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An Investigation on the Antiproliferative and Antibacterial Activity of Silver Nanoparticles of *Quercus infectoria and Daucus carota subsp sativum*

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Authors' contributions

This work was carried out in collaboration among all authors. Author SP performed the experimental analysis and wrote the protocol. Author JA designed the study and managed the literature search and wrote the first draft of the manuscript. Authors JA and SAS managed the conduct of the study. All authors read and approved the final manuscript.

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ABSTRACT

Plant mediated fabrication of nanoparticles and nanomaterials are gaining momentum as it is eco-friendly and cost-effective. In the present study, we synthesis of Silver nanoparticles using aqueous extract of *Quercus infectoria* nuts and *Daucus carota subsp sativum* leaves. The surface plasma resonance at 417 and 450 nm for *Q. infectoria* and *D. carota* respectively confirmed the formation of AgNPs. Scanning Electron Microscopic (SEM) confirmed the spherical shape of the nanoparticles, which had an average size of 67.5 nm and 49.2 nm for *Q. infectoria* nanoparticles (QAgNPs)and *D. carota* nanoparticles (DAgNPs). The elemental composition by Energy-Dispersive X-ray analysis of the nanoparticle showed an atomic percentage of silver as 73.64 % and 75.93% for *Q. infectoria* and *D. carota*.FT- IR analysis of the plant extracts and synthesized silver nanoparticles showed the presence of various functional groups. The total antioxidant activity of QAgNPs was 81.18% and that of DAgNPs was 73.36%. The QAgNPs and DAgNPs exhibited antibacterial activity against *B. subtilis, E. coli* and *S. aureus*. The percentage of cell viability for QAgNPs and DAgNPs assessed using HeLa cells was 21.1% and 6% respectively.

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Keywords: Silver nanoparticles; characterization; antibacterial activity; antioxidant activity; cell viability.

1. INTRODUCTION

Nanotechnology is a rapidly emerging technology having a wide variety of potential applications in waste management, optics and electronics, solar energy and sensing technology [1]. It also finds its application in chemical technology such as water purification, catalysis,(and) cosmetics [2,3,4]. Nanomaterials are used in the field of medicine for drug delivery, diagnosis, wound healing, development of antimicrobial agents [5]. There are many physical and chemical methods employed for the preparation of nanoparticles. These methods are costly and toxic to the environment [6]. Biosynthesis of silver nanoparticles is a bottom-up approach of nanoparticles synthesis. The antioxidants or reducing properties of the phytochemicals present in the plants are responsible for the reduction of metal compounds. Methods employed for the biosynthesis of metal nanoparticles are considered to eco- friendly, biocompatible, non- toxic and clean [7].

Among the noble metals, silver is the most preferred metal of choice in the field of biological systems and medicines. It has its own disinfectant property which is employed for hygienic and medical purposes like, treatment of mental illness, nicotine addiction and other infectious diseases like syphilis and gonorrhoea. Silver nanoparticles can also be used for increasing shelf life of fruits[8], in dental materials [9], cosmetics [10], water treatment [11] and for coating stainless steel used in medical devices [12].

Quercus infectoria commonly known as Aleppo Oak , belongs to the family *Fagaceae.* It is a small tree, native of Greece, Asia Minor and Iran. The extract is claimed to be highly beneficial for postpartum women to restore the elasticity of the uterine wall. It is also known to possess astringent, antidiabetic, local anesthetic, antiviral, antimicrobial, larvicidal (refrencess) and anti-inflammatory activities. The nut galls are pharmacologically documented on their antiamoebic, anticarcinogenic and anti-inflammatory activities, to treat skin infections and gastrointestinal disorders. The galls mainly contain a mixture of 50-70% gallotannin, gallic acid, ellagic acid, starch and glucose as the principle components. Other constituents include syringic acid, methyl gallate, β- sitoserol, amentoflavone, hexamethylether, isocryptomerin, methyl oleanate, methyl betulate and hexagallocyl glucose [13]. These are used as medicines for treating fungal infections mostly on hair, skin and nails.

Daucus carota subsp. sativus commonly known as carrot belongs to the family *Apiaceae*. The plant was introduced in Spain by the Moors in the 8th century. It is a biennial plant. Carrot leaves are harvested young in high- density plantings, before significant root development, and typically used in salads. The leaves of *D. carota subsp. Sativus* are highly nutritive, rich in protein, vitamin and minerals. The vitamin C present in the leaves is six times more than that present in the root. The leaves also have good antiseptic and astringent property. The leaves can be used as a good remedy as antibacterial, antifungal against *Mycocentrospora acerina* and *Cladosporium cladosporiodes* and for anti-inflammatory purposes. The leaves are said to contain compounds such as pyrrolidine [14]. The leaves can be used as a good remedy as antibacterial, antifungal and anti- inflammatory purposes. Polyacetylenes such as falcarinol and falcarinol can be found in carrots where they show cytotoxic activities [15].

The present study aimed to synthesize silver nanoparticles using nut galls of *Q. infectoria* and leaves of *D. carota*. To the best of our knowledge this is a first report on synthesis of silver nanoparticles from leaves of *D. carota.* We evaluated the antibacterial and antioxidant activity of the nanoparticles. The antiproliferative effect of the nanoparticles on HeLa (Human Breast cancer cell lines (MCF-7) and Vero) cell lines was (ere-delete) studied by MTT assay.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Samples

Fresh, healthy nut galls of *Q. infectoria* were purchased from local siddha medicinal shop in Chennai. Fresh leaves of *D. carota* were obtained from farms in Ooty, The Nilgiris. The leaves were washed repeatedly with running tap water to remove any surface contaminants and finally in distilled water. It was shade dried at room temperature and made into a fine powder. 2g of *Q. infectoria* powder was added to 100 mL distilled water and 10g of *D. carota* leaf powder

was added to 100 mL distilled water and heated at 90℃ for 20 minutes. The mixture was cooled to room temperature and filtered using Whatman no 1 filter paper. The filtered extracts was then stored at 4℃ for further use [16].

2.2 Synthesis of Silver Nanoparticles

0.5 mL *Q. infectoria* extract was added to 100 mL of silver nitrate solution (1mM) and 2 mL of *D. carota* extract was added to 100 mL of silver nitrate solution (1mM). The mixture was heated at 90℃ for 30 minutes. The change of colour from pale yellow to black indicated the formation of silver nanoparticles [17]. Further, the reaction mixtures were centrifuged at 10,000 rpm for 10 minutes and washed with double distilled water. The centrifugation process is repeated for 4- 5 times and the pellet was allowed to dry in hot air oven. The dried powder is used for further characterization studies.

2.3 Characterization of Silver Nano-particles

The formation of silver nanoparticles is monitored by measuring the UV- vis spectra of the reaction mixture at a wavelength of 300- 500nm. The shape of silver nanoparticles is examined by Scanning Electron Microscopy (SEM). The presence of elemental silver is analyzed by energy dispersive spectroscopy attached to SEM.

2.4 Fourier Transform Infra-Red Spectrophotometer (FTIR)

Both aqueous extracts and synthesized silver nanoparticles of *Quercus infectoria* and *Daucus carota* were subjected to FTIR analysis. The characteristic peak values and their functional groups were recorded [18].

2.5 Phytochemical Analysis

The aqueous *Q. infectoria* and *D. carota* extracts were test for the various phytocompounds such as phenols, flavonoids, tannins, carbohydrates, steroids, quinines, coumarins, phlobatannins, amino acids, alkaloids, glycosides, vitamin C, saponins, triterpenes and proteins [18,19].

2.6 Total Phenolic Content

The total phenolic content of the aqueous plant extract, QAgNPs and DAgNPs were estimated using Folin- Ciocalteau method. 500 µL of distilled water was taken in a test tube and 100 µL of Folin- Ciocalteau reagent and extract (1000µg/ ml) was added. The solution mixture was incubated for 6 minutes. To this, 1.25 mL of 7% Sodium Carbonate was added. After incubation, 3 mL of distilled water was added and left for an incubation period of 90 minutes. The absorption was measured at 765nm [20].

2.7 Antioxidant Activity

2.7.1 Total antioxidant content

Aqueous plants extracts and QAgNPs and DAgNPs (200-1000µg/mL) were taken and the volume was made up to 1 mL using distilled water. 4mL of a mixture of 28mM Sodium Phosphate, Ammonium Molybdate and Sulphuric acid were added to the solution. In a similar way, a Tannic acid standard was prepared. The samples were kept in a boiling water bath for 30 minutes. The absorbance of the samples was read spectrophotometrically at 695nm [20].

$$\text{Scavenging effect (\%)} = \frac{\text{Absorbance of Control- Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.7.2 Radical Scavenging Activity by DPPH(2,2- diphenyl- 2- picrylhydrazyl hydrate)

The free radical scavenging activity of the aqueous plant extracts, QAgNPs and DAgNPs were measured by using the DPPH method. An aliquot (200- 1000µg/ mL) of AgNPs or control and (1000- 1800µL) of H_2O was mixed with 2 mL of 0.2 N DPPH in absolute methanol. The mixture was vortexed vigorously and allowed to stand at room temperature for 30 minutes in dark. The absorbance of the mixture was measured spectrophotometric ally at 517 nm[19], and the free radical scavenging activity was calculated using the following equation:

Scavenging effect (%) = [1- {absorbance of sample/ absorbance of control}] x 100

2.8 Antibacterial Activity

The bacterial strains *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus* were used to determine the antibacterial activity of the aqueous plant extracts and synthesized Silver nanoparticles. The antibacterial activity was determined using Muller- Hinton agar by agar

well diffusion method. 100µl of working stock culture was spread with sterile cotton swab and holes were made with stainless steel cylinders. The aqueous plant extracts, QAgNPs and DAgNPs were added at a concentration of 100µg/mL individually in 2 different wells. The cultures were then allowed to grow overnight in the incubator at 37℃ for 24 hours. The antibacterial activity of the sample was assessed by measuring the zone of inhibition.

2.9 Antiproliferative Activity

Cell viability was determined by using the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan. HeLa cell lines were treated with biosynthesized AgNPs (dispersed in water). The treated cells were incubated for 24h at 37℃. 100µL of MTT solution was added to each AgNPs treated well and incubated for 4h. Then 100µL of dimethyl sulphoxide (DMSO) was added to each well, and the absorbance values were determined by spectrophotometer at 490 nm [21].

Cell viability % = (Absorbance sample– Absorbance blank) / (Absorbance control – Absorbance blank) x 100

3. RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of Silver Nanoparticles

The color of the solution changed distinctively from light yellow to dark brown for *Q. infectoria* and light brown to black for *D. carota* on heating, suggesting the formation of Ag nanoparticles [22]. The Ag nanoparticles formation by reducing Ag^{+} ions during exposure of the extracts was investigated by UV- Vis spectroscopy. UV- Vis Spectra showed a strong Surface Plasmon Resonance (SPR) band at 415 nm for *Q. infectoria* (Fig. 1a) and at 425 nm for *D. carota* (Fig. 1b). According to Mei theory, spherical nanoparticles show only a single SPR band and the number of bands increases by the diversity of particles shape [23]. As single peak were obtained it can be confirmed that spherical nanoparticles were formed [24], reported a absorption peak between 400- 450nm for the silver nanoparticles obtained from Oak fruit hull [25], obtained a distinct peak at 442nm from Nanoparticles of *Pimpinella anisum* seeds.

SEM image of the AgNPs reveals cluster of spherical bead like structures (Fig. 2a & 2b). The average size of AgNPs from *Q. infectoria* was 67.5 nm and that of *D. carota* 49.2 nm. The EDAX analysis of the synthesized silver nanoparticles showed the presence of elemental silver in *Q. infectoria* and *D. carota* to be 73.64% and 75.93% (Fig. 3a & 3b) [26],reported the particle size of 90nm, and a Silver peaks at 3 keV due to SPR by EDX analysis in *Prunus japonica*.

3.2 FT- IR Analysis

The FT-IR spectrum of *Q. infectoria* is shown in the Fig. 4a. The broad band appearing at 3411cm^{-1} is assigned for O-H stretching vibration indicating the presence of hydroxyl group. The bands at 2920 and 2851cm^{-1}can be assigned to asymmetric and symmetric stretching vibration of –CH2 group. The band at 1729cm^{-1} correspond to the C=O stretching representing the presence of lignin in *Q. infectoria* leaves. The characteristic bands observed at 1627 and 1582 cm^{-1} corresponds to COO-(Carboxylate) group and aromatic skeletal vibration and OH deformation which may be due to the presence of pectin and lignin in *Q. infectoria* leaves. The sharp absorption peak at 1384 cm^{-1} assigned to the C-H deformation and the C-O stretch which may be due to the presence of the absorption peak at 1103cm^{-1} correspond to C-O or C-O-C (Ether) stretching vibration. The FT-IR Spectrum of QAgNPs was shown in Fig. 4b.The peak at 3373cm^{-1} was due to the stretching of the O-H band of amino groups or H groups indicating the presence of alcohols or phenols. The absorption peak at 1710cm^{-1} was due to the C=O stretch of the carbonyl. The sharp and strong characteristic peak observed at 1613cm^{-1} assigned to the stretching vibration of (NH) C=O group. The band developed at 1324cm^{-1} contributes for C-C and C-N stretching. Absorption peaks at 1450 and 1202cm-1 corresponds to the C-H deformation and C-O stretch vibrations. A number of bands are showed between 1083cm^{-1} to 869cm^{-1} due to C-O, C-N stretching vibrations of alcohols, ethers, esters and amines. Thus the presence of functional group implied that the *Q. infectoria* extract is responsible for the synthesis of silver nanoparticles.

FT-IR spectrum of *D. carota* leaves revealed the presence of band around 3411 cm-1 was due to stretching vibration of O–H bond in hydroxyl groups. The peaks observed at 2920 and 1385 cm-1 were assigned to the stretching and bending vibration of C–H bond in methyl groups, respectively. The peak at 1630cm-1 was due to stretching vibration of COO- groups. The strong C–O band at 1109cm-1 was due to the C-O-C

ether linkage which also confirms the lignin structure of the *D. carota* leaves (Fig. 4c). FT-IR spectrum of QAgNPs exhibits a strong band at 3399cm-1 which is due to the presence of O-H group of amino or H group indicating the presence of alcohol or phenols. QAgNPs exhibited prominent peaks at 2930, 1603 and 1383cm^{-1}. The spectra showed sharp and strong absorption band at 1603cm^{-1} assigned to the stretching vibration of (NH) C=O group. The presence of sharp peak at 2930cm^{-1} was assigned to C-H stretching vibration. The band developed at 1385cm^{-1} indicates the presence of C-C and C-N stretching groups. The presence of absorption peaks ranging from 1198 to 612cm^{-1} indicates the presence of stretch vibrations of C-O-C and C-O groups respectively (Fig. 4d). [27] reported that alcohols and aldehydes from *Petroselinum crispum* played the role of a reducing and capping agent, resulting in the formation and stabilization of Silver nanoparticles.

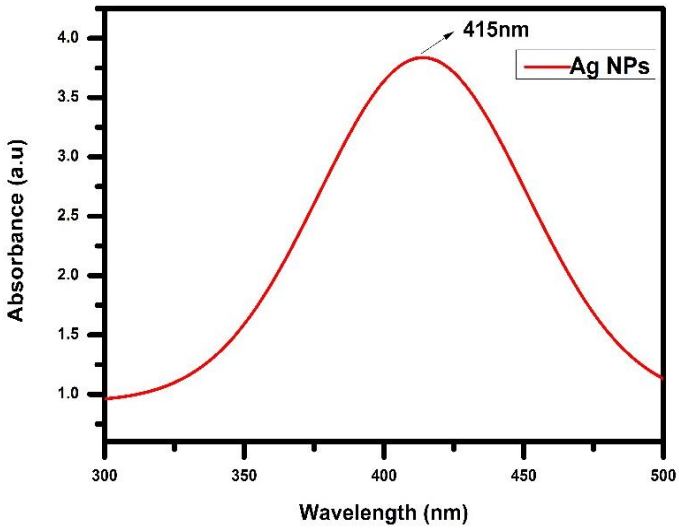

Fig. 1a. UV- Vis Spectra of synthesized Silver nanoparticles of *Q. infectoria*

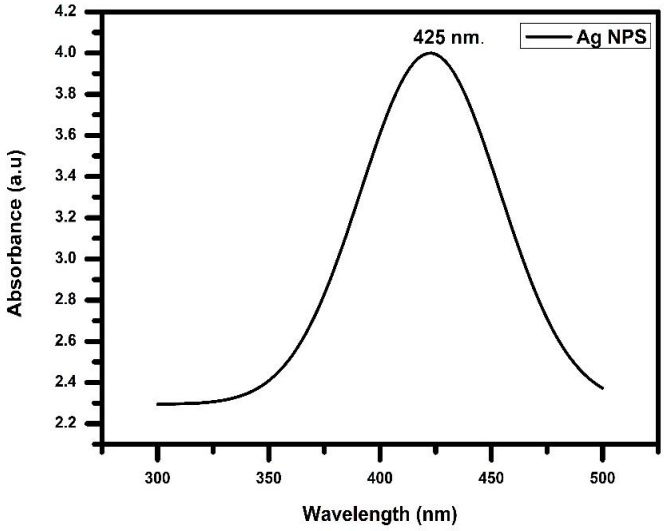

Fig. 1b. UV- Vis spectra of synthesized Silver nanoparticles of *D. carota*

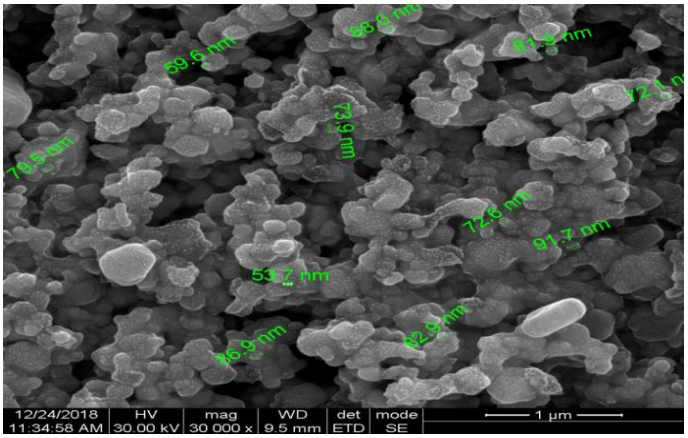

Fig. 2a. SEM image of AgNPs from *Q. infectoria*

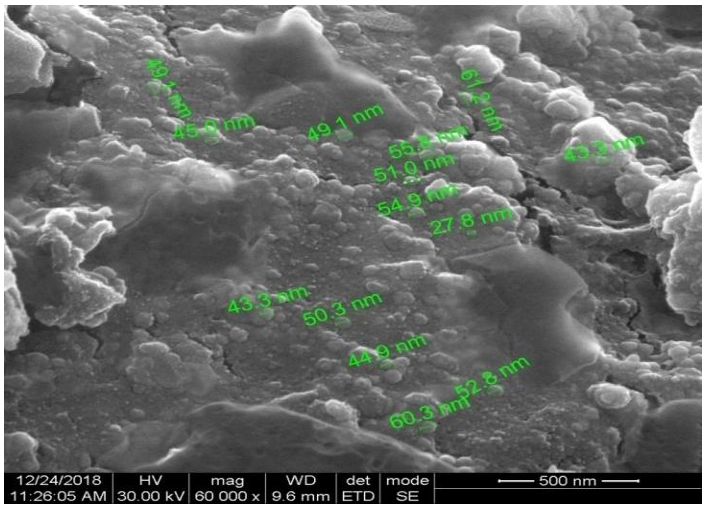

Fig. 2b. SEM image of AgNPs from *D. carota*

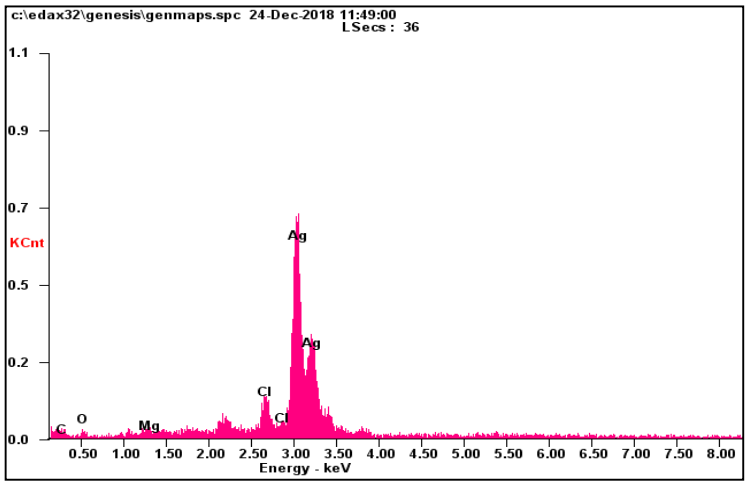

Fig. 3a. EDX of AgNPs from *Q. infectoria*

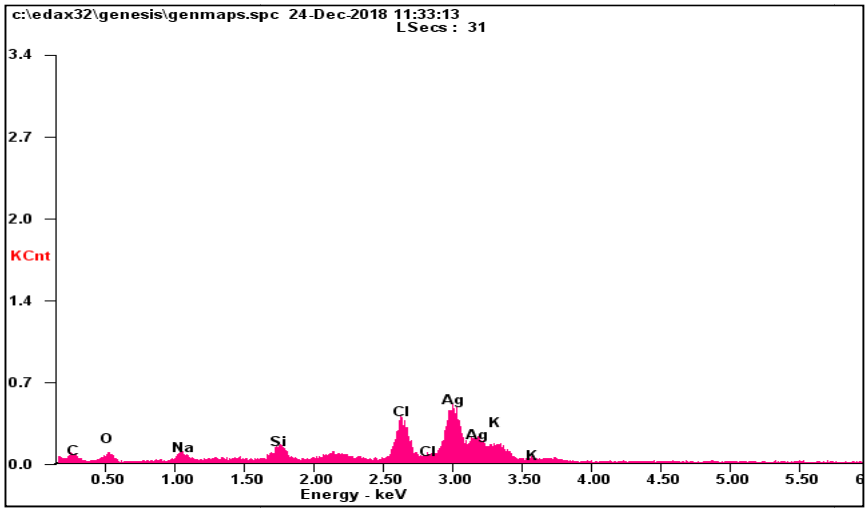

Fig. 3b. EDX of AgNPs from *D. carota*

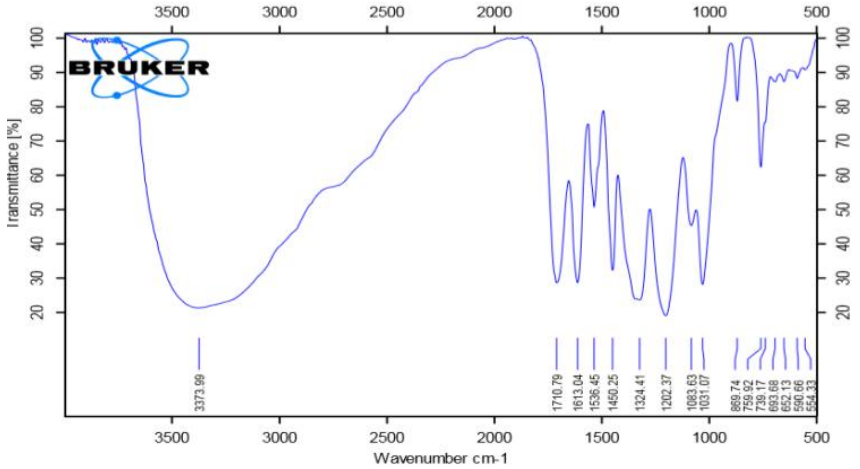

Fig. 4a. Fourier Transformed Infrared Spectroscopy of *Quercus infectoria* plant extract

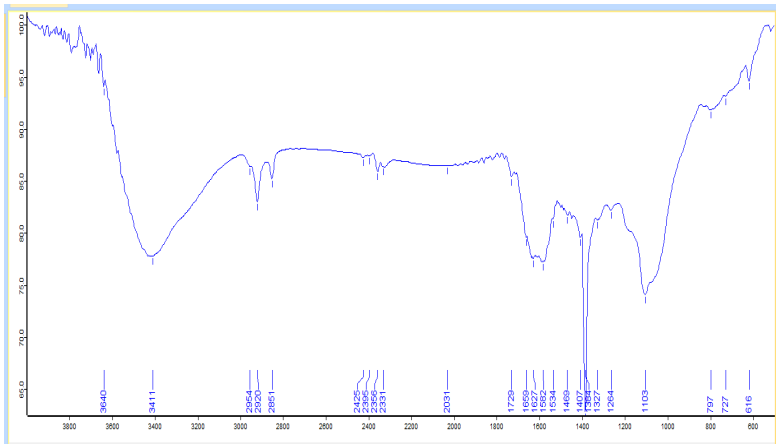

Fig. 4b. Fourier transformed infrared spectroscopy of AgNPs of *Quercus infectoria*

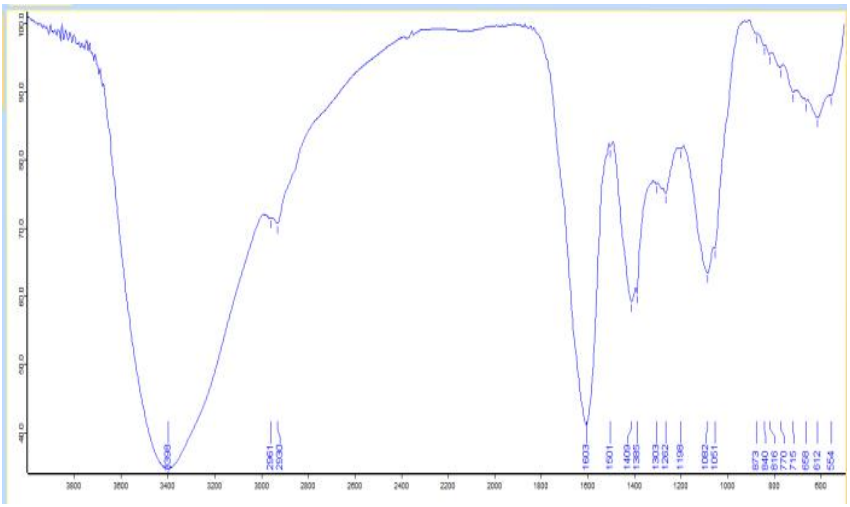

Fig. 4c. Fourier transformed infrared spectroscopy of *Daucus carota* plant extract

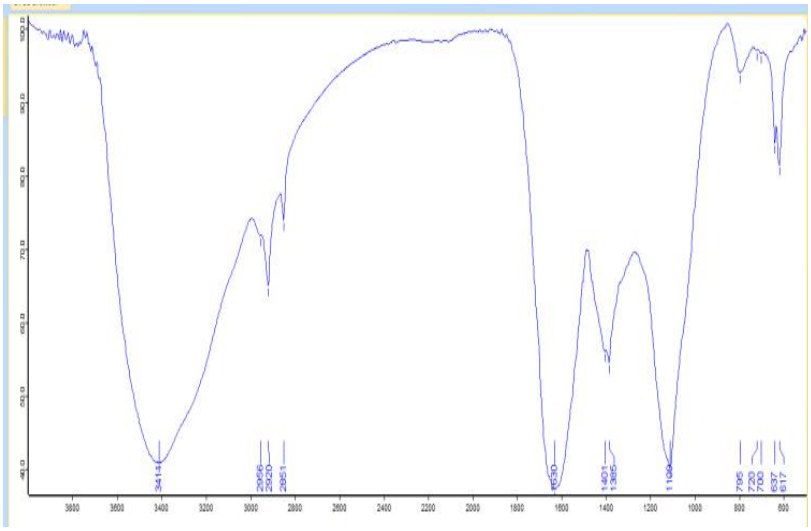

Fig. 4d. Fourier Transformed Infrared Spectroscopy of AgNPs from *Daucus carota*

3.3 Phytochemical Analysis

The present study has shown the presence of carbohydrates, tannins, flavonoids, phenols, alkaloids, glycosides, vitamin C, saponins in the aqueous extract of *Q.infectoria* and carbohydrates, tannins, flavonoids, phenols, steroids, quinines, coumarins, amino acids, alkaloids, vitamin C, saponins, triterpenes, proteins in the aqueous extract of *D. carota.* Similar results were reported by [28] for the phytochemical analysis of *Q. infectoria.* Neil *et al.,* 2011 reported the presence of alkaloids; [29], reported the presence of phenolics, carotenoids, polyacetylenes, and ascorbic acid in *D. carota*.

3.4 Total Phenolic Content

The total phenolic content was estimated in the sample and NPs using tannic acid.as standard. The total phenolic content in aqueous extracts & AgNP of *Q. infectoria* was 28.2mg Tannic acid /g and 23.3 mg Tannic acid /g respectively. In the case of *D. carota* the aqueous extract had 24.5mg Tannic acid /g and the DAgNPs had 28.47mg Tannic acid /g of total phenol (Fig. 5). The important and prominent phenolic compounds in Q. infectoria are phydroxybenzoic acid (PHBA), pyrogallol, catechol, caffeine, catechein, e-vanillic acid, gallic acid, cinnamic acid and many more [30].Hydroxycinnamic acid

and its derivatives were the major phenolic compounds in *D. carota* [31]. Phenolic compounds are effective reducing agents in nanoparticle synthesis [32,33].

3.5 Antioxidant Assay

3.5.1 Total antioxidant content

The total antioxidant content in the plant extract of *Q. infectoria* (89.75%) whereas QAgNPs showed 81.18% at a concentration of 1000µg/ ml (Fig. 6a). The total antioxidant content in *D. carota* and DAgNPs was 73.19% and 73.36% respectively (Fig. 6b). Ajay *et al.,* 2017 reported maximum antioxidant content of 62.5% at a concentration of 1000µg/ ml in Silver nanoparticles synthesized from *Cymbopogon citratus.*

3.5.2 Radical scavenging activity by DPPH

DPPH was stable compound and accepts hydrogen or electrons from AgNPs. It produces a violet colour in ethanol which turns yellow on addition of the samples which contains antioxidant compounds. The maximum percentage inhibition of 71.6% with an IC_{50} value of 6.64 µg/mL was observed in *Q. infectoria* AgNPs at a maximum concentration of 1000µg/mL (Fig. 7a). In the case of *D. carota*, maximum inhibition percentage was higher at 53.08% with an IC_{50} value of 8.83µg/mL at a maximum concentration of 1000µg/mL in the plant extract (Fig. 7b). This may be due to the higher content of phenolic compounds when compared to *Q. infectoria*. A positive correlation between phenolics (polyphenols, flavonoids, tannins) and IC50 values of the DPPH exist indicating that polyphenolics play an important role in free radical scavenging [34]. [35] where the plant extracts and silver nanoparticles synthesized from *Chenopodium murale* showed a maximum scavenging activity of 65.43% and 59.67% respectively[26]. Reported maximum radical scavenging activity of 55% in Silver nanoparticles synthesized from *Prunus japonica.*

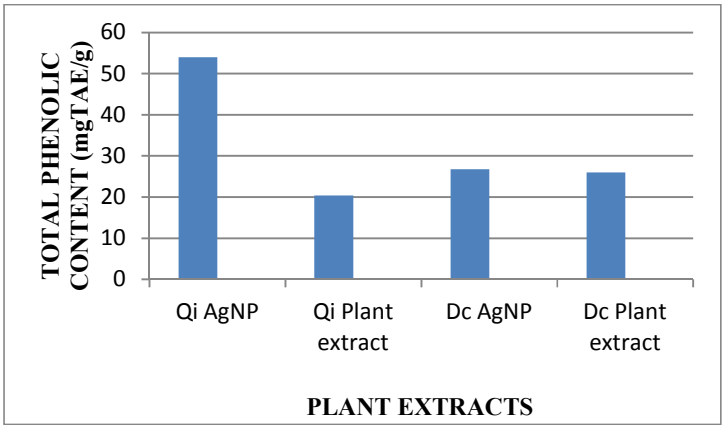

Fig. 5. Total Phenolic Content

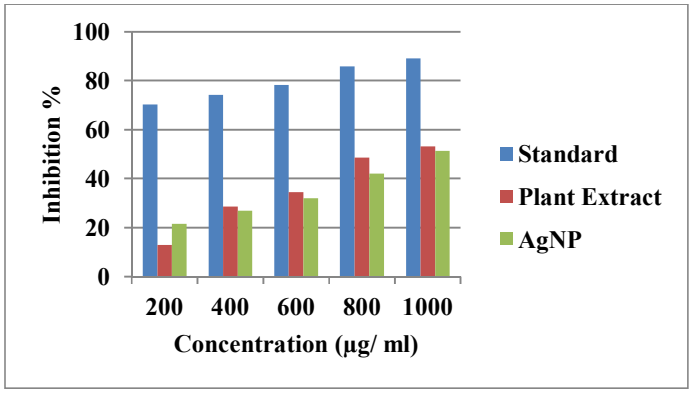

Fig. 6a. Total Antioxidant Content of *Quercus infectoria* plant extract and its AgNPs

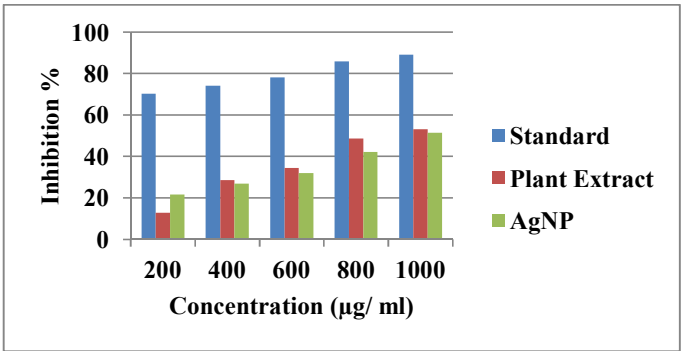

Fig. 6b. Total Antioxidant Content of *Daucus carota* plant extract and its AgNPs

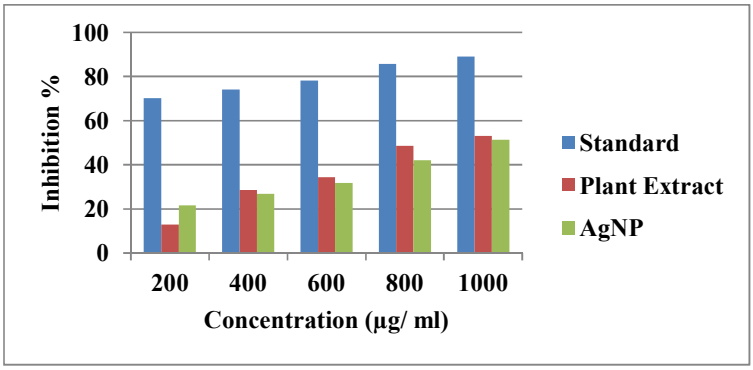

Fig. 7a. Antioxidant activity – DPPH Assay of plant extracts and AgNPs of *Quercus infectoria*

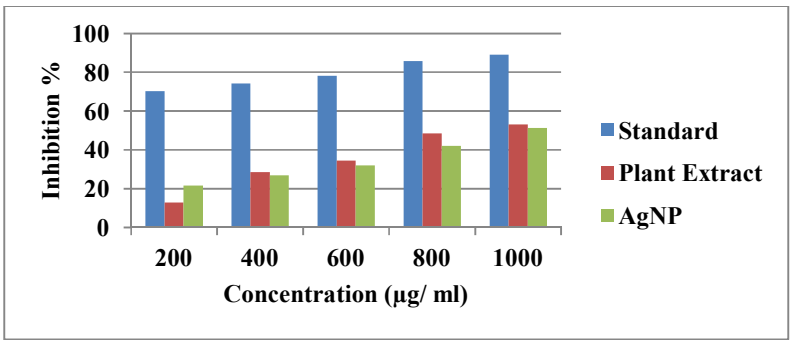

Fig. 7b. Antioxidant activity - DPPH Assay of plant extracts and AgNPs of *Daucus carota*

3.6 Antibacterial Activity

A maximum zone of inhibition of 31mm and 30mm was seen against *E. coli* treated with silver nanoparticles (100µg/ ml) of *D. carota* and *Q. infectoria.* The plant extracts of *D. carota* showed a maximum zone of inhibition of 29mm against *E.coli* whereas *Q. infectoria* showed 28mm against *B. subtilis*. A zone of inhibition of 17mm was observed against *S. aureus* treated with silver nanoparticles synthesized from *D. carota*. The bactericidal activity of the AgNPs may be due to the attachment of the AgNPs to the bacterial cell wall and the generation of free radicals. The AgNPs penetrate the cell membrane by disturbing the membrane permeability and causes intracellular leakage of ATP and thereby the death of the cell [36]. The positive charged ions (Ag+) have the tendency to bind with the phosphorous and sulphur present in DNA and RNA and disrupt its function [37,38,25] reported a maximum zone of inhibition of 17mm against *Staphylococcus aureus* treated with Silver nanoparticles synthesized from *Pimpinella*

anisum seeds at a concentration of 50µg/ ml [39], reported a zone of inhibition of 17mm against *Bacillus subtilis* and *Staphylococcus aureus* treated with Silver nanoparticles synthesized from *Quercus infectoria*. [27] reported a maximum zone of inhibition of 14.15mm against *Escherichia coli* treated with silver nanoparticles synthesized from *Petroselinum crispum.*

3.7 Antiproliferative Activity

The percentage of cell viability of HeLa cell lines was 21.1% and 6% for the silver nanoparticles of *Q. infectoria* and *D. carota*. Anticancer activity of silver NPs are attributed to increase in the intracellular Reactive Oxygen species (ROS) generation and nullification of the membrane potential in mitochondria leading to DNA fragmentation, arrest of cell cycle and apoptosis [40]. *Nigella sativa* AgNPS were effective for Hepatocellular carcinoma HepG2 cell lines [41]. Krishnan et al., 2016 reported AgNPS from *Piper nigrum* to have anticancer activity against MCF-7 and Human Pharynx cancer cell lines (Hep-2). AgNPS from *Pimpinella anisum* seeds were cytotoxic to human neonatal skin stromal cancer cells [42].

4. CONCLUSION

In the present study, Silver nanoparticles were synthesised using the plant extract of *Q. infectoria* and *D. Carota.* The formation of the particles was confirmed by *t*he surface plasma resonance at 417 and 450 nm. SEM analysis showed that the particles were spherical in shape. The antioxidant content was exhibited by the plant extract of *Q. infectoria* was higher when compared to Silver nanoparticles. In contrast the total antioxidant content Silver nanoparticles synthesized from *D. carota* was was higher compared to the plant extract. The Silver nanoparticles synthesized from *Q. infectoria* and *D. carota* exhibited good antibacterial activity against *B. subtilis, E. coli* and *S. aureus*. They also showed cytotoxicity on HeLa cell lines. The green synthesis of Silver nanoparticles can be tested as drugs for pharmacological applications.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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