Green Synthesis of Silver Nanoparticles Using Aqueous Rhizome Extract of *Corallocarpus Epigaeus* for Biomedical Applications

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ABSTRACT

We report on silver nanoparticles synthesized with the *Corallocarpus epigaeus* rhizome extract and their characteristics. The synthesized phytochemical compound acts as both a stabilizing and a reducing agent. We systematically characterize the synthesized silver nanoparticles. UV-Vis spectra confirmed the formation of green synthesized silver nanoparticles from the *Corallocarpus epigaeus* rhizome. Scanning electron microscopy and high-resolution transmission electron microscopy images exhibited indicated spherical and randomly distributed silver nanoparticles. The synthesized nanoparticles of the *Corallocarpus epigaeus* rhizome aqueous extract showed bacteriostatic activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus cereus*, and *Staphylococcus aureus*. The synthesized silver nanoparticles from *Corallocarpus epigaeus* rhizome aqueous extract displayed fungicidal activity of *Mucor species*, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus species*. Furthermore, the silver nanoparticles exhibited a cytotoxic activity of inhibiting HepG2 cell proliferation in the MTT cytotoxicity test.

Keywords: *Corallocarpus epigaeus*, UV irradiation, Ag nanoparticles, Biomedical

1. Introduction

Metal nanoparticles have been extensively used in numerous recent technological applications [1–3]. Nanoparticles exhibit important optical, electronic, catalytic, and catalytic attributes owing to their high surface area to volume ratio, as compared to their bulk [4,5]. In particular, silver nanoparticles are one of the most frequently engineered materials utilized in numerous technological and industrial applications. Several approaches are available for the synthesis of these silver nanoparticles. These approaches include reduction in solutions, thermal decomposition of silver compounds, microwave assisted synthesis, laser mediated synthesis, and the biological reduction method [6]. Among these, plant-mediated synthesis of silver nanoparticles is considered a conservation technique [7]. The attractive attributes of silver nanoparticles frequently depend on their size and shape. The morphological properties of less aggregated, small, and spherical-shaped silver nanoparticles afford better performance, which in turn indicates a higher potential for applications [8]. These nanoparticles have been employed to prevent wound infections, and as wound dressing by adding them into topical creams and as antimicrobial agents. They can also be used as anticancer agents [9–11].

Recently, metal nanoparticles obtained from plants and plant-derived materials have gained interest for numerous technological applications [12–16]. This type of synthesis is more adaptable than the microbe-mediated nanoparticles synthesis process. Synthesized metal nanoparticles originating from plants and plant-derived materials eliminate the need for culture maintenance and are easy to han-

dle. In particular, the green synthesis of silver nanoparticles is more advantageous compared to other methods. This process does not necessitate requirements such as high pressure, energy, temperature, and toxic chemicals [17,18]. Silver nanoparticles can also be synthesized from other plant parts [19,20]. These metabolites function as bioactive compounds and are capable of acting as both capping and reducing agents. This eliminates the requirement of the addition of any other chemical agents to the synthesized nanoparticles [21].

Corallocarpus epigaeus (Rottl.&Wild) Clarke belongs to the *Cucurbitaceae* family and is a type of tendril-bearing climbing herb. The root is yellowish white and has a bitter taste. It has laxative properties and is used as treatment for syphilitic rheumatism and in the later stages of dysentery [22]. *Corallocarpus epigaeus* (Rottl. & Wild) Clarke is a prostrate or climbing monoecious plant found in tropical countries such as India, Ceylon, Deccan, and in the Peninsula region [23]. The tuber extract of *Corallocarpus epigaeus* exhibits a higher inhibitory effect than the leaf and stem. Further, antimicrobial substances are produced in response to an infection [24]. This also serves as an effective remedy for diabetes, herpes [25], anthelmintic [26], rheumatism, and snake bites. The decoction of the root has proved beneficial in cases of chronic mucous enteritis. The phytochemical characterization of *Corallocarpus epigaeus* has shown that it contains numerous phytochemical compounds with properties of antimicrobial, antifouling, and antioxidant activities [27–30]. However, detailed reports on this plant material have not been published thus far.

In the present study, we synthesized silver nanoparticles with the *Corallocarpus epigaeus* (Rottl.&Wild) Clarke aqueous rhizome extract

Figure 1. Photograph of (a) *Corallocarpus epigaeus* rhizome and (b) *Corallocarpus epigaeus* chopped rhizome.

using UV-irradiation. The synthesized Ag nanoparticles morphology results were analyzed via scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). Subsequently, the presence of silver nanoparticles was determined using UV-Vis spectroscopy. The resultant nanoparticles were tested for their antibacterial activity against selected bacterial cultures and their antifungal activity against certain selected fungal cultures; their cell cytotoxicity was also assessed.

2. Experimental details

2.1. Materials

The rhizome of *Corallocarpus epigaeus* (Rottl. & Wild) Clarke was purchased from Ayurvedic (Kelambakkam, Chennai, India), and it was authenticated by Prof. P. Jayaraman, Retired Professor, Presidency College, Chennai, India. Silver nitrate (AgNO_3) was purchased from Thermo Fisher Scientific (India). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was purchased from Invitrogen (India), and acridine orange was purchased from Sigma – Aldrich (India).

Bacterial cultures *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*, and fungal cultures *Rhizopus species*, *Mucor species*, *Aspergillus niger*, *Aspergillus flavus* were obtained from IBMS (Institute of Basic Medical Sciences, Taramani, Chennai, India). Potato dextrose agar, rose Bengal agar, Mueller Hinton agar and Mueller Hinton broth (HI Media, India) were used in the present study.

2.2. Method

The dust and debris on the collected rhizomes were washed using running tap water followed by distilled water. Figure 1 shows the photograph of *Corallocarpus epigaeus* and chopped rhizome. The rhizome skin was peeled off and cut into small pieces. Then, it was ground using a mortar and pestle (40 g of sample in 100 ml of distilled water). Wattman filter paper was used to filter the grounded paste.

The silver nitrate solution was mixed with the *Corallocarpus epigaeus* rhizome extract at ratios of 9:1, 8:2, 7:3, 6:4, and 5:5. Then, 1, 3, and 5 mM of silver nitrate with plant extract were prepared at ratios of 9:1, 8:2, 7:3, 6:4, and 5:5, respectively, as shown in Fig. 2. The mixed solutions were UV irradiated (253.7 nm) for 5, 10, and 15 min with their respective controls. Figure 3 shows the photograph of the UV treated samples. After irradiation, the solution at 6:4 dilution was centrifuged at 12000 rpm for 15 min at 4 °C. The above step was repeated twice to remove unreacted compounds. The pellets were collected and stored for further characterization and analyses.

UV-irradiating synthesis was conducted at a wavelength of 253.7 nm and time intervals of 5, 10, and 15 min. Among these solutions, plant extract at the ratio of 6:4 with 5 mM silver nitrate exhibited dark

Figure 2. Photograph of (a) *Corallocarpus epigaeus* plant rhizome extract, (b) *Corallocarpus epigaeus* plant rhizome extract and 1 mM AgNO_3 , (c) *Corallocarpus epigaeus* plant rhizome extract and 3 mM AgNO_3 , and (d) *Corallocarpus epigaeus* plant rhizome extract and 5 mM AgNO_3 .

Figure 3. (a) UV irradiated silver nitrate (AgNO_3), (b) *Corallocarpus epigaeus* rhizome extract, and (c) *Corallocarpus epigaeus* rhizome extract + Ag.

brown color. The result was obtained in 15 min. The solution was subsequently centrifuged at 12000 rpm for 10 min at 4 °C. The overall yield of the extract was 100 µg/ml, and the experiments were carried out with this yield.

2.3. Characterizations

Employing the gold palladium coating method, the samples were coated on a clean glass plate and analyzed. After the preparation, sample plates were examined, and the size and shape of the particles was determined using SEM (TESCAN VEGA3). The silver nanoparticle morphology was assessed using high resolution transmission electron microscopy. The structural characterization of Ag nanoparticles was performed using X-ray diffraction (Rigaku Smart Lab 3kW, Japan). The bonding configuration of the silver nanoparticles was studied using an FTIR spectrometer (Bruker, Germany). The biologically syn-

Figure 4. SEM images of *Corallocaepus epigaeus* plant rhizome extract at (a) low and (b) high magnification; *Corallocaepus epigaeus* plant rhizome extract with silver at (c) low and (d) high magnification.

thesized silver nanoparticles were observed systematically, i.e., reduction of Ag^+ ions during different time intervals using the UV-Visible spectrophotometer (Shimadzu, UV-2450). The absorption maximum was recorded before and after the synthesis of the nanoparticles using the *Corallocaepus epigaeus* rhizome extract.

2.4. Antibacterial test

The culture of the test microorganisms (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus cereus*, and *Staphylococcus aureus*) were inoculated in the Mueller-Hinton broth and incubated at 37 °C. After incubation, in the case of similar turbidity, it was used for inoculum. Discs with a diameter of 6 mm were prepared by using Whatmann No. 1 filter paper. The discs were then placed in a Petri-dish and sterilized in a hot air oven. Using sterile micropipette tips, 5 μl of each *Corallocaepus epigaeus* and *Corallocaepus epigaeus* with silver nanoparticles and AgNO_3 was dispensed into the corresponding sterilized discs. The ingredients were weighed and suspended in 500 ml of distilled water and heated to a boil. The pH of the media was adjusted to 7.4, after which it was cooled and poured into the Petri dishes. The plates were allowed to set, and they were stored at ambient temperature.

A sterile swab was moistened with the broth culture, after which it was pressed against the sides of the tubes to remove excess fluids and was swabbed evenly over the MHA medium. After swabbing, the surface was allowed to dry. The prepared filter paper discs were placed in the previously prepared agar plates. To complete contact with the agar surface, each disc was pressed down. They were evenly distributed, such that they were no closer than 24 mm from each other. Each plate was examined after 24 h of incubation at 37 °C. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones and discs with complete inhibition were measured.

2.5. Antifungal test

Khatami *et al.* [31] employed a method to examine the antifungal potency of nanoparticles against selected fungi. Here, this method was employed with slight modifications. Graded concentrations (1 and 5 mg/mL) of *Corallocaepus epigaeus* with silver nanoparticles were incorporated into potato dextrose agar (PDA) plates, and 6 mm agar

Figure 5. HRTEM image of *Corallocaepus epigaeus* plant rhizome extract with silver nanoparticles at (a) low, (b) high magnification, and (c) SAED pattern.

plugs of 48 h cultures of *Aspergillus flavus*, *Rhizopus species*, *Mucor species*, and *Aspergillus niger* were placed on the PDA plates containing nanoparticles and PDA plates without nanoparticles (control). The plates were incubated for 72 h at 28 ± 2 °C. The millimeter range of fungal diameter growth was measured. The percentage of growth inhibition is obtained according to the following equation:

$$\text{Growth inhibition} = (D_{\text{control}} - D_{\text{test}}) / D_{\text{control}} \times 100 \%$$

2.6. Cytotoxicity test

HepG2 cells collected from NCCS (National Centre For Cell Science, Pune) were cultivated in Rose well Park Memorial Institute medium (RPMI), supplemented with 10 % fetal bovine serum, penicillin/streptomycin (250 U/ml), gentamycin (100 $\mu\text{g}/\text{ml}$), and amphotericin B (1 mg/ml) from Sigma Chemicals, MO, USA. All cells cultivated were maintained at 37 °C in a humidified atmosphere of 5 % CO_2 . The cells were allowed to proliferate for more than 24 h before use. The sample concentrations were recorded.

Figure 6. XRD pattern of (a) *Corallocarpus epigaeus* rhizome extract and (b) *Corallocarpus epigaeus* rhizome extract with silver nanoparticles.

The conventional MTT reduction test was used to measure cell viability, as described previously with slight modifications. In summary, HepG2 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 μ l of RPMI with 10 % fetal bovine serum. RPMI containing various concentrations of the test sample was added and incubated for 48 h. Cells were incubated with MTT (10 μ l, 5 mg/ml) at 37 °C for 4 h after treatment and then with DMSO at room temperature for 1 h. The 595 nm plates were read on a scanning multi-well spectrophotometer.

$$\text{Cell viability (\%)} = \left(\frac{\text{Test Optical Density}}{\text{Control Optical Density}} \right) \times 100\%$$

$$\text{Cytotoxicity (\%)} = 100 - \text{Cell viability (\%)}$$

3. Results and discussion

Figure 4 shows the SEM images of *Corallocarpus epigaeus* and *Corallocarpus epigaeus* with silver nanoparticles at low and high magnifications. The particles were measured in micro size (1–6 μ m). The typical sizes of *Corallocarpus epigaeus* and *Corallocarpus epigaeus* with silver particles are approximately 5 μ m. The size of the particles is observed to be larger, due to the biomaterial being dried and processed for SEM analysis.

Figure 7. FTIR spectrum of *Corallocarpus epigaeus* rhizome extract and *Corallocarpus epigaeus* rhizome extract with silver nanoparticles.

Figure 8. UV-Vis spectrum of (a) *Corallocarpus epigaeus* rhizome extract and (b) *Corallocarpus epigaeus* rhizome extract with silver nanoparticles.

Figure 9. Antimicrobial activity of (1) *Corallocarpus epigaeus* rhizome extract, (2) *Corallocarpus epigaeus* plant rhizome extract with silver nanoparticles, (3) AgNO₃ on (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, (c) *Proteus mirabilis*, (d) *Staphylococcus aureus*, (e) *Bacillus cereus*, and (f) Mueller-Hinton agar control plate.

Figure 10. Antimicrobial activity test (Bar Graph): Inhibitory zone diameter for *Corallocarpus epigaeus* rhizome extract, *Corallocarpus epigaeus* plant rhizome extract with silver nanoparticles, and AgNO₃ on *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*.

Figures 5(a) and 5(b) show the synthesized silver nanoparticles viewed at low and high magnifications via HRTEM. The images obtained using HRTEM show that the synthesized silver nanoparticles have a spherical morphology with smaller sizes (10 - 90 nm). Because the prepared silver nanoparticles were used for HRTEM analysis, we were able to observe the sizes of the silver nanoparticles in the range of 10–90 nm, which differs significantly from SEM observations (as dried materials are used for SEM analysis). The overall morphology of the silver nanoparticles is more clearly observed at a random distribution of the sizes, as shown in the HRTEM images. Figure 5(c) clearly depicts the lattice fringes of the SAED patterns of silver nanoparticles, which confirm the polycrystalline nature of the material. As far as biomedical applications are concerned, the formations are of uniform size and without any agglomeration of Ag nanoparticles.

The XRD patterns of pristine and sample silver nanoparticles are shown in Figs. 6(a) and 6(b), respectively. The diffraction peaks show that the synthesized *Corallocarpus epigaeus* plant rhizome-Ag nanoparticle has a crystalline nature. Figure 6(b) shows the XRD results, with predominant diffraction patterns at 32.5°, 39°, 48°, 58°, 68°, and 78° corresponding to metallic Ag(122), Ag(111), Ag(200),

Figure 11. Antifungal activity of *Corallocarpus epigaeus* with silver nanoparticles against *Mucor* species, *A. niger*, *A. flavus*, and *Rhizopus* species on PDA plate with low and high nanoparticle concentrations and control plate without nanoparticles.

Ag(142), Ag(220), and Ag(311) planes, respectively. The presence of unreacted plant extracts is likewise observed through diffraction peaks.

Changes in the functional groups were obtained using FTIR spectroscopy. Figure 7 shows the FTIR spectrum of the Ag nanoparticles extracted from plant and pristine plant. The characteristic transmittance spectrum of Ag nanoparticles can thus be assigned. The peak around 500–1700 cm⁻¹ represents the sample synthesized from the plant extract. The peak at approximately 1700 cm⁻¹ (amide I) indicates the presence of silver nanoparticles due to the existing amino group. The appearance of the CH₂ groups in the final product is marked by the CH₂ transmittance band in the 2000–2250 cm⁻¹ region [32]. The stretching of the –OH group can be obtained through the presence of a broad peak between 3400–3500 cm⁻¹.

UV-Vis spectroscopy is a useful technique for the determination of nanoparticle synthesis. The highest absorption peak was observed for a wavelength of 420–460 nm, representing the characteristic peak for silver nanoparticle synthesis, as shown in Fig. 8.

The *Corallocarpus epigaeus* plant rhizome extract and *Corallocarpus epigaeus* with silver nanoparticles exhibited bactericidal activity against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*, as shown in Fig. 9. The most significant activity of the *Corallocarpus epigaeus* plant rhizome extract was obtained for *Escherichia coli* (13 mm). Similar inhibitory activities of *Corallocarpus epigaeus* were acquired for *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Staphylococcus aureus* (7 mm).. For instance, Priyavardhini *et al.* reported that chloroform and acetone extract showed similar activity against *Staphylococcus aureus* (9.33 and 9.67 mm at 100 % concentrations). Similar activity was also observed for chloroform and methanol extract against *Pseudomonas aeruginosa* (7.03 and 7.06 mm at 100 % concentration) [33]. Higher activity was reported for the aqueous seaweed extract of *Hypnea muciformis*. Ag nanoparticles were fairly toxic to *Bacillus cereus*, *E. coli*, and *Staphy-*

Table 1. Antimicrobial activity test and diameter of inhibition zone.

Extract	Zone of inhibition (mm)				
	<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>
Plant	7 ± 0.1	7 ± 0.1	13 ± 0.1	7 ± 0.1	6 ± 0.1
Plant + silver	12 ± 0.1	11 ± 0.1	17 ± 0.1	11 ± 0.1	11 ± 0.1
Silver nitrate	13 ± 0.2	10 ± 0.1	10 ± 0.1	8 ± 0.1	11 ± 0.1

Figure 12. MTT cytotoxicity test of HepG2 cell line for 100 µg test samples: (a) Control, (b) *Corallocaepus epigaeus*, and (c) *Corallocaepus epigaeus* with silver nanoparticles.

lococcus aureus, with inhibition zones of 26, 22, and 24 mm, respectively [34]. No activity against *Bacillus cereus* was observed for the *Corallocaepus epigaeus* plant rhizome extract; however, significant antibacterial activity was observed for *Corallocaepus epigaeus* with Ag nanoparticles against *Bacillus cereus* (11 mm). The same type of zone size was also measured for *Proteus mirabilis* and *Staphylococcus aureus* (11 mm), as shown in Table 1. Figure 10 illustrates the antimicrobial activity zone diameter. *Corallocaepus epigaeus* with Ag nanoparticles displayed optimistic results for all the strains; however, the diameter of the inhibitory zone was larger in *Escherichia coli* (17 mm) than in *Corallocaepus epigaeus*, as shown in Table 1. The diameter of the inhibitory zone for *Pseudomonas aeruginosa* (12 mm) was measured. The aqueous rhizome extract (*Corallocaepus epigaeus*) was only used as a control, which displays slight inhibitory actions compared to *Corallocaepus epigaeus* with silver nanoparticles. The silver nitrate solution, which was used as a positive control in this study, showed bactericidal activity for all strains. The inactivation of the proteins is generally believed to occur as the metal nanoparticles react with proteins by combining thiol groups. Recently, the microbiological and chemical experiments were attributed to the interaction of the silver ion with thiol groups playing a crucial role in bacterial inactivation [35]. This suggests that the synthesized silver nanoparticles can be used to treat

Figure 13. MTT cell viability test of HepG2 cell line using control, solvent control, CE (*Corallocaepus epigaeus*), and CE + Ag (*Corallocaepus epigaeus* with silver nanoparticles).

diseases caused by the abovementioned organisms.

Figure 11 shows the antifungal activity of *Corallocaepus epigaeus* with silver nanoparticles at two different concentrations. The PDA medium incorporated with 1 mg/ml of *Corallocaepus epigaeus* with silver nanoparticles displayed lower growth inhibition of 58.3 % and 52.6 % for *Mucor species* and *Aspergillus niger*, respectively. A poor growth inhibition of 46 % was recorded for *Aspergillus flavus*, and no inhibition was recorded for *Rhizopus species*. The PDA medium incorporated with 5 mg/ml of *Corallocaepus epigaeus* with silver nanoparticles exhibited a higher growth inhibition of 83, 77, and 80.5 % for *Mucor species*, *Aspergillus niger*, and *Aspergillus flavus*, respectively. For example, Lateef *et al.* reported that PA-Ag nanoparticle concentrations of 100 and 150 mg/ml exhibit a growth inhibition of 65.22–66.67 % against *Aspergillus niger* [36]. Further, Devi *et al.* reported that the aqueous seaweed extract of *Hypnea muciformis* Ag nanoparticles at different concentrations of 10, 20, 30, and 40 µl possessed higher antifungal activities with inhibition zones of 24, 26, and 30 mm, respectively [34]. A slightly moderate growth inhibition of 68.7 % was observed for *Rhizopus species*. The millimeter range of the fungal growth diameter was measured, and the growth inhibition percentage was calculated and tabulated, as listed in Table 2. Nanoparticles have the ability to disrupt both membranes and fungal cell walls, leading to the leakage of intracellular essential materials, which may cause death [37]. The experimental results indicate that fungal mycelium growth was inhibited at higher concentrations of the silver nanoparticles.

The MTT cytotoxicity test was performed, and the resulting HepG2 cell line images are shown in Fig. 12. The MTT test was utilized for survival determination measurements, and the concentrations required for cell viability and cytotoxicity were determined, as shown in Figs. 13 and 14, respectively. The effect of the samples on the reproduction of HepG2 was expressed as the % cell viability. Tukappa *et al.* reported

Table 2. Antifungal activity of *Corallocaepus epigaeus* plant rhizome extract with silver nanoparticles on *Mucor species*, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus species*.

<i>Corallocaepus epigaeus</i> with silver nanoparticles	Growth inhibition % (mm)			
	<i>Mucor species</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Rhizopus species</i>
PDA medium with 1 mg/ml	58.3	52.6	46	0
PDA medium with 5 mg/ml	83	77	80.5	68.7

Figure 14. MTT cytotoxicity test of HepG2 cell line using control, solvent control, CE (*Corallocarpus epigaeus*), and CE + Ag (*Corallocarpus epigaeus* with silver nanoparticles).

that the dose dependence of % cytotoxicity of methanol extract of *Rumex vesicarius* L. increases with rising concentrations [38]. At low concentrations of methanol, a decrease in viability was found through HepG2, and likewise, an eventual decline was observed tested at higher concentrations. The % inhibition of cell proliferation at 62.5, 125, 250, 500, and 1000 µg/ml was found to be 21.57 ± 0.7 , 32.68 ± 1.0 , 41.69 ± 0.6 , 47.92 ± 0.9 , and 70.21 ± 0.6 , respectively. The percentage of cell inhibition of *Corallocarpus epigaeus* increased with the concentrations of the test compounds, whereas *Corallocarpus epigaeus* with Ag nanoparticles displays slightly higher activity of inhibition than *Corallocarpus epigaeus*. Figure 14 shows that the concentration of aqueous extract of *Corallocarpus epigaeus* on HepG2 was 22.47 µg/ml, and the concentration of aqueous extract of *Corallocarpus epigaeus* with silver nanoparticles on HepG2 was 24.64 µg/ml. On increasing the concentration of both *Corallocarpus epigaeus* and *Corallocarpus epigaeus* with silver nanoparticles, the HepG2 cell may result in higher inhibitions. Thus, the extracts have potent activities against human liver carcinoma. Phytochemical constituents, such as flavonoids, are the major components responsible for potential cytotoxic activity [39]. The flavonoids have cytotoxic activity owing to the presence of phenolic groups [40].

Our future research plan involves the isolation and evaluation of these active compounds to elucidate the exact mechanism of action. The cytotoxicity activity against HepG2 cells was demonstrated; hence, further studies including assay for apoptosis, DNA damage, and detection of gene expression (real time-PCR) followed by flow cytometry may be carried out to use *Corallocarpus epigaeus* and *Corallocarpus epigaeus* with silver nanoparticles as an anticancer agent.

4. Conclusions

The plant rhizomes of *Corallocarpus epigaeus* were used to prepare silver nanoparticles. UV-irradiation was adapted to the synthesis of silver nanoparticles from the *Corallocarpus epigaeus* plant rhizome extract at the ratio of 6:4 at 15 min time intervals. Morphological features of silver nanoparticles synthesized from *Corallocarpus epigaeus* were analyzed using SEM and HRTEM. *Corallocarpus epigaeus* and *Corallocarpus epigaeus* with silver nanoparticles exhibit bactericidal activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus cereus*, and *Staphylococcus aureus*. *Corallocarpus epigaeus* with silver nanoparticles displayed encouraging results for all the strains; however, the diameter of the inhibitory zone was found to be larger in *Escherichia coli* in *Corallocarpus epigaeus*. The

silver nanoparticles possessed antifungal properties. Hence, *Corallocarpus epigaeus* with silver nanoparticles was studied to determine its antifungal properties; fungicidal activity was revealed against *Mucor species*, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus species*. PDA medium incorporated with 1 mg/ml *Corallocarpus epigaeus* with silver nanoparticles displayed lower mycelial growth inhibition on *Mucor species*, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus species* with 58.3, 52.6, 46, and 0 %, respectively. PDA medium incorporated with 5 mg/ml of *Corallocarpus epigaeus* with silver nanoparticles displayed higher mycelial growth inhibition on *Mucor species*, *Aspergillus niger*, and *Aspergillus flavus* with 83, 77, and 80.5 %, respectively. Slightly lower inhibition was found in *Rhizopus species* with 68.7 % when compared to other fungal mycelium. Further, a cytotoxic activity of inhibiting the HepG2 cell proliferation was revealed. The MTT test with *Corallocarpus epigaeus* on HepG2 yields the cytotoxicity percentage value as 22.47 µg/ml and *Corallocarpus epigaeus* with silver nanoparticles on HepG2 yields the cytotoxicity percentage value of 24.64 µg/ml.

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