Green Synthesis and Characterization of Silver Nanoparticles Using Rice (*Oryza sativa*) and Spent Coffee (*Coffea robusta*) Grounds from Agricultural Waste[§]

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⁶Special Issue: Turning agricultural waste into useful biochemicals and biofuels through biochemical engineering and biotechnological processing

SUMMARY

Research background. Silver nanoparticles (AgNPs) were synthesised using agricultural waste and green synthesis, a sustainable alternative to traditional synthesis techniques that require hazardous chemicals and extensive processing. The AgNPs were produced using spent coffee (*Coffea robusta*) grounds and rice (*Oryza sativa*) husks, both common agricultural wastes rich in bioactive substances such as proteins, flavonoids and phenolic acids that act as natural reducing agents.

Experimental approach. The formation and stability of AgNPs were confirmed using various methods. UV-Vis spectroscopy showed surface plasmon resonance (SPR) peaks at 450 nm, indicating the formation of AgNPs, while Fourier transform infrared spectroscopy (FTIR) identified functional groups responsible for the bio-reduction and stabilisation of the nanoparticles. X-ray diffraction spectroscopy (XRD) confirmed the crystalline, face-centred cubic structure. Zeta potential analysis showed a stable dispersion and particle size analysis showed a consistent size distribution. The antibacterial activity of AgNPs was evaluated by testing their effectiveness against both Gram-positive and Gram-negative bacteria.

Results and conclusions. The AgNPs were synthesised using spent coffee grounds and rice husks, which are rich in biomolecules that serve as effective reducing and stabilising agents. FTIR analysis identified functional groups involved in the reduction and stabilisation of nanoparticles, while XRD confirmed their face-centred cubic (FCC) crystalline structure. Zeta potential measurements showed stable dispersions with particle sizes of AgNPs obtained using spent coffee grounds of approx. 187 nm and of AgNPs obtained using rice husks of 198 nm. The synthesised AgNPs also showed strong antibacterial activity against both Gram-positive and Gram-negative bacteria.

Novelty and scientific contribution. AgNPs were obtained by green synthesis using agricultural waste such as spent coffee grounds and rice husks as natural reducing and stabilising agents. This study highlights the innovative use of biomolecule-rich materials to generate stable AgNPs with strong antibacterial properties and provides a sustainable basis for further development of nanotechnological applications.

Keywords: agricultural waste; green synthesis; rice husks; spent coffee grounds; silver nanoparticles

INTRODUCTION

Nanotechnology has become a transformative field dominated by silver nanoparticles (AgNPs) due to their exceptional properties and diverse applications (1). Conventional AgNP synthesis often involves hazardous chemicals and complex processes that cause significant environmental concerns (2). Therefore, green synthesis has gained attention as a sustainable alternative that uses environmentally friendly materials as reducing agents (3). Agricultural waste rich in biomolecules with reducing capabilities offers a promising platform for AgNP production (4), which adheres to the principles of sustainable development and circular economy (5). Agricultural wastes rich in proteins, phenols and flavonoids act as biological reducing agents in the biological synthesis of AgNPs (6) and this approach aids waste management by transforming waste into valuable products. It supports resource conservation by utilising waste materials efficiently, thereby addressing the environmental challenges associated with traditional methods for nanoparticle production (7). Although various bio-based approaches have been investigated, the potential of rice husks and spent coffee grounds as precursors for AgNP synthesis remains relatively understudied.

Coffee is a popular non-alcoholic beverage obtained from the roasted seeds of the Coffea plant (8). Coffee consumption is increasing globally (9) and the coffee industry generates significant amounts of waste, including spent coffee grounds (SCG). SCG are a rich source of biologically active compounds, such as flavonoids, phenolic compounds and phytonutrients, but can also contribute to environmental pollution due to high amounts of tannins and caffeine (10). The polyphenolic compounds in SCG, including chlorogenic acids and melanoidins, interact with metal salt solutions to form metal atoms through a reduction mechanism (11). Additionally, coffee beans contain two main alkaloids, caffeine and trigonelline, and some other compounds such as adenine, xanthine, hypoxanthine and guanine, which can facilitate the formation of nanostructures through oxidative coupling (12).

Rice (Oryza sativa) husks are a by-product of the rice milling industry often discarded as agricultural waste (13). Due to its abundance and renewability, rice husks offer a significant opportunity for sustainable nanoparticle synthesis (14). It is a rich source of biologically active compounds, such as phenolic acids and flavonoids, which can facilitate the reduction of metal ions to produce AgNPs (15,16). The AgNPs synthesised using rice husks are safe for humans and the environment and have been shown to have higher reducing power and the ability to scavenge reactive oxygen species (ROS), which makes them potential antioxidant compounds (17). The use of SCG and rice husks as reducing agents provides a cost-effective, sustainable and eco-friendly approach to AgNP production. This method contributes to the sustainable utilisation of agro-waste and a circular bioeconomy, thus helping to mitigate the environmental impact associated with traditional nanoparticle synthesis.

The main focus of this study is to develop a sustainable and environmentally friendly method to synthesise silver nanoparticles (AgNPs) using agricultural waste materials. By utilising rice husks and coffee grounds as reducing agents, we aimed to synthesise nanoparticles and then characterise their properties. This characterisation is essential for evaluating the effectiveness and quality of the produced nanoparticles. Additionally, we investigated the potential benefits of AgNPs in various fields, including antimicrobial, catalytic and biomedical applications. Furthermore, this study paves the way to the advancement of green nanotechnology by demonstrating the feasibility and sustainability of using agricultural wastes for nanoparticle synthesis.

MATERIALS AND METHODS

Preparation of spent coffee grounds

Spent coffee ground (SCG) extract was prepared from Coffea robusta beans obtained from Zig Bakery and Cake, Gong Badak, Terengganu, Malaysia. First, the SCG was rinsed with deionized water and filtered to remove excess impurities. The cleaned SCG was then dried in a universal oven (UN55; Memmert, Schwabach, Germany) at 60 °C overnight until complete desiccation, then blended in a blender (MX--GM1011; Panasonic, Shah Alam, Malaysia) to obtain a fine dry powder. A total of 15 g of powdered SCG was mixed with 150 mL deionized water and 30 mL absolute ethanol (8.33 % m/V) in a 5:1 mass per volume ratio for the extraction of bioactive compounds. This mixture was stirred and heated at 80 °C for 35 min in a water bath (WNB 7; Memmert). After the mixture was cooled down, it was centrifuged at $769 \times q$ for 15 min using a high-speed floor-top refrigerated centrifuge (CR22N; Hitachi, Hitachinaka, Japan) and then filtered to separate the solid particles from the liquid phase and freeze-dried to preserve the material for further use.

Preparation of rice husk extract

Rice husks were sourced from MAAS Agros Technology in Kajang, Selangor. A mass of 500 g sample of rice husks was rinsed with deionized water. The rinsed rice husks were dried overnight in a universal oven at 60 °C. The dried rice husk was then powdered to a fine powder in a blender (MX-GM1011; Panasonic).

For the extraction, 300 g of rice husk powder was combined with 1000 mL of deionized water (3:10 m/V ratio) and heated at 60 °C for 30 min in a water bath. After heating, the mixture was centrifuged at 769×g for 15 min (CR22N; Hitachi), followed by filtration and then it was collected and freezedried using a freeze dryer (Genesis 35EL Pilot freeze dryer; SP Scientific VirTis, Gardiner, New York, NY, USA) for further use.

Synthesis of silver nanoparticles using SCG extract

The SCG extract was added to 10 mM silver nitrate (Bendosen Laboratory Chemicals, Bendosen, Norway) solution in a 1:1 mass per volume ratio. This mixture was homogenised and stored in a dry, dark place for 5 h (Fig. S1). The change in the colour of the solution indicated the formation of nanoparticles. These changes were observed and recorded hourly from 0 to 5 h.

Synthesis of silver nanoparticles using rice husk extract

Silver nitrate solution of 10 mM was prepared from solid $AgNO_3$. The freeze-dried rice husk extract was mixed with the $AgNO_3$ solution in a 1:4 volume ratio (Fig. S2). The reaction mixtures were heated at 75 °C for 15 min in a water bath and then stored in the dark for 96 h to prevent photodegradation. The colour change of the solution indicated nanoparticle

formation. The colour changes were observed and recorded hourly.

UV-Vis spectroscopy

The absorbance of the solution containing nanoparticles was determined using a UV-Vis spectrophotometer (UV-1800, 240 V; Shimadzu, Kyoto, Japan) in a wavelength range from 350 to 650 nm (*18*). The reduction of silver ions obtained by green synthesis was recorded using the UV-Vis spectrum of the reaction of the mixture of silver nanoparticles.

Structural analysis of AgNPs

The hydrodynamic size of particles (Z average), polydispersity index (PDI) and zeta potential of AgNPs were measured by dynamic light scattering (DLS) (Litesizer 500; Anton Paar, Graz, Austria) (*18*).

The chemical functional groups of synthesised AgNPs were analysed using Fourier transform infrared spectroscopy (FTIR) (IR Traces-100; Shimadzu) in the spectral range of 4000–400 cm⁻¹ (*18*).

The profile of freeze-dried AgNPs at 20 and -80 °C was characterised with the X-ray diffraction (XRD) (SmartLab X-ray diffractometer; Rigaku, Tokyo, Japan) using voltage of 40 kV and current of 30 mA (*18*).

Antimicrobial assay

The agar disc diffusion method was used to evaluate the antimicrobial activity of silver nanoparticles using rice husk and spent coffee grounds. The synthesised AgNPs showed strong antibacterial properties against Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) (Fig. S3). These bacteria were cultured and streaked on the nutrient agar. Using a densitometer, the streaked bacteria were used to prepare the suspension of 0.5 McFarland standard (equivalent to 1.5.108 CFU/mL). The prepared bacterial inoculum was uniformly swabbed on the Mueller-Hinton agar (MHA) plate. The concentrations of 400, 200 and 100 mg/mL of AqNPs, obtained with the addition of rice husk or spent coffee ground extract, were added to the sterile plain disc and placed onto an MHA plate. The zone of inhibition was measured using a vernier calliper after incubating for 24 h at 37 °C. The gentamicin disc was the positive control and distilled water was the negative control, both used to assess the effectiveness of the antimicrobial assay.

Statistical analysis

All the data are presented as mean value±standard deviation (S.D.). ANOVA and Tukey's honestly significant difference (HSD) test was used for the statistical analysis with the IBM SPSS Statistics v. 29.0 software, setting the significance at p<0.05 (*19,20*).

RESULTS AND DISCUSSION

Biosynthesis and spectroscopic analysis of AgNPs derived from agricultural waste

The synthesis of silver nanoparticles (AgNPs) was successfully achieved through a green approach utilising extracts from Oryza sativa (rice husk) and spent coffee grounds as reducing agents. This environmentally friendly method involved the reduction of Ag⁺ ions to AgNPs, visually indicated by a distinct change of colour from light yellow to brownish black (21). The colour intensity of rice husk extract in 10 mM AgNO₃ solution increased from 0 to 96 h (Fig. S4a) of storage, and that of spent coffee grounds in 10 mM AgNO₃ from 0 to 5 h (Fig. S4b). The mechanism, attributed to the excitation of electrons via surface plasmon resonance (SPR), confirmed the formation of AqNPs (22). Phenolic compounds from the plant extracts contributed a vital role in this bio-reduction process, acting as both reducing and stabilising agents for the nanoparticles (23). Optimization of parameters such as metal ion concentration, temperature, and reaction time was essential for achieving optimal AgNP formation (24). The interaction of silver ions with phenolic acids from rice husk and coffee grounds was confirmed by the change of colour from light yellow to brownish black, indicating increased stable AgNP formation. Similarly, Vasyliev et al. (25) found that the blackcurrant pomace extract contained phenolic compounds that may have contributed to the improved stability of AgNPs. The extracts were freezedried before being combined with AqNO₃ for synthesis. Freeze-drying removes 98 % of water from the samples, allowing long-term storage while preserving the bioactive phenolic acids responsible for AgNP formation (26).

The UV-Vis spectra were used to determine the structure of the AgNPs by analysing their free surface electron plasmon oscillations. The SPR peak, indicating AgNP formation, typically appears in the visible range (350–650 nm), which depends on particle size, shape and environment (*27*). **Fig. 1a** shows that a weak or nearly absent SPR peak at 450 nm was observed for AgNPs obtained from rice husks after 96 h, suggesting the formation of AgNPs. The peaks of phenolic acid components in the spectra obtained for AgNPs were observed at approx. 430 nm, as reported by Liu (*15*).

The concentration of AgNPs is directly proportional to the intensity of the absorption peak, a higher concentration of nanoparticles or larger nanoparticles result in a higher absorption peak (28). The increasing absorbance with time suggested the growth of larger particles, indicating a bathochromic shift as the SPR peak shifted towards longer wavelengths (29). In the synthesis of AgNP using SCG, a rapid colour change from light to dark brown within 30 min confirmed the formation of AgNPs, with the peak at 450 nm increasing with time and reaching maximum absorbance after 4 h (Fig. 1b). Similarly, silver nanoparticles obtained using spent coffee grounds showed a peak at 450 nm (30).

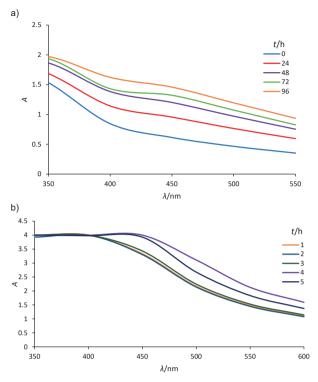


Fig. 1. UV-Vis spectra of silver nanoparticles (AgNPs) obtained with the addition of: a) rice husk and b) spent coffee ground (SCG) extracts

The UV-Vis analysis confirmed AgNP synthesis using both rice husks and SCG extracts, with a consistent SPR peak around 450 nm. These results agree with previous studies that confirm SPR bands in similar ranges (*30*). Spherical metal nanoparticles can only produce a single SPR band, while anisotropic particles may generate two or more SPR bands depending on their shape (*18*). In this study, a single SPR peak was observed, which indicated that the synthesised silver nanoparticles were spherical. This study highlights the feasibility of using sustainable, cost-effective and environmentally friendly methods for AgNP synthesis using agricultural wastes, with potential applications in various fields.

Analysis of functional groups of AgNP by FTIR

FTIR analysis was carried out to identify the functional groups and biomolecules involved in the bio-reduction of AgNPs derived using rice husks and SCG. This method provides critical insights into the surface chemical composition and reactive sites of the AgNPs that are essential for understanding their surface reactivity (*31*). The FTIR spectra of AgNPs obtained using rice husks in Fig. 2a show several significant absorption peaks. The significant peak at 3332.99 cm⁻¹ (O-H stretching) indicates the presence of carboxylic acids (*32*). The O-H stretching probably originates from the phenolic O-H groups in the lignin structure of rice husks (*33,34*). Additional prominent peaks were observed at 2931.80 cm⁻¹, corresponding to CH₂ stretching, 2360.87 cm⁻¹ (alkynes or ammonium), 1635.64 cm⁻¹ (C-N and C-C stretching, associated

with proteins or amides), 1319.31 cm⁻¹ (N=O stretching, nitro compounds), 1081.41 cm⁻¹ (C-N stretching, amines) and 648.08 cm⁻¹ (C-Cl stretching, alkyl groups) (18,35). A broad band at 3286 cm⁻¹ and a peak at 2924 cm⁻¹ for AgNPs obtained with SCG, as shown in Fig. 2b, indicate different properties (36). The peak at 3286 cm⁻¹ corresponds to N-H and O-H stretching vibrations and the peak at 2924 cm⁻¹ corresponds to asymmetric stretching of the C-H bond in methyl groups (29). The broad band at 3286 cm⁻¹ suggests the presence of -OH groups in alcohols and phenolic compounds, which function as capping and stabilising agents. For the rice husk-derived AgNPs, the polyphenols and polysaccharides act as capping and stabilising agents (37) with the peaks at 1357 and 1273 cm⁻¹ associated with the bending vibrations of C-H bonds in methyl and methylene groups, which also affect the size, shape and stability of the AgNPs. The absorption peak at 663.51 cm⁻¹ indicated C-Cl stretching vibrations, reflecting the presence of halogen compounds that act as stabilising agents to prevent nanoparticle agglomeration (38). Various functional groups were present in rice husk and SCG extracts, as confirmed by FTIR analysis, thus this agricultural waste is a potential resource for the green synthesis of silver nanoparticles, an approach that contributes to the advancement of sustainable and environmentally friendly nanotechnology.

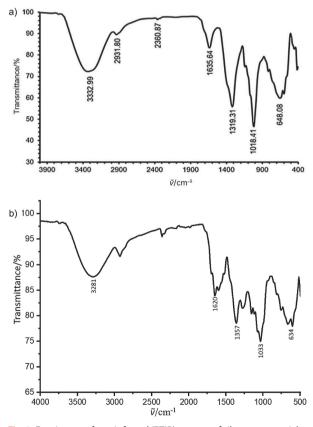


Fig. 2. Fourier transform infrared (FTIR) spectra of silver nanoparticles (AgNPs) obtained with the addition of: a) rice husk and b) spent coffee ground extracts

X-ray diffraction spectroscopy analysis of silver nanoparticles synthesised using rice husk and spent coffee grounds

The molecular and crystalline structures of AgNPs were analysed using X-ray diffraction spectroscopy (XRD), which provides critical insights into the crystalline structure and phase identification of materials. XRD analysis of the molecular and crystalline AqNP structures provided valuable information about the physicochemical properties and degree of crystallinity of the synthesised AqNPs (39). The XRD pattern of the AgNPs obtained fusing rice husks (Fig. 3a) showed distinct peaks at 38°, 43°, 64° and 77°, corresponding to (111), (200), (220) and (311) planes, respectively, confirming the presence of a face-centred cubic (FCC) structure (40), i.e. the synthesised nanoparticles were crystalline. This is consistent with previous studies reporting similar XRD patterns for Ag-NPs synthesised with apricot and blackcurrant pomace extracts, and rice husks subjected to acid-alkali pretreatment (15,24). In addition, the XRD analysis of AgNPs obtained from SCG (Fig. 3b) showed prominent peaks in the 2θ range from 20° to 80°, with distinct peaks at 27.24°, 38.01° (111), 41.11° (200), 64.3° (220) and 77.3° (311) corresponding to the planes of Bragg's reflection and confirming a face-centred cubic crystalline structure (38). Notably, the peak at 38.01° indicates Ag⁰ with an FCC structure and confirms the effective reduction of Ag⁺ ions with both SCG and rice husks (40). Overall, these results show the successful formation of crystalline silver nanoparticles using agricultural waste and highlight their potential for sustainable and environmentally friendly applications in nanotechnology (18).

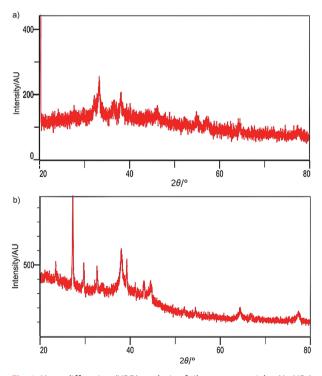


Fig. 3. X-ray diffraction (XRD) analysis of silver nanoparticles (AgNPs) obtained with the addition of: a) rice husk and b) spent coffee ground extracts

Particle size and zeta potential

The analysis of AgNPs produced using rice husk and SCG extracts shows that the AgNPs synthesised using rice husks had a larger average size of (198.6±3.6) nm (Fig. 4a), which is attributed to the complex chemical composition of rice husks and affects the formation of nanoparticles. Furthermore, these particles had a zeta potential of (-21.2±2.0) mV and a polydispersity index (PDI) of 26.9 % (Fig. 4b). The AgNPs obtained using SCG had an average size of 187 nm (Fig. 4c), typical for biologically synthesised nanoparticles. However, their stability was moderate, as shown by a zeta potential of -11.8 mV and a PDI of 22.1 % (Fig. 4d), which indicates insufficient electrostatic repulsion and thus the potential for aggregation of particles. The multiple peaks in the particle diameter distribution of rice husks indicate polydispersity and the negative

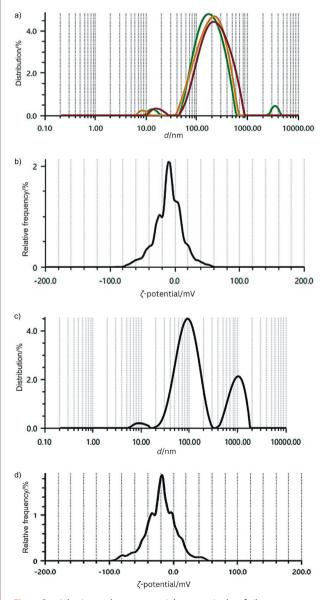


Fig. 4. Particle size and zeta potential, respectively, of silver nanoparticles (AgNPs) obtained with the addition of: a) and b) rice husk and c) and d) spent coffee ground extracts

charge of the particles tends to increase stability by inhibiting aggregation (41). The negatively charged surface of the nanoparticles contributes to the anionic capping agents, including polyphenols and flavonoids from blackcurrant and apricot pomace extracts, which are coordinated to the surface of the silver nanoparticles (24). Optimisation of the synthesis conditions is crucial to obtain AgNPs with the desired size and stability for specific applications. Previous studies have shown that both the concentration of silver nitrate and the pH of the reaction medium significantly influence the properties of the resulting nanoparticles. An increased pH accelerates the reduction of Aq⁺ ions, resulting in smaller AqNPs due to accelerated crystallisation. Additionally, a higher pH can weaken the aggregation of nanoparticles by fully charging the particle surface, and thus increasing electrostatic repulsion. Conversely, a higher AgNO₃ concentration can also lead to the formation of smaller nanoparticles (41).

Antimicrobial analysis of AgNPs

Organic nanoparticles provide thermal stability, tensile strength, extended shelf life and antibacterial properties (42). AgNPs have antibacterial activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains (39). Accordingly, this study showed that AgNPs obtained using rice husk and SCG exhibited similar antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 1 and Table 2). Silver nanoparticles obtained

from rice husks have antimicrobial activity against bacteria at a concentration of 400 mg/mL. The maximum zone of growth inhibition against *Staphylococcus aureus* and *Bacillus subtilis* is 10 and 12 mm, respectively, as shown in **Table 1**. The inhibition zone against *Pseudomonas aeruginosa* and *Escherichia coli* is 11 mm for both bacteria at 400 mg/mL. In addition, Ag-NPs obtained using spent coffee grounds (**Table 2**) have maximum antimicrobial activity against bacteria at a concentration of 400 mg/mL. The maximum growth inhibition zone against *S. aureus* and *B. subtilis* is 9 and 12 mm, respectively. The inhibition zones against *P. aeruginosa* and *E. coli* are 9 and 11 mm at 400 mg/mL.

The antimicrobial efficacy of AgNPs is significantly influenced by their physicochemical properties, including shape, size, concentration and colloidal state (43). These properties allow AgNPs to interact with or penetrate cell walls and membranes to exert their antimicrobial effect (39). In a recent study, rice husks were found to inhibit the growth of *S. aureus*, *Escherichia coli* and *Salmonella enterica*, demonstrating their potential as an antimicrobial agent (44). In addition, rice husk extract was effective against clinical strains of *S. aureus* isolated from skin wound infections (17,45). It has also been reported that SCG samples were more effective against Gram-positive than Gram-negative bacteria (46). The silver nanoparticles synthesised using both rice husks and SCG showed strong antimicrobial properties and their potential for biomedical applications (47,48).

Table 1. Antibacterial activity of silver nanoparticles (AgNP) obtained with the addition of rice husks against both Gram-positive and Gram-negative bacteria

Organism		γ/(mg/mL)						
	Gentamicin	Rice husk AgNP			Rice husk extract	AgNO ₃	Control	
		400	200	100	10	1.7		
	d(inhibition zone)/mm							
Pseudomonas aeruginosa	21.4±0.7	(10.4±0.7) ^a	(9.0±0.7) ^{ab}	(8.4±0.7) ^b	0	7.0±0.0	0	
Escherichia coli	20.1±0.2	(11.0±0.0) ^a	(9.0±0.0) ^{ab}	(8.0±0.0) ^b	0	7.0±0.0	0	
Staphylococcus aureus	21.0±0.0	(9.4±0.7)ª	(8.4±0.7) ^{ab}	(7.4±0.7) ^b	0	7.0±0.0	0	
Bacillus subtilis	22.0±0.0	(10.4±0.7) ^a	(9.4±0.7) ^{ab}	(9.4±0.7) ^b	0	7.4±0.7	0	

Data are shown as mean value \pm S.D. (N=3). Tukey's honestly significant difference (HSD) test indicates that different letters in superscript denote significant differences within the concentration at p<0.05

Table 2. Antibacterial activity	y of spent coffee ground silver nano	particles (AgNP) exhibited ag	gainst both Gram-positive and Gr	am-negative bacteria

Organism		γ/(mg/mL)						
	Gentamicin	Spent coffee grounds AgNP			Spent coffee ground extract	AgNO ₃	Control	
		400	200	100	10	1.7	-	
		d(inhibition zone)/mm						
Pseudomonas aeruginosa	21.4±0.7	(9.0±0.0) ^a	(8.0±0.0) ^b	(7.0±0.0) ^c	0	7.0±0.0	0	
Escherichia coli	20.0±0.0	(10.4±0.7) ^a	(8.0±0.7) ^b	(7.0±0.7) ^c	0	7.0±0.0	0	
Staphylococcus aureus	22.0±0.0	(9.4± 0.7)ª	(8.0±0.0) ^b	(7.0± 0.0) ^c	0	7.0±0.0	0	
Bacillus subtilis	22.0±0.0	(9.4±0.7) ^a	(8.4±0.7) ^b	(7.4± 0.7) ^c	0	7.4±0.7	0	

Data are shown as mean value \pm S.D. (N=3). Tukey's honestly significant difference (HSD) test shows that different letters in superscript represent significant differences within the concentration at p<0.05

CONCLUSIONS

In conclusion, this study successfully demonstrated a green synthesis method for the production of silver nanoparticles (AgNPs) using agricultural waste, namely spent coffee grounds (SCG) and rice husks. The results showed that both rice husk and SCG extracts act as reducing and stabilising agents by utilising their abundance in biomolecules, which includes flavonoids, phenols and other organic components. In the synthesis with both rice husks and SCG, AgNP production was confirmed by the significant colour change during the process and the appearance of surface plasmon resonance peaks at around 450 nm. The potential of these waste materials as bio-reductants was further demonstrated by FTIR analysis, which revealed the precise functional groups involved in the reduction and stabilisation processes. The crystalline face-centred cubic structure of the produced Ag-NPs, demonstrated by X-ray diffraction studies, is consistent with the known properties of silver nanoparticles. Zeta potential indicated stable nanoparticle dispersions, and particle analysis showed diameters of about 187 nm for AgNPs produced using SCG and 198 nm for AgNPs produced using rice husks. By recycling agricultural waste, this study demonstrates that rice husks and SCG can be used as affordable and sustainable precursors for AgNP synthesis, which is consistent with the ideas of circular economy. The produced AgNPs showed promising antibacterial properties, suggesting potential applications in the environmental and biomedical fields. The production of environmentally friendly, resource-efficient nanoparticles fosters green nanotechnology.

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CONFLICT OF INTEREST

The authors declare that they have no known financial or personal conflicts of interest that could have affected the work presented in this paper.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at www.ftbcom.hr.

AUTHORS' CONTRIBUTION

N. Nallusamy and N.A. Mohd Kamal Rufadzil contributed to the investigation, the writing of the original draft, and participated in reviewing and editing the manuscript. W.N.D. Wan Fauzi and H.D. Mohd Jefri were involved in the writing of the original draft, as well as reviewing and editing. J. Bala Murally and J.Z. Liam contributed to the investigation and the writing of the original draft. S. Ramakrishna and A.A.A. Amirul were responsible for the conceptualization, visualization, and supervision of the study. S. Vigneswari contributed to the conceptualization, visualization, project administration, and funding acquisition, as well as reviewing and editing the manuscript.

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REFERENCES

 Muthukumar J, Shukla G, Bhattacharyya S, Pathak R, Kumar L, Chidambaram R. Nanotechnology applications in food packaging. In: Sharma A, Vijayakumar PS, Prabhakar EPK, Kumar R, editors. Nanotechnology applications for food safety and quality monitoring. Cambridge, MA, USA: Academic Press; 2023. pp. 301–20.

https://doi.org/10.1016/b978-0-323-85791-8.00004-5

- Ritu, Verma KK, Das A, Chandra P. Phytochemical-based synthesis of silver nanoparticle: Mechanism and potential applications. J BioNanoSci. 2023;13(3):1359–80. https://doi.org/10.1007/s12668-023-01125-x
- Singh H, Desimone MF, Pandya S, Jasani S, George N, Adnan M, et al. Revisiting the green synthesis of nanoparticles: Uncovering influences of plant extracts as reducing agents for enhanced synthesis efficiency and its biomedical applications. Int J Nanomed. 2023;18:4727–50. https://doi.org/10.2147/ijn.s419369
- 4. Singh S, Tiwari H, Verma A, Gupta P, Chattopadhaya A, Singh A, et al. Sustainable synthesis of novel green-based nanoparticles for therapeutic interventions and environmental remediation. ACS Synth Biol. 2024;13(7):1994–2007. https://doi.org/10.1021/acssynbio.4c00206
- Omran BA, Baek KH. Valorization of agro-industrial biowaste to green nanomaterials for wastewater treatment: Approaching green chemistry and circular economy principles. J Environ Manag. 2022;311:114806.
 https://doi.org/10.1016/j.jopumap.2022.114806

https://doi.org/10.1016/j.jenvman.2022.114806

- Mat Yusuf SNA, Mood CNAC, Ahmad NH, Sandai D, Lee CK, Lim V. Optimization of biogenic synthesis of silver nanoparticles from flavonoid-rich *Clinacanthus nutans* leaf and stem aqueous extracts. Roy Soc Open Sci. 2020;7(7):200065. https://doi.org/10.1098/rsos.200065
- 7. Mir RH, Maqbool M, Mir PA, Hussain MS, Wani SUD, Pottoo FH, Mohi-Ud-Din R. Green synthesis of silver nanoparticles and their potential applications in mitigating cancer.

Curr Pharm Des. 2024;30(31):2445–67.

https://doi.org/10.2174/0113816128291705240428060456

- Tarigan EB, Wardiana E, Hilmi YS, Komarudin NA. The changes in chemical properties of coffee during roasting: A review. IOP Conf Ser: Earth Environ Sci. 2022;974:012115. https://doi.org/10.1088/1755-1315/974/1/012115
- Freitas VV, Borges LLR, Vidigal MCTR, dos Santos MH, Stringheta PC. Coffee: A comprehensive overview of origin, market, and the quality process. Trends Food Sci Technol. 2024;146:104411.

https://doi.org/10.1016/j.tifs.2024.104411

 Arya SS, Venkatram R, More PR, Vijayan P. The wastes of coffee bean processing for utilization in food: A review. J Food Sci Technol. 2022;59(2):429–44.

https://doi.org/10.1007/s13197-021-05032-5

 Chen XE, Mangindaan D, Chien HW. Green sustainable photothermal materials by spent coffee grounds. J Taiwan Inst Chem Eng. 2022;137:104259.

https://doi.org/10.1016/j.jtice.2022.104259

 Ashihara H. Biosynthetic pathways of purine and pyridine alkaloids in coffee plants. Nat Prod Commun.2016;11(7): 1047–54.

https://doi.org/10.1177/1934578x1601100742

 Kordi M, Farrokhi N, Pech-Canul MI, Ahmadikhah A. Rice husk at a glance: From agro-industrial to modern applications. Rice Sci. 2023;31(1):14–32.
https://doi.org/10.1016/j.regi.2023.08.005

https://doi.org/10.1016/j.rsci.2023.08.005

14. Taiye MA, Hafida W, Kong F, Zhou C. A review of the use of rice husk silica as a sustainable alternative to traditional silica sources in various applications. Environ Prog Sustain Energy. 2024;43(6):e14451.

https://doi.org/10.1002/ep.14451

 Liu YS, Chang YC, Chen HH. Silver nanoparticle biosynthesis by using phenolic acids in rice husk extract as reducing agents and dispersants. J Food Drug Anal. 2018;26(2): 649–56.

https://doi.org/10.1016/j.jfda.2017.07.005

16. Maity A, Nath P, Bagchi D, Debnath S, Tabassum H, Sinha S, Chakraborty A. Formation of lipid corona on Ag nanoparticles and its impact on Ag⁺ ion dissolution and aggregation of Ag nanoparticles against external stimuli. Chem Phys Impact. 2024;8:100445.

https://doi.org/10.1016/j.chphi.2023.100445

 Burlacchini G, Sandri A, Papetti A, Frosi I, Boschi F, Lleo MM, Signoretto C. Evaluation of antibacterial and antibiofilm activity of rice husk extract against *Staphylococcus aureus*. Pathogens. 2024;13(1):80.

https://doi.org/10.3390/pathogens13010080

18. Mouriya GK, Mohammed M, Azmi AAA, Khairul WM, Karunakaran T, Amirul AA, *et al*. Green synthesis of *Cicer arietinum* waste derived silver nanoparticle for antimicrobial and cytotoxicity properties. Biocatal Agric Biotechnol. 2023; 47:102573.

https://doi.org/10.1016/j.bcab.2022.102573

- IBM SPSS Statistics for Windows, v. 29.0.2.0, IBM Corp, Armonk, NY, USA; 2023. Available from: https://www.ibm. com/support/pages/downloading-ibm-spss-statistics-29020.
- Vigneswari S, Chai JM, Kamarudin KH, Amirul AAA, Focarete ML, Ramakrishna S. Elucidating the surface functionality of biomimetic RGD peptides immobilized on nano-P(3HB-co-4HB) for H9c2 myoblast cell proliferation. Front Bioeng Biotechnol.2020;8:567693.

https://doi.org/10.3389/fbioe.2020.567693

- 21. Liaqat N, Jahan N, Khalil-Ur-Rahman N, Anwar T, Qureshi H. Green synthesized silver nanoparticles: Optimization, characterization, antimicrobial activity, and cytotoxicity study by hemolysis assay. Front Chem. 2022;10:952006. https://doi.org/10.3389/fchem.2022.952006
- 22. Sahu SK, Kushwaha A, Pradhan U, Majhi P, Shukla AK, Ghorai TK. Sustainable green synthesis of *Hedychium coronarium* leaf extract-stabilized silver nanoparticles and their applications: colorimetric sensing of Sn²⁺ and Hg²⁺, record antifungal and antimicrobial properties. Nanoscale Adv. 2024;6(21):5361–74.

https://doi.org/10.1039/d4na00443d

23. Villagrán Z, Anaya-Esparza LM, Velázquez-Carriles CA, Silva-Jara JM, Ruvalcaba-Gómez JM, Aurora-Vigo EF, et al. Plant-Based extracts as reducing, capping, and stabilizing agents for the green synthesis of inorganic nanoparticles. Resources. 2024;13(6):70.

https://doi.org/10.3390/resources13060070

24. Soyucok A, Kabak B, Tosun B. Optimization of synthesis reaction parameters of AgNPs derived from *Laser trilobum* plant for foodborne pathogens. Food Bioprocess Technol. 2024;17:3800–12.

https://doi.org/10.1007/s11947-024-03359-3

- 25. Vasyliev G, Vorobyova V, Skiba M, Khrokalo L. Green synthesis of silver nanoparticles using waste products (apricot and black currant pomace) aqueous extracts and their characterization. Adv Mater Sci Eng. 2020;2020:4505787. https://doi.org/10.1155/2020/4505787
- Krakowska-Sieprawska A, Kiełbasa A, Rafińska K, Ligor M, Buszewski B. Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. Molecules. 2022;27(3):730.

https://doi.org/10.3390/molecules27030730

27. Mota DR, Da Silva Martini W, Pellosi DS. Influence of Ag size and shape in dye photodegradation using silver nanoparticle/ZnO nanohybrids and polychromatic light. Environ Sci Pollut Res. 2023;30(20):57667–82.

https://doi.org/10.1007/s11356-023-26580-7

28. Mohamed T, El-Motlak MH, Mamdouh S, Ashour M, Ahmed H, Qayyum H, Mahmoud A. Excitation wavelength and

colloids concentration-dependent nonlinear optical properties of silver nanoparticles synthesized by laser ablation. Materials. 2022;15(20):7348.

https://doi.org/10.3390/ma15207348

- 29. Abdelmigid HM, Morsi MM, Hussien NA, Alyamani AA, Sufyani NMA. Comparative analysis of nanosilver particles synthesized by different approaches and their antimicrobial efficacy. J Nanomater. 2021;2021:2204776. https://doi.org/10.1155/2021/2204776
- Zuorro A, Iannone A, Miglietta S, Lavecchia R. Green synthesis of silver nanoparticles using spent coffee ground extracts: process modelling and optimization. Nanomaterials. 2022;12(15):2597.

https://doi.org/10.3390/nano12152597

31. Torres-Rivero K, Bastos-Arrieta J, Fiol N, Florido A. Metal and metal oxide nanoparticles: An integrated perspective of the green synthesis methods by natural products and waste valorization: Applications and challenges. In: Verma SK, Das AK, editors. Comprehensive Analytical Chemistry. Biosynthesized Nanomaterials, vol. 94. Amsterdam, The Netherlands: Elsevier; 2021. pp. 433–69.

https://doi.org/10.1016/bs.coac.2020.12.001

- *32.* Anguraj A, Michael HSR, Sugumaran S, Madhusudhanan GR, Sivaraman RK. A comparative study on biosynthesized silver nanoparticles from *H. undatus* fruit peel and their therapeutic applications. Discover Nano. 2024;19:49. https://doi.org/10.1186/s11671-024-03995-w
- 33. Alkhulaifi MM, Alshehri JH, Alwehaibi MA, Awad MA, Al-Enazi NM, Aldosari NS, *et al*. Green synthesis of silver nanoparticles using *Citrus limon* peels and evaluation of their antibacterial and cytotoxic properties. Saudi J Biol Sci. 2020;27(12):3434–41.

https://doi.org/10.1016/j.sjbs.2020.09.031

34. Yadav AK, Singh S, Sen R, Jha P, Tungare K, Bhori M, et al. Biosorption and bioreduction of Cr (VI) by rice husk and toxicity analysis on zebrafish embryos. Int J Environ Sci Technol. 2023;20(1):397–410.

https://doi.org/10.1007/s13762-021-03826-w

35. Kokila T, Ramesh PS, Geetha D. Biosynthesis of silver nanoparticles from Cavendish banana peel extract and its antibacterial and free radical scavenging assay: A novel biological approach. Appl Nanosci 2015;5:911–20. https://doi.org/10.1007/c12204_015_0401_2

https://doi.org/10.1007/s13204-015-0401-2

 Wattananon S, Narakaew S, Chaisena A. Molecularly imprinted polymers based on konjac for selective caffeine adsorption in aqueous solution. J Turk Chem Soc A: Chem. 2023;10(2):359–70.

https://doi.org/10.18596/jotcsa.1194200

37. Suman TY, Radhika Rajasree SR, Kanchana A, Elizabeth SB. Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using *Morinda citrifolia* root extract. Colloid Surface B. 2013;106:74–8.

https://doi.org/10.1016/j.colsurfb.2013.01.037

- Khan MZ, Tareq FK, Hossen MA, Roki M. Green synthesis and characterization of silver nanoparticles using *Coriandrum* sativum leaf extract. J Eng Sci Technol. 2018;13(1):158–66.
- 39. De Oliveira PFM, Torresi RM, Emmerling F, Camargo PHC. Challenges and opportunities in the bottom-up mechanochemical synthesis of noble metal nanoparticles. J Mater Chem A. 2020; 8(32):16114–41.

https://doi.org/10.1039/d0ta05183g

- 40. Renuka R, Thilagavathi T, Dhanalakshmi A, Uthrakumar R, Kaviyarasu K, Yewale MA, *et al.* Biological synthesis of silver nanoparticles using *Senna auriculata* flower extract for antibacterial activities. Luminescence. 2024;39(9):e4894. https://doi.org/10.1002/bio.4894
- Bruna T, Maldonado-Bravo F, Jara P, Caro N. Silver nanoparticles and their antibacterial applications. Int J Mol Sci. 2021;22(13):7202.

https://doi.org/10.3390/ijms22137202

- 42. Qiao Y, Kang J, Song C, Zhou N, Zhang P, Song G. Further study on particle size, stability, and complexation of silver nanoparticles under the composite effect of bovine serum protein and humic acid. RSC Adv. 2024;14(4):2621–32. https://doi.org/10.1039/d3ra06159k
- 43. Mulenos MR, Lujan H, Pitts LR, Sayes CM. Silver nanoparticles agglomerate intracellularly depending on the stabilizing agent: Implications for nanomedicine efficacy. Nanomaterials. 2020;10(10):1953.

https://doi.org/10.3390/nano10101953

- 44. Dar P, Waqas U, Hina A, Anwar J, Dar A, Khan Z, Shafqat T. Biogenic synthesis, characterization of silver nanoparticles using *Multani mitti (Fullers Earth)*, tomato (*Solanum lycopersicum*) seeds, rice husk (*Oryza sativa*) and evaluation of their potential antimicrobial activity. J Chem Soc Pak. 2016:38(4): 665–74.
- 45. Flores-Contreras EA, González-González RB, Pizaña-Aranda JJP, Parra-Arroyo L, Rodríguez-Aguayo AA, Iñiguez-Moreno M, et al. Agricultural waste as a sustainable source for nanoparticle synthesis and their antimicrobial properties for food preservation. Front Nanotechnol. 2024;6:1346069. https://doi.org/10.3389/fnano.2024.1346069
- 46. Monente C, Bravo J, Vitas AI, Arbillaga L, De Peña MP, Cid C. Coffee and spent coffee extracts protect against cell mutagens and inhibit growth of food-borne pathogen microorganisms. J Funct Food. 2015;12:365–74. https://doi.org/10.1016/j.jff.2014.12.006
- Muriady, Meilina H, Faisal M. Antibacterial activity of liquid smoke powder from rice husk. Int J GEOMATE. 2022;23(95): 89–96.

https://doi.org/10.21660/2022.95.7522

 Naganthran A, Verasoundarapandian G, Khalid FE, Masarudin MJ, Zulkharnain A, Nawawi NM, *et al.* Synthesis, characterization and biomedical application of silver nanoparticles. Materials. 2022;15(2):427.

https://doi.org/10.3390/ma15020427