to the mixture. Pink colour formation indicates the presence of the anthraquinones.

2.4.2 Detection Of Phenols

Ferric chloride test: Extracts were treated with a few drops of a solution of ferric chloride. Bluish black colour formation shows presence of phenol.

2.4.3 Detection Of Saponins

For 5ml of distilled water, about 0.2 g of the extract was shaken. Frothing formation (creamy appearance of small bubbles) indicates the presence of saponins.

2.4.4 Detection Of Tannins

A small amount of extract was mixed with water and placed on a bath of water. The mixture was filtered, and to the filtrate, ferric chloride was added. The presence of tannins is confirmed by the appearance of dark green coloration.

2.4.5 Detection Of Carbohydrates

0.2gm filtrate was boiled on water bath with 0.2ml each of Fehling solution A and Fehling solution B. A red precipitate indicates the presence of sugar.

2.4.6 Detection Of Oils And Resins

Test solution was applied on filter paper. It developed a transparent appearance on the filter paper. It indicates the presence of oils and resins.

2.5 Quantitative Phytochemical Analysis

2.5.1 Estimation Of Alkaloids

Determination of alkaloids was done using standard process[13]. *D. quercifolia* sample of weight 5 g taken in a 250 ml beaker and 200 ml of 10 percent acetic acid in methanol was added and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was applied to the extract in drop wise until the precipitation was complete. The entire solution was allowed to settle, and the precipitate was collected, washed and filtered with diluted ammonium hydroxide. The residue is the alkaloid which was measured and dried.

2.5.2 Estimation Of Flavonoids

Ten grams of *D. quercifolia* plant samples have been harvested repeatedly at room temperature with 100ml of 80 per cent aqueous methanol. The mixture was then filtered into a pre-weighed 250ml beaker with a filter paper. The filtrate initially weighed was moved and kept in a water bath and allowed to evaporate till it dried. The flavonoid percentage content was calculated by difference in weight[14].

2.5.3 Synthesis Of Silver Nano-Particles

0.1 M of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 1 ml of methanolic extract of *D.quercifolia* with 9 ml of 1 mM silver nitrate was combined. Methanol leaf extracts from the *D.quercifolia* and silver nitrate solution were used as the control throughout the experiment.[15.] About 200 ml of the solution were centrifuged at 18,000 rpm for 25 min. The pellets obtained were processed at 40°C. The supernatant was heated at temperature maintained between 50°c and 95°c. During the heating process, a change in colour of the solution was observed.

2.5.4 *Analytical Characterization*

In-order to confirm the formation of Silver Nano particles in *D. quercifolia,* absorption studies were performed on a Perkin-Elmer UV-visible spectrophotometer, Lambda 35, Germany) the spectra were taken between 340 nm and 480 nm in different time intervals up to 24 Hrs. The chemical composition of the synthesized silver nanoparticles was analyzed with a FTIR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer). Fourier transform infrared spectroscopy (FTIR) is just one of the superlative analytical tool that allows functional groups to be identified in the aqueous bark extract and generated SNs. The solutions were dried at 75°C and KBr was used to characterize the pellet method with dried powders in the range 4000-400 $cm^{-1.}$

2.5.5 Antimicrobial Studies

The antimicrobial activity was tested using the disc diffusion method[16]. *In Vitro* antimicrobial activity was screened using Mueller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media onto sterile petri dishes. The well diffusion agar method[17] done with some modification. The dextrose agar (SDA) of Sabouraud was used for fungal cultivations. The culture medium was inoculated in Sabourauds dextrose broth with the fungal strains suspended separately. A total disc of 8 mm in diameter was kept in the agar and filled with extracts from plants. Generic antibiotics (Fucanazole, concentration 1 mg / ml) have been used as positive control and fungal plates have been incubated for 72 hours at 37°C. The observed diameters of the inhibition zone were measured. The plates were allowed to solidify for 5 minutes, and 0.1 percent inoculum suspension was swabbed evenly and drying allowed for 5 minutes. The 40 mg concentration of the extract was placed on a 6 mm sterile disc. The loaded disk was placed on the medium surface and the extract was allowed to diffuse for 5 minutes, while the plate was held for incubation at 37°C for 24 hours. At the end of the incubation, inhibition zones formed around the disc were measured in mm with a transparent ruler.

2.6 Antioxidant Activity

2.6.1 *Evaluation Of Total Antioxidant Capacity*

In an Eppendorff tube, an aliquot of 0.1ml of the sample solution containing a sample in DMSO was mixed with 1ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). For 90min, the tubes were capped and incubated at 95°C in a water bath. The samples were cooled to room temperature, and each solution was calibrated at 695 nm for absorption. The total antioxidant power was expressed as an ascorbic acid equivalent in mM[18].

2.6.2 Reducing Power Assay

An Oyaizu[19] method was developed to test the reducing power. With 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1 percent potassium ferricyanide, the above sample including extract together with Ascorbic acid solutions was spiked. The mixture was then held for 20min in a water-bath of 50°C. The resulting solution was easily cooled, spiked with 2.5ml of 10 per cent trichloro acetic acid, and centrifuged for 10 min at 3000rpm. The supernatant (5ml) was then Combined with 5ml distilled water and 1ml ferric chloride of 0.1 per cent. After a 10min reaction the absorbance was

measured at 700 nm. The higher the absorbance, the greater the reduction capacity. The power reduction assay was expressed in terms of equal Ascorbic acid per gram of dry weight basis.

3. RESULTS AND DISCUSSION

Extracts of *D. quercifolia* extract was prepared using Methanol, Ethyl acetate, and Petroleum Ether solvents by using Soxhlet extraction method. Compared to other two extracts (Ethyl acetate and Petroleum Ether), higher yield was obtained in methanol extract.

3.1 Phytochemical Analysis Of *Drynaria Quercifolia*

The comprehensive phytochemical study of *D. quercifolia* rhizome extracts of Methanol, Ethyl acetate, and Petroleum Ether was performed. The methanol extract had major constituents such as alkaloids, flavonoids, terpenoids, saponin, and carbohydrates (Table 1). Ethyl acetate extract had revealed flavonoids, terpenoids, oils & resins. Carbohydrate, Oils & Resins were present in the petroleum ether extract. The methanol extract from *D. quercifolia* observed higher phytochemical activity. Recent studies have been observed on the phytochemistry of medicinal plants, especially on components such as leaves and stems, etc[20,-23]. Phytochemicals are a large group of compounds which act as major antioxidants, especially plant phenolics[24].

Table 1 Qualitative phytochemical analysis of *D. quercifolia* extract

Phytochemicals	Observations	Extracts		
		Methanol	Ethyl acetate	Pet. Ether
Alkaloids Mayer's test	Cream *colour* Reddish brown solution/ precipitate	+	-	-
Flavonoids H_2SO_4 test	Yellow orange Reddish brown / Orange *colour* precipitate	+	+	-
Steroids Liebermann-Burchard test	Violet to blue or Green *colour* formation	-	-	-
Terpenoids Salkowski test	Reddish brown precipitate	+	+	-
Arthroquinone Borntrager's test	Pink *colour*	-	-	-
Phenols Ferric chloride test	Deep blue to Black *colour* formation White precipitate	-	-	-
Saponin	Stable persistent	+	-	-
Tannin	Brownish green / Blue black	-	-	-
Carbohydrates	Yellow / brownish / blue / green *colour*	+	+	+
Oils & Resins	Filter paper method	-	+	+

Note: "+" indicates presence and "-" indicates absence

The methanol extract of *D. quercifolia* was subjected to phytochemical quantitative analysis. Total alkaloid content and flavonoid content were found to be 0.496g, 0.237 g respectively (Table 2). Alkaloids have shown a cytotoxic effect on tumor cell lines, emphasizing their role in the prevention of cancer, neurodegenerative diseases, chronic inflammation, etc.,[25]. Medicinal benefits of flavonoids in plants include antioxidant and anti-inflammatory activities[26,27].

Table 2 Quantitative Analysis of *D. quercifolia*

S.No.	Phyto constituents	Methanol Extracts (g)
1.	Alkaloids	0.496 ± 0.05
2.	Flavonoids	0.237 ± 0.12

3.2 *Antioxidant Activity*

With the increased concentration, the antioxidant activity of the extract increased. The total antioxidant activity of *D.quercifolia* methanol rhizome extract was evaluated at various extract concentrations. For increased extract concentrations, the overall antioxidant activity increased. The frequencies were 497.48 µg / ml, 497.75 µg / ml, 506.13 µg / ml and 513.16 µg / ml, respectively (Table 3).Increased absorption can show an increase in energy reduction[28], while decreased compound ability can indicate potential antioxidant property. The reduction capacity of Fe^{+3} to Fe^{+2} have already been determined using the technique described (reduction effect) [29]. A compound's reduced capacity is also a strong indicator of its future antioxidant activity[30]. There was a comparison with dietary antioxidants, such as ascorbic acid. Extracts with reduced energy prove that they are electron donors and can minimize the oxidized intermediates of lipid peroxidation procedures to function as main and secondary antioxidants[31]. Test was conducted to determine the power reduction of the *D.quercifolia* methanol rhizome extract. With increasing extract concentration, the percentage of decreased power activity increases.

Table 3 Reducing power Assay of *D. quercifolia*

S.no.	Sample (µl)	% IC_{50}	IC_{50}
1.	31.25	32.65	

			632.06
2.	250	37.41	
3.	500	44.90	
4.	750	53.06	
5.	1000	62.59	

3.3 Antimicrobial Activity

Bacterial strains *B.subtilis, S.typhi* and *S.aureus* and fungal strains *C.lunata, C.albicans* and *A.niger* were used to study the antimicrobial activity from the synthesized *D. quercifolia* methanol extract. The *in vitro* antimicrobial activity showed that the methanol extract had substantial activity against all the microorganisms studied, especially on high-concentration of *S.typhi, S.aureus* and *C.lunata* (60 µl), but there was activity in the lower concentrations also. The results of antimicrobial activity from the synthesized *D. quercifolia* methanol extract showed excellent effect on all organisms. The maximum inhibition zone on *C.lunata* (20 mm) was observed in the rhizome extract in methanol synthesized with a concentration of 60 µl and a minimum inhibition zone observed on *C.albicans* (14 mm) and *A.niger* (14 mm) (Table 4). The antimicrobial activity of nanoparticles also depends on the nanoparticle shapes. This attribute can be further verified by using various formed nanoparticles to study the inhibition of bacterial growth[32]. According to Pal,[33] the smaller nanoparticles displayed bacterial inhibition with concentration of silver ion 1 as large as 10^{-3} M.

Table 4 Antimicrobial activity of *D. quercifolia* containing silver nanoparticles

S.No.	Organisms	Control	Methanol		Silver nanoparticles	
			30 µl	**60** µl	**30** µl	**60** µl
1.	*B.subtilis* (ATCC No: 39090)	22	09	15	11	16
2.	*S.typhi* (ATCC No: 6539)	24	09	20	10	18
3.	*S.aureus* (ATCC No: 6538)	23	08	18	08	17
4.	*C.lunata* (ATCC No: 34600)	24	11	21	11	20
5.	*C.albicans* (ATCC No: 10231)	24	12	15	10	14
6.	*A.niger* (ATCC No: 20611)	22	09	16	11	14

3.4 Spectral Studies Of Silver Nanoparticles

The plasmon surface vibrations of these SNs provided a peak of 420 nm and suggested a reducing $AgNO_3$ to SNs. It is known that the optical properties of the metal nanoparticles are strongly dependent on their shape and size[34]. In a 100 ml flask 1 mm silver nitrate solution was prepared. 1 ml of plant extract was combined with a silver nitrate 9 ml of 1 mM.

3.5 Uv-Vis Analysis

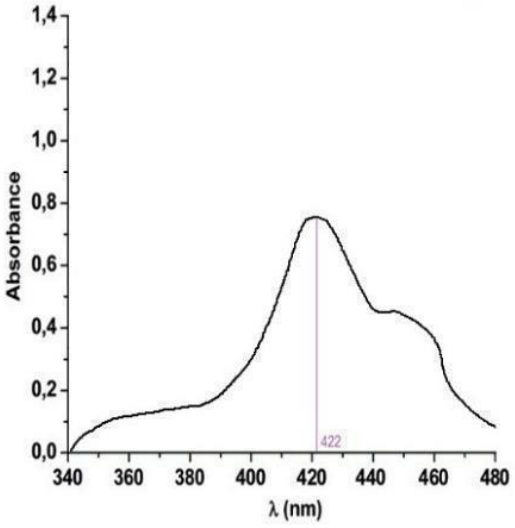

Fig 1 UV-Vis spectroscopy of *D. quercifolia* Synthesized Methanol Extract

Over the entire experiment, the leaf methanol extracts from the *D. quercifolia* and silver nitrate solution were used as a buffer. The final solutions were centrifuged for 25 minutes at 18,000 rpm. The pellets collected had been stored at -40˚c. The supernatant was heated at 50˚c to 95˚c. During the heating process a change in solution colour was observed. The UV-VIS analysis of the silver nanoparticles synthesized using methanol extract of *D. quercifolia* was done. It was seen that the maximum absorption was obtained at 422 nm in the visible range (Figure 1).

3.6 FTIR Analysis

The FTIR data of the silver nanoparticles revealed that in the =C–H stretch (alkenes) occurred at 3062.27 cm-1, C-H Stretch (alkanes) at 2921.80, N–O asymmetric stretch (nitro

group) at 1591.30 cm-1, C–N stretch (aliphatic amines) at 1104.32 cm-1. C-O (alkoxy) occurred at 1076.56 cm-1, O–H bend (carboxylic acids) at 926.27 cm-1 and ortho disubstituted aromatic group at 743.92 cm-1. –C≡C–H: C–H bend (alkynes) and C–Br stretch (alkyl halides) occurred at 644.03 cm-1 and 527.45 cm-1 respectively (Figure 2).

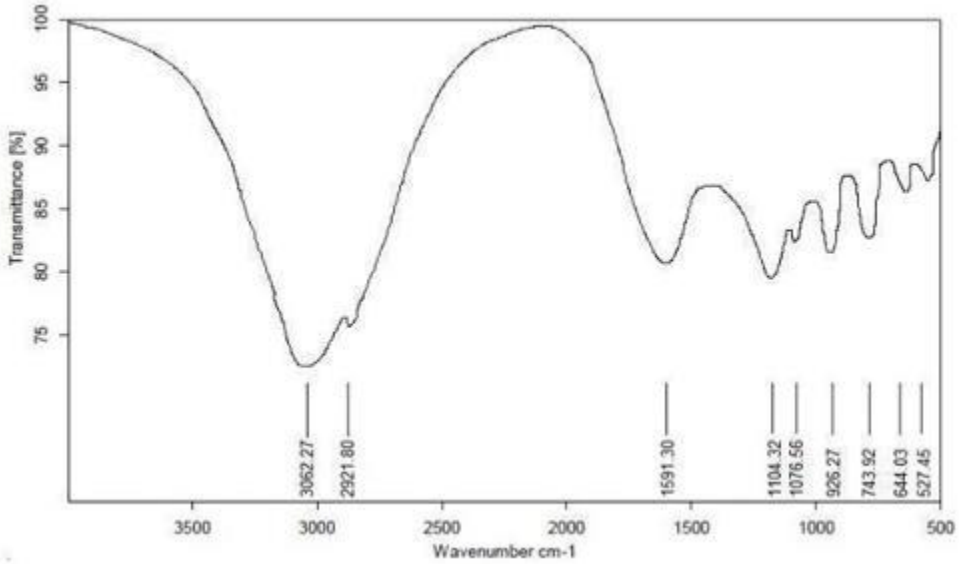

Fig 2 FTIR Analysis of *D. quercifolia* Synthesized Methanol Extract

3.7 XRD Analysis

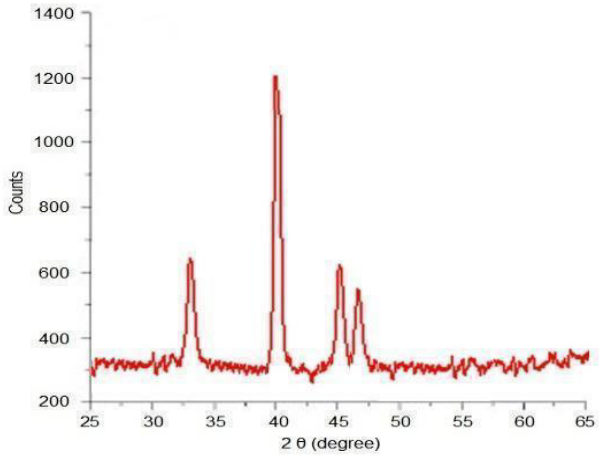

Fig 3 XRD Analysis of *D. quercifolia* Synthesized Methanol Extract

XRD patterns of ZnO synthesized from leaf extract are shown in Figure 3. The diffraction peaks at 2θ 33.17, 40.62, 45.03 and 47.43 correspond to (211), (111), (200), (103), planes respectively. As indicated, all of the diffraction patterns are well indexed to cubic ZnO structure. The estimated lattice constants have values a=b= 0.9054 Å and c= 6.68Å, which are close to the standard values (JCPDS no. 04-0783). According to the XRD data, the mean crystalline sizes (D) of the Silver nanoparticles are calculated using Debye Scherrer's formula.

$$D = \frac{K\lambda}{\beta \, Cos \, \theta} \text{ Å}$$

Where, λ=1.5418 Å is the wavelength of the X-ray radiation used. The term θ is the Bragg diffraction angle and β is the full width at half its maximum intensity of diffraction pattern (FWHM) in radian. The obtained sizes are 69.381, 57.82, 92.51 and 126.15 nm.

3.8 SEM Analysis

The SEM analysis of the silver nanoparticles synthesized from methanol extract of *D. quercifolia* reveal that the particles are cubic in structure (Figure 4). Silver nanoparticles (AgNPs) appear yellowish brown in colour in aqueous medium as a result of surface plasmon vibrations[35]. As the leaf extracts were added to aqueous silver nitrate solution, the colour of the solution changed from faint light to yellowish brown to reddish brown and finally to colloidal brown indicating AgNP formation. Lalitha[36], Simghal[37], Philip and Unni[38] confirmed the completion of reaction between leaf extract and $AgNO_3$. The silver nanoparticles were synthesized in both crude extract and methanol extract of plant samples. The colour changes observed indicates the formation of silver nanoparticles[39,40].

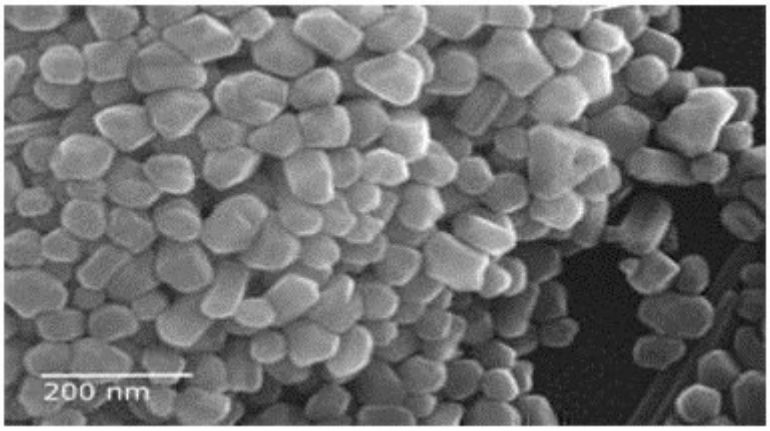

Fig 4 SEM Analysis of *D. quercifolia* Synthesized Methanol Extract

4. CONCLUSION

The present investigation brings out adequate data on the phytochemical composition in different extracts like methanol, ethyl acetate and petroleum ether. The Methanol extract (Alkaloid, Flavonoid, Terpenoids, Saponin and Carbohydrates) shows higher phytoconstituents when compared to other two extracts. The synthesized silver nanoparticles and its antibacterial activity of methanol extract of *D.quercifolia* were studied. From the study it is concluded that more number of phytochemical constituents are present in methanol extract when compared to other two extracts. As for the antibacterial activity, the methanol extracts show differences in the activity, depending on the concentration of the extract. Synthesized silver nanoparticles show highest antimicrobial activity at higher concentration (60µl) against six different organisms tested. The synthesized nanoparticles are characterized by UV (422 nm), FTIR, XRD and SEM. The antioxidant activity is also found to be higher in methanol extract of *D.quercifolia*. Finally, it is concluded that the *D.quercifolia* is a source of bioactive compounds with potential applications, revealing activity to control pathogens.

5. AUTHOR'S CONTRIBUTION STATEMENT

Dr. Rajagopal Kanagalatha and Geetha Chinnusamy conceptualized and gathered the data regarding this work and also analyzed these data and necessary inputs were given towards the designing of the manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared of none.

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