Photo-mediated optimized synthesis of silver nanoparticles using the extracts of outer shell fibre of Cocos nucifera L. fruit and detection of its antioxidant, cytotoxicity and antibacterial potential

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Abstract
Presently, photo-mediated optimized synthesis of SNPs (CS-AgNPs) was carried out with the help of aqueous extracts of coconut (Cocos nucifera) outer shell fibre. Green synthesis of CS-AgNPs was undertaken under laboratory light conditions and characterized by several standard techniques such as UV–visible spectrophotometer (UV–Vis), X-ray diffraction pattern (XRD), Fourier transform infrared (FT-IR), and scanning electron microscopy (SEM) images and energy dispersive spectroscopy (EDX). UV–Vis spectra displayed a surface plasmon resonance peak at 468 nm equivalent to CS-AgNPs, and the FT-IR spectra confirmed the association of biological molecules from the extract in the synthesis process. The SEM image data confirmed the round and circular nature of CS-AgNPs. The EDX data presented the elemental configuration with a solid peak at 3 KeV that matched with the Ag. The synthesized CS-AgNPs exhibited substantial cytotoxicity potential against the HepG2 cells with (effective concentration (IC50) value of 15.28 mg/ml along with robust antioxidant potential, with respect to its 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging (IC50 of 96.39 mg/ml) and reducing assay (IC0.5 of 209.96 mg/ml). The CS-AgNPs demonstrated encouraging antimicrobial potential against four different pathogenic bacteria and one Candida sp. with inhibition zone diameter ranged between 8.87 and 13.07 mm. Overall, the existing investigation suggested that CS-AgNPs can be an attractive, cost-effective, and environment-friendly candidate for its possible uses in the food, cosmetics, and therapeutic fields.

1. Introduction
Currently, nanotechnology has appeared as the utmost anticipated revolutionary technology that encompasses a variety of disciplines including biology, physics, chemistry, material science, material chemistry, etc. with numerous applications like pharmacology, biomedical to human care, and comfort (Gupta and Chauhan, 2017). Among several nanomaterials type, the metallic nanomaterials are perceived as the utmost accomplished material due to its huge volume to surface area ratio, particle size, charge, surface functionalization, etc. (Gupta and Chauhan, 2017). Silver nanoparticles (SNPs) are the most studied nanomaterial because of its uses as antimicrobial, antioxidant, anti-inflammatory, and anticancer, etc. agent (Zamiri et al., 2011; Ahmad et al., 2003). And for this purpose, the SNPs synthesis has garnered much attention and numerous technologies are established for the manufacture of eco-friendly, low cost and biocompatible SNPs. Considering the circumstance that the physical and chemical synthesis process is extremely hazardous and expensive (Rajeshkumar and Bharath, 2017), the focus is on the green synthesis approach and utilization of biological resources for the successful manufacture of SNPs. The green synthesis of SNPs follows the bottom-up approach where the main reaction is a reduction process and it is useful...
not only for its low environmental effect as compared to the physical and chemical techniques, but also it produces a large amount of well-defined and safe to use nanoparticles (Patra and Baek, 2014). Plant materials, including fruit, flower, leaves, roots, seeds, etc. and their extracts have been efficaciously considered as the source of reducing agent in the production process (Pirtarighat et al., 2019; Patra and Baek, 2017; Das et al., 2020). Whereas, using the food waste materials for the above-said purpose is very limited. The food/agricultural discarded things are termed as the bio-wastes and generally dumped, resulting in the increasing environmental issues causing health problems and environmental contaminations as these are the breeding ground of insects and pests. The utilization of these wastes in the manufacture of different types of nanomaterials could serve as an alternate process for bio-waste management.

Coconut (Cocos nucifera (L.) (family Arecaceae), and commonly called coconut, coco, coco-da-bahia, or coconut-of-the-beach, is a major crop in many countries including India, Sri Lanka, Indonesia, Malaysia, Philippines, Brazil, Thailand, Mexico, Vietnam, Tanzania (Sinsinwar et al., 2018; Lima et al., 2015). The whole plant is utilized for various purposes including for food, oil, materials in textile industries, and small-scale decoration industries. In the case of the coconut fruit, the inside white pulp and the coconut water are eaten up or used in the manufacture of different products including the coconut oil, whereas the whole shell fibre is thrown out as waste material. Besides, the virgin coconut oil and coconut flour have been reported to be used as food (cooking oil, bakery, snacks, and noodles) and in cosmetics (moisturizer, cream), etc. (Yalegama and Chavan, 2006; Satheesh, 2015; Karandeep et al., 2019).

Further, several beneficial effects of the whole shell fibre of coconut have been reported in the literature (Lima et al., 2015). Coconut shell fibre has been used as an ingredient of tea for the treatment of diarrhoea in Brazil and amenorrhoea in Haiti (Esquenazi et al., 2002; Weniger et al., 1986). In the folklore medicine, the extracts of the whole shell fibre is reported to be treated against many diseases such as Venereal diseases treatment in Trinidad (Wong, 1976); antipyretic, kidney inflammation in Guatemala (Cáceres et al., 1987); Diuretics and gonorrhea treatment in Peru (Ramirez et al., 1988); Urogenital inflammation caused by Trichomonas vaginalis in Mexico (Calzada et al., 2007); Amenorrhoea and dysmenorrhoea in Trinidad (Wong, 1976); for treatment against diabetes in Jamaica (Mitchell and Ahmad, 2006), and asthma in Haiti, Peru (Hope et al., 1993; Ramirez et al., 1988). The cream made from the whole outer shell is used in the treatment of abscesses, dermatitis, and burns injuries in the Guatemala (Cáceres et al., 1987), and Haiti (Weniger et al., 1986). It has been assessed that many industries using the coconut as the raw material dispose of around 3.18 million tons of the coconut waste (coconut shell) only in one year in India (Sinsinwar et al., 2018), and this amounts to an increase of 60% of agricultural waste to the environment. Considering only one country generates and if all coconut producing and consuming countries are considered, then the amount of these wastes generated each year can be imagined how much devastation it would be causing to this environment. But, these coconut shells were stated to possess numerous active compounds including the lignin, cellulose, and phenols, tannins, flavonoids, triterpenes, etc., with multiple beneficial properties (Sinsinwar et al., 2018; Rodrigues et al., 2008; Rodrigues and Pinto, 2007; Thebo et al., 2016; Lima et al., 2015; Sagar et al., 2018) and also there are numerous traditional claims of this plant part used in the treatment of numerous diseases (Lima et al., 2015). Besides, the gas chromatography and mass spectroscopy (GC–MS) analysis of the coconut shell has revealed the presence of compounds such as dodecanoic acid, tetradecanoic acid, n-Hexadecanoic acid, 9-Octadecenoic acid and Squalene (Dhanya et al., 2018). Hence, considering the above points, with an attempt to utilize these agricultural wastes in the synthesis of SNPs, the current investigation aimed to synthesize SNPs with the outer shell fibre aqueous extracts as the source of reducing agent and evaluating its biological potentials in terms of the anticancer, antioxidant and antimicrobial activity.

2. Materials and methods

2.1. Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), butylated hydroxytoluene (BHT), nutrient broth, nutrient agar, cephallexin used in the study were purchased from Sigma Aldrich, USA. HepG2 cells were procured from Cell Line Bank, South Korea. EZ-Cytox kit was purchased from DoGenBio Co., Ltd., South Korea. Dulbecco’s Modified Eagle Medium was purchased from Welgene, South Korea.

2.2. Sample preparation and nanoparticle synthesis

Fresh green coconut (Cocos nucifera L.) (Fig. 1A) were bought from a local supplier of Vietnamese food items in Seoul, Republic of Korea. The outer part of the coconut fruit was washed with tap water and cleaned. The non-edible outer covering (shell) fibre was peeled out, cut into small pieces, weighed and around 50 g boiled with 250 ml of distilled water for 20 min with continuous stirring. The filtrate (CS extract) was preserved for further use in the biosynthesis of NPs. The manufacture of SNPs was undertaken using photo mediated optimized laboratory light condition with the aqueous CS extract as the reducing agent and the AgNO3 as the precursor compound. About 100 ml AgNO3 (1 mM) was taken in 2 separate sets of the conical flask (250 ml) and then the addition of PS extract (10 ml) was carried out with constant stirring in a magnetic stirrer following standard procedure (Patra and Baek, 2015). One set was kept under an optimized laboratory light condition with constant exposure of laboratory light, while another set was kept in complete dark condition. At regular intervals, aliquots of the mixture solution were taken out from each flask separately and scanned using UV–Vis absorption spectroscopy and its color change is recorded. The reactions were continued till 24 h followed by centrifugation at 10,000 rpm (for 30 min). The pellets were then collected, followed by its washing with distilled water to remove impurities.

2.3. Physico-chemical properties of the synthesized AgNPs

The synthesized SNPs (CS-AgNPs) were characterized using UV–Visible spectroscopy (UV–Vis), X-ray powder diffraction (XRD), Fourier–transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) spectroscopy using standard procedures (Patra and Baek, 2015). The synthesized CS-AgNPs was scanned by UV–Vis spectrophotometer (Multiskan GO; Thermo Scientific, Waltham, MA, USA) in the range of 300 and 700 nm at regular interval for 6 hr and the color change is also recorded. The XRD spectra were taken using the XRD machine (X’Pert MRD; PANAnalytical, Almelo, The Netherlands) following the standard procedures (Patra and Baek, 2015) for predicting the phase variety and nature of the SNPs. The FI-IR spectral analysis for the CS-AgNPs and CS extract was done by FT-IR spectrophotometer (Spectrum Two™ FT-IR Spectrometer; PerkinElmer, Waltham, MA, USA) at wavelengths (400–4000 cm⁻¹) for prediction of possible functional groups associated with the bio-reduction and equilibrium of SNPs (Basavegowda et al., 2017). SEM (S-4200; Hitachi, Japan) and EDX (EDS; EDAX Inc., Mahwah,
NJ, USA) machine was used to study the surface morphology and elemental configuration of the CS-AgNPs.

2.4. Biopotential of the synthesized CS-AgNPs

The cytotoxicity activity of the CS-AgNPs was assessed against HepG2 cells by using a standard procedure as described by Patra et al. (2018) followed by determination of the viable nature and surface nature of the tested cells by trypan blue exclusion assay and by scrutiny under an inverted microscope (Faedmaleki et al., 2014a).

The antioxidant activity of CS-AgNPs and the butylated hydroxytoluene (BHT), taken as the standard compound was estimated in terms of their DPPH (1,1-diphenyl-2-picrylhydrazyl) activity and reducing power potential using standard procedures (Patra et al., 2018). The effective concentration (IC50 value for DPPH activity and IC0.5 value for reducing power) was calculated by the standard procedure.

Further, the antimicrobial perspective of CS-AgNPs and the butylated hydroxytoluene (BHT), taken as the standard compound was estimated in terms of their DPPH (1,1-diphenyl-2-picrylhydrazyl) activity and reducing power potential using standard procedures (Patra et al., 2018). The effective concentration (IC50 value for DPPH activity and IC0.5 value for reducing power) was calculated by the standard procedure.

The diameter of inhibition zones was measured after 24 h following incubation at 37 °C. The minimum inhibitory concentration (MIC) was calculated in μg/ml by the two-fold serial dilutions method (Kubo et al., 2004). Cephalexin was used as a positive control and 5% dimethyl sulfoxide in which the sample solution was prepared was taken as the negative control. The antimicrobial index of the acquired results was also interpreted using the following formula (Ghasemi et al., 2003).

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\text{Antimicrobial index} = \frac{\text{inhibition zone by CS-AgNPs}}{\text{inhibition zone by Cephalexin}} \times 100
\]

2.5. Statistical analysis

All the tests were repeated thrice and the outcomes are depicted as the mean value with the standard deviation. Statistical investigation such as one-way ANOVA and Duncan’s multiple range test was undertaken for each experiment by SPSS statistical software (IBM SPSS Statistics Version 25.0, IBM Corp., Armonk, NY, USA).

3. Results

CS-AgNPs synthesis was carried out using the aqueous CS extract (prepared from fresh coconut shell) (Fig. 1A) as the reducing agent under laboratory conditions. The color change from greenish-yellow to brownish color was detected in the tested sample at regular intervals trailed by its absorbance scan using the UV–Vis spectrophotometer (Fig. 1B). The synthesized CS-AgNPs under the optimized laboratory photo condition displayed an absorbance maxima at 447 nm whereas, in the case of the dark exposure, no change was witnessed in the absorbance scan of the UV–Vis spectrophotometer (Fig. 1B). Later on, the powdered CS-AgNPs synthesized in the optimized laboratory photo condition was only characterized for its crystalline nature by the XRD spectroscopy (Fig. 2A) that exhibited four diverse deflection peaks at the 2θ angles of 38.62, 45.33, 66.83 and 76.33 that corresponded to the
with the reference compound BHT (Fig. 4). The IC50 value of CS-AgNPs in a concentration-dependent manner as related to the DPPH and reducing power potential is shown in Fig. 2B. The results of the SEM and EDX examination of the CS-AgNPs is presented in Fig. 2C. The SEM image displays the quite round like structure of the AgNPs, conversely, these were not clear and distinct. The EDX analysis showed the elemental configuration of the CS-AgNPs with a solid peak at 3 KeV that matched to Ag (53.00 wt%), thus endorsing the synthesized particles are the SNPs. Besides the EDX data displayed the existence of other compounds, carbon (8.89%) and oxygen (38.11%) (Fig. 2C, inset).

The bio-potential of the manufactured CS-AgNPs under the optimized laboratory photo condition was assayed by the cytotoxicity, antioxidant and antimicrobial tests. The cytotoxicity property of the CS-AgNPs was verified against the HepG2 cells. The results showed a concentration mediated cell death with an IC50 value of 15.28 μg/ml (R2 = 0.8883). The photo of the morphology as obtained by an inverted microscope showed that there was limited spreading and floating of cells (black arrow) gradually increased with the increase in the cell death mediated by the increased dose of CS-AgNPs, while the control cells were healthy and alive (white arrow) (Fig. 3). The antioxidant perspective of CS-AgNPs investigated by the DPPH and reducing power potential is shown in Fig. 4. The results showed promising DPPH scavenging (51.87% at 100 μg/ml) and reducing power activity (0.165 at 100 μg/ml) of the CS-AgNPs in a concentration-dependent manner as related to the reference compound BHT (Fig. 4). The IC50 value of CS-AgNPs and the BHT for the DPPH activity was 96.39 μg/ml and 35.67 μg/ml respectively (Table 1). The IC50 values for the CS-AgNPs and the BHT for the reducing power assay were found out to be 209.96 μg/ml and 609.32 μg/ml respectively (Table 1). The antimicrobial activity results of CS-AgNPs against four pathogenic bacteria and one Candida species are presented in Table 2. CS-AgNPs extract is effective against all the five pathogens with the diameter of inhibition zones ranged between 8.87 and 13.07 mm (Table 2), while the standard cephalexin, taken as the positive control, showed inhibition zones ranged between 13.11 and 14.79 mm and the dimethyl sulfoxide (DMSO, negative control), didn’t show any inhibition action against any of the tested pathogen. Among the five tested pathogens, the CS-AgNPs was more active against the L. monocytogenes with 13.07 mm diameter of inhibition zone followed by 12.44 mm against the C. albicans as compared to 13.72 mm and 14.79 mm of inhibition zones for both L. monocytogenes and C. albicans respectively by the cephalexin taken as the positive control. Besides, the MIC for CS-AgNPs against the pathogens was found out to be around 100 μg/ml (Table 2) as we have not tested the concentration >100 μg/ml. The antimicrobial index was also calculated that ranged between 67.66% and 95.26% for all the tested pathogens (Table 2).

4. Discussion

Biologically synthesized SNP has fascinated much consideration in the recent scenario because of its potential use in many fields including the food, medical and pharmaceutical sectors (Mousavi et al., 2018; Patra et al., 2018; Masum et al., 2019). Thus many efficient and cost-effective process for the synthesis of the SNPs has been tested and developed using numerous plant and other biological materials (Behravan et al., 2019; Jegadeeswaran et al., 2012; Patra and Baek, 2017; Patra et al., 2018; Félix-Domínguez et al., 2017). Among these, the utilization of food and agricultural wastes in the production of SNPs is more fascinating and important due to two prominent reasons, i. successful application of these waste material in the manufacture of a useful product with numerous applications; ii. An operative instrument for waste management during the present time, where management of biological waste is a burning issue. Currently, the outer coconut shell, which is a chief food/agricultural waste material in the manufacture of a useful product with numerous applications; ii. An operative instrument for waste management during the present time, where management of biological waste is a burning issue. Currently, the outer coconut shell, which is a chief food/agricultural waste material in the Indian subcontinent has been utilized in the manufacture of CS-AgNPs. It is stated that these coconut shell fibre possess a diversity of chemical compounds including lignin, cellulose, phenols, tannins, flavonoids, triterpenes, etc. with countless natural properties (Sinsinwar et al., 2018; Rodrigues et al., 2008; Rodrigues and Pinto, 2007; Thebo et al., 2016; Lima et al., 2015; Sagar et al., 2018), Hence it is assumed that the CS-AgNPs manufactured by the aqueous extract of coconut shell (Fig. 1A), might have been surrounded by these compounds and could be beneficial in further applications particularly in the food sector as a component of antibacterial food
packaging materials, together with the potential uses in biomedical and pharmaceutical sectors. Among the various green synthesis processes, the photo-mediated production of nanoparticles has proven as an efficient and environmentally friendly process as evident for previously published literature (Kumar et al., 2017; Amaladhas et al., 2013; Quaresma et al., 2009; Sahu et al., 2013). Hence, currently, an optimized laboratory photo condition was adopted to synthesize SNPs (Fig. 1B).

The green synthesis of CS-AgNPs was visually established when there is a color change of the reaction mixture from greenish-yellow to brownish color. Further, the UV–VIS absorption spectra showed a surface plasmon resonance (SPR) band at 447 nm due to the excitation of free electrons (Fig. 1B), which corroborated previous claims (Patra and Baek, 2016; Mousavi et al., 2018). Among the 2 sets (1 in laboratory photo condition and another in complete dark), the sample kept under the laboratory photo condition showed significant absorption peaks, which corresponds to the photo mediated synthesis of SNPs while the sample kept under the dark didn’t show any significant rise in the absorption peaks (Fig. 1B). This clarifies that the reaction was photocatalytic in nature and it corroborates with previous findings (Patra et al., 2018; Kumar et al., 2017; Sumitha et al., 2018; Sahu et al., 2013). The XRD spectroscopy data unveiled four distinct diffraction peaks at 2\(\theta\) angles corresponded to (111), (200), (220), and (311) (Fig. 2A) which are in accordance to face-centered cubic (fcc) phase of Ag0 standard (JCPDS Card no. 04-0783) (Jagtap and Bapat, 2013; Morris et al., 1981). Further, the FT-IR results also showed slight alterations in the peaks of the CS extracts and the CS-AgNPs (Fig. 2B). The 3336.86 cm\(^{-1}\) bands of the CS extract was shifted to 3337.12 cm\(^{-1}\) in the case of the CS-AgNPs, which are the O—H stretch of the H-Bond to the free hydroxyl moiety of phenol groups and N—H stretch of the primary and secondary amines (Coates,
2006); similarly, the 1636.06 cm⁻¹ band of the CS extract was shifted to 1636.97 cm⁻¹ in case of the CS-AgNPs which are basically the N-H bend of the primary amines; and 627.24 cm⁻¹ band of the CS extract was shifted to 594.38 cm⁻¹ in case of the CS-AgNPs (Fig. 2B). The morphological nature of the bio-synthesized CS-AgNPs was predicted by the SEM-EDX analysis (Fig. 2C). Round and spherical shape of CS-AgNPs were visible from the SEM image and the EDX analysis showed the existence of maximum percentage of Ag (53.00%) in the AgNP along with carbon and oxygen, since CS extracts was used in the synthesis process as the reducing agents and the bioactive compounds CS extract might have capped to the surface of CS-AgNPs rendering strength and stabilization to the NPs.

Following the description of CS-AgNPs, its biological potential was investigated using cytotoxicity, antioxidant and antimicrobial assays. The CS-AgNPs displayed significant cytotoxicity action against the HepG2 cells (Fig. 3). The IC₅₀ value of CS-AgNPs was found out to be 15.28 μg/ml (R² = 0.8883). A possible reason for the significant cytotoxicity effect of CS-AgNPs might be that, because of their reduced size, they could have entered the inside of the cell and have accumulated there resulting in causing injury to the internal organelles of the cell resulting in the death of the cells with various immunological and electrostatic responses (Faedmaleki et al., 2014b; Rajkumar et al., 2019; Patra et al., 2018; Patil Shriniwas and Kumbhar Subhash, 2017). Additionally, the bio-synthesized CS-AgNPs demonstrated significant DPPH and reducing power activity (Fig. 4, Table 1), which can be accredited to the presence of a range of compounds including phenols, flavonoids, triterpenes in the coconut shell (Sinsinwar et al., 2018; Rodrigues et al., 2008; Rodrigues and Pinto, 2007; Thebo et al., 2016; Lima et al., 2015; Sagar et al., 2018), whose aqueous extract was used as the reducing agent in the manufacturing procedure. Additionally, the CS-AgNPs also demonstrated promising antimicrobial activity against four pathogenic bacteria and one candida sp. (Table 2), which indicates its capability for its application in the cure of bacterial and candida infection and also in a number of biomedical applications such as antibacterial products, wound dressing, antibacterial coatings and many more. Apart from these, the synthesized AgNps could act as a possible applicant in the smart food packaging system as an antibacterial protecting agent, antibacterial food packaging materials to prevent the spoilage of food materials from the action of pathogenic bacteria. A possible mode of action of the CS-AgNPs is due to the existence of Ag⁺ ions, which inhibits the bacterial and candida progression through the destruction of the respiratory enzyme and the electron transport mechanisms and also through the interfering with the DNA function (Li et al., 2006; Gupta and Chauhan, 2017). The antimicrobial index ranged between 67.66% and 95.26% for all the tested pathogens (Table 2) which further confirm the usability of the CS-AgNPs in various antimicrobial applications (Ghasemi et al., 2003).

5. Conclusion

The utilization of food/agriculture waste in the manufacture of SNPs has been attempted in the current investigation. And thus this study provides a single one-step approach for the synthesis of SNPs using aqueous coconut shell fibre extract as the reducing agent, which is economical, non-toxic, and cost-effective along with the existence of maximum percentage of Ag (53.00%) in the AgNP along with carbon and oxygen, since CS extracts was used in the synthesis process as the reducing agents and the bioactive compounds CS extract might have capped to the surface of CS-AgNPs rendering strength and stabilization to the NPs.

Fig. 4. Antioxidant effect of CS-AgNPs. Variance in the superscript letters indicates statistical variance at P < 0.05.

Table 1

| Pathogen | DPPH activity IC₅₀ value (μg/ml) | Reducing Power IC₀.₅ value (μg/ml) |
|----------|---------------------------------|-----------------------------------|
| CS-AgNP | 96.39                           | 209.96                            |
| BHT     | 35.67                           | 609.32                            |

Table 2

| Pathogen  | MIC (μg/ml) | Positive control | Negative control | Antimicrobial index (%) |
|-----------|-------------|------------------|------------------|-------------------------|
| E. coli O157:H7 ATCC 23514 | >100         | 13.14±1.05       | 0                | 72.30                   |
| E. feacium DB01 | >100         | 13.11±0.31       | 0                | 67.66                   |
| P. acnes ATCC 6919 | >100         | 13.72±0.49       | 0                | 95.26                   |
| L. monocytogenes ATCC 33090 | 100          | 14.79±0.33       | 0                | 84.11                   |
| C. albicans ATCC 10231 | 100          | 14.79±0.33       | 0                | 84.11                   |

*Positive control – Cephalexin.
**Negative control – 5% dimethyl sulfoxide.
Variance in the superscript letters indicates statistical variance at P < 0.05. NA- no activity.

"Antimicrobial index (%) = inhibition of sample/inhibition of antibiotics X 100."
with an effective strategy for the control of biological wastes. The green synthesized CS-AgNPs were categorized using UV–VIS, XRD, FT-IR, and SEM-EDX. The FTIR results displayed the participation of several bioactive compounds from the CS extract particularly the hydroxyl groups accountable for the rapid decrease of ions, resulting in the formation of CS-AgNPs. The XRD spectra displayed the crystallite nature of the synthesized AgNPs and the SEM and EDX results showed its round structure and elemental composition. Furthermore, the CS-AgNPs demonstrated promising cytotoxicity potential against the HepG2 cells along with antioxidant scavenging activity in terms of its DPPH scavenging and reducing power and antimicrobial activity against four pathogenic bacteria and one candida species. Overall, the results suggest possible utilization of CS-AgNPs in biomedical, food, and pharmaceutical fields including bacterial, candidal, and cancer treatment, antibacterial food packaging materials, etc.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R., Sastry, M., 2003. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids Surf. B: Biointerfaces 28, 313–318.
Amaladhas, T.P., Usha, M., Naveen, S., 2013. Sunlight induced rapid synthesis and kinetics of silver nanoparticles using leaf extract of Achyranthes aspera L. And their antimicrobial applications. Adv. Mater. Lett. 4, 779–785.
Basavegowda, N., Mishra, K., Thombal, R.S., Kaliraj, K., Lee, Y.R., 2017. Sonocchemical green synthesis of yttrium oxide (Y2O3) nanoparticles as a novel heterogeneous catalyst for the construction of biologically interesting 1,3-thiazolidin-4-ones. Catal. Lett. 147 (10), 2630–2639.
Behravan, M., Hossein Panahi, A., Najghadeh, A., Ziaei, M., Mahdavi, R., Mirzapour, A., 2019. Facile green synthesis of silver nanoparticles using Berberis vulgaris grown in vitro and their antimicrobial activity against the pathogenic bacteria and Max oxyrea strain RS-2 of rice bacterial brown stripe. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.00820.
Mitchell, S., Ahmad, M., 2006. A review of medicinal plant research at the University of the West Indies, Jamaica. West Indian Med. J. 55, 243.
Mousavi, B., Tafvizi, F., Zakeri Bostanabad, S., 2018. Green synthesis of silver nanoparticles using Artemisia turcomanica leaf extract and the study of antioxidant and anti-inflammatory effects on gastric cancer cell line (AGS). Artif. Cells Nanomed. Biotechnol. 1–12.
Patra, J.K., Baek, K.-H., 2014. Green nanobiotechnology: factors affecting synthesis and characterization techniques. J. Nanomater. 2014, 219.
Patra, J.K., Baek, K.-H., 2015. Novel green synthesis of gold nanoparticles using Citrullus lanatus rind and investigation of proteasome inhibitory activity, antibacterial, and antioxidant potential. Int. J. Nanomed. 10, 7253.
Patra, J.K., Baek, K.-H., 2016. Biosynthesis of silver nanoparticles using aqueous extract of silty hairs of corn and investigation of its antibacterial and antifungal synergistic activity and antioxidant potential. IET Nanobiotechnol. 10 (5), 326–333.
Kubo, I., Fujita, K.-I., Kubo, A., Nihei, K.-I., Ogura, T., 2004. Antibacterial activity of polyphenolics from Cocos nucifera L. (Arecaeaceae). A phytochemical and pharmacological review. Brazil. J. Med. Biol. Res. = Revista brasileira de pesquisas medicas e biologicas 48, 593–964.
Kumar, V., Mohan, S., Singh, D.K., Verma, D.K., Singh, V.K., Hasan, S.H., 2017. Photo-mediated optimized synthesis of silver nanoparticles for the selective detection of iron(III), antibacterial and antioxidant activity. Mater. Sci. Eng., C 71, 1004–1019.
Le, Y., Liang, P., Yao, C., Song, Q., Newton, E., 2006. Antimicrobial effect of surgical masks coated with nanoparticles. J. Hosp. Infect. 62, 58–63.
Lim, E.B.C., Souza, C.N.S., Meneses, L.N., Ximenos, N.C., Santos Júnior, M.A., Vanhooren, P.G.S., Lima, N.B.C., Patrocínio, M.C.A., Macedo, P.D., Vasconcelos, S.M.M., 2015. Cocos nucifera (L.) (Areaceae) A phytochemical and pharmacological review. Brazil. J. Med. Biol. Res. = Revista brasileira de pesquisas medicas e biologicas 48, 593–964.
Pritarigath, S., Ghanadinia, M., Baghotsi, S., 2019. Green synthesis of silver nanoparticles using the plant extract of Salvia spicigera grown in vitro and their antibacterial activity assessment. J. Nanomater. Chem. 9, 1–9.
Quaresma, P., Soares, L., Contar, L., Miranda, A., Osório, I., Carvalho, P.A., Franco, R., Pereira, E., 2009. Green photocatalytic synthesis of stable Au and Ag nanoparticles. Green Chem. 11, 1889–1893.

Rajeshkumar, S., Bharath, L., 2017. Mechanism of plant-mediated synthesis of silver nanoparticles—a review on biomolecules involved, characterisation and antibacterial activity. Chem. Biol. Interact. 273, 219–227.

Rajkumar, T., Sapi, A., Das, G., Debnath, T., Ansari, A., Patra, J.K., 2019. Biosynthesis of silver nanoparticle using extract of Zea mays (corn flour) and investigation of its cytotoxicity effect and radical scavenging potential. J. Photochem. Photobiol., B 193, 1–7.

Ramirez, V., Mostacero, L., Garcia, A., Mejia, C., Pelaez, P., Medina, C., Miranda, C., 1988. Vegetales empleados en medicina tradicional Norperuana. Banco Agrario del Peru & NACL Univ Trujillo, Trujillo, Peru.

Rodrigues, S., Pinto, G.A.S., 2007. Ultrasound extraction of phenolic compounds from coconut (Cocos nucifera) shell powder. J. Food Eng. 80, 869–872.

Rodrigues, S., Pinto, G.A.S., Fernandes, F.A.N., 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (Cocos nucifera) shell powder by response surface methodology. Ultrason. Sonochem. 15, 95–100.

Sagar, N.A., Pareek, S., Sharma, S., Yahia, E.M., Lobo, M.G., 2013. Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Compr. Rev. Food Sci. Food Saf. 17, 512–531.

Sahu, N., Soni, D., Chandrashekhar, B., Sarangi, B.K., Satpute, D., Pandey, R.A., 2013. Synthesis and characterization of silver nanoparticles using Cynodon dactylon leaves and assessment of their antibacterial activity. Bioprocess. Biosyst. Eng. 36, 999–1004.

Satheesh, N., 2015. Review on production and potential applications of virgin coconut oil. Ann. Food Sci. Technol. 16, 115–126.

Sinsinwar, S., Sarkar, M.K., Suriya, K.R., Nithyanand, P., Vadhivel, V., 2018. Use of agricultural waste (coconut shell) for the synthesis of silver nanoparticles and evaluation of their antibacterial activity against selected human pathogens. Microb. Pathog. 124, 30–37.

Sumitha, S., Varanmithi, S., Shalini, S., Chinni, S.V., Gopinath, S.C.R., Anbu, P., Bahari, M. R., Harish, R., Kathiresan, S., Ravichandran, V., 2018. Phyto-mediated photo catalysed green synthesis of silver nanoparticles using durio zibethinus seed extract: Antimicrobial and cytotoxic activity and photocatalytic applications. Molecules 23 (12), 3311. https://doi.org/10.3390/molecules23123311.

Thebo, N.K., Simair, A.A., Mangrio, G.S., Ansari, K.A., Bhutto, A.A., Lu, C., Sheikh, W.A., 2016. Antifungal potential and antioxidant efficacy in the shell extract of Cocos nucifera (L.) (Areaceae) against pathogenic dermal mycosis. Medicine 3, 2–12.

Weniger, B., Rouzier, M., Daguilh, R., Henrys, D., Henrys, J., Antin, R., 1986. Traditional medicine in the Central Plateau of Haiti. 2. Ethnopharmacological inventory. J. Ethnopharmacol. 17, 13–30.

Wong, W., 1976. Some folk medicinal plants from Trinidad. Econ. Bot. 30, 103–142.

Yalegama, L., Chavan, J., 2006. Studies on utilization of coconut flour as a source of cell wall polysaccharides.

Zamiri, R., Azmi, B., Sadrolhosseini, A.R., Ahangar, H.A., Zaidan, A., Mahdi, M., 2011. Preparation of silver nanoparticles in virgin coconut oil using laser ablation. Int. J. Nanomed. 6, 71.