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BIOSYNTHESIS OF SILVER NANOPARTICLES USING PALM OIL MILL EFFLUENT (POME) EXTRACT

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ABSTRACT

Silver nanoparticles (AgNP) possess broad biocidal activities, making them ideal for antibacterial, antiviral and anti-inflammatory formulations. A green synthesis method using palm oil mill effluent (POME) extract as both a stabilising and reducing agent was developed, eliminating the need for hazardous chemicals, with a 1.5 mM $AgNO_3$ solution used as the precursor for the synthesis. Preliminary profiling of POME extract was performed, including quantification of total phenolic, flavonoid and tannin contents. The synthesis was optimised using one factor at a time (OFAT) approach and characterised with vis-spectroscopy, fourier transform infrared (FTIR) and dynamic light scattering (DLS). Liquid Chromatography-Mass Spectrometry (LC-MS) was used to identify organic compounds containing hydroxyl, carbonyl and amine groups as possible reducing agents. Under optimised conditions of 6 mL extract and 1.5 mM $AgNO_3$ incubated at room temperature for 14 hr, AgNP exhibited a surface plasmon resonance (SPR) peak at 440 nm. FTIR spectra revealed significant hydroxyl and carboxyl groups essential for reducing Ag^+ to Ag^0. The synthesised POME-AgNP were crystalline, spherical in shape and 21 nm average size, with a low polydispersity index (PdI = 0.295) indicating monodispersity. A zeta potential of -19.3 mV demonstrated good stability against agglomeration and oxidation. Overall, the green AgNP synthesis method using POME extract proved successful, offering a sustainable alternative to conventional chemical and physical methods.

Keywords: bioactive compounds, biosynthesis, LC-MS, POME, silver nanoparticles.

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INTRODUCTION

Nanotechnology offers a transformative approach to advancing technology across various disciplines, including science, medicine and engineering. Among nanomaterials, metallic nanoparticles and their composites are broadly explored for various applications due to their exceptional properties. These include enhanced mechanical and thermal stability, a high surface-to-volume ratio and an abundance of reactive sites on their surfaces, making them highly desirable for a wide range of applications (Alvarez *et al.*, 2018; Haleem *et al.*, 2023; Liu *et al.*, 2023; Savolainen *et al.*, 2010). With growing awareness of the environmental impact of traditional manufacturing processes, there is an increasing demand for sustainable and eco-friendly methods for nanoparticle synthesis and application. Green synthesis addresses this need by offering a range of techniques that minimise the use of hazardous chemicals, reduce energy consumption and promote the use of renewable

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resources. This approach has the potential to revolutionise various sectors and provide sustainable solutions to pressing global challenges (Barabadi *et al.*, 2021; Vankudoth *et al.*, 2022).

In the energy sector, metallic nanoparticles offer significant opportunities, such as the use of nanomaterials in solar cells to enhance light absorption and improve energy conversion efficiency, leading to cleaner and more efficient energy sources (Dastafkan *et al.*, 2015; Dolatabadi *et al.*, 2022). In medicine, it enables the production of biocompatible nanoparticles for drug delivery, diagnostics and therapeutics, reducing toxicity and enhancing efficacy (Kumari *et al.*, 2021a; Wasilewska *et al.*, 2023). For environmental remediation, green-synthesised nanoparticles can be applied in water purification, pollutant detection and the cleanup of contaminated sites (Zahoor *et al.*, 2021). Additionally, in agriculture, green nanotechnology contributes to crop improvement, pest control and nutrient delivery systems (Acharya & Pal, 2020).

Among emerging metallic nanomaterials, silver nanoparticles (AgNP) have gained significant interest due to their outstanding physicochemical properties (De Matteis *et al.*, 2023). Their unique surface chemistry, composition, morphology, shape, size distribution and reactivity in solution make them highly desirable for a range of applications, including as antioxidants, anticancer agents, antimicrobials, larvicides and in the degradation of dyes for water treatment (Jaast & Grewal, 2021; Mondal *et al.*, 2024). Typical method used to synthesise AgNP mainly uses chemical agents that are hazardous and toxic, which adversely affect our environment (Iravani *et al.*, 2014). Other than toxic side effects, chemical methods commonly use volatile chemical reactants that are very hazardous to the environment. The reducing agents such as sodium borohydrate and hydrazine derivatives, are highly noxious, causing air pollution. Other examples include volatile solvents like aromatic amines and thiols that have high vapour pressure (Zhang *et al.*, 2021). The presence of chemical substances on the surface of AgNP can compromise their effectiveness and hinder their biocompatibility as such in future medical applications. Thus, there is a pressing need to adopt environmentally friendly techniques that harness the power of renewable resources to ensure the sustainable use of AgNP (Wasilewska *et al.*, 2023). Recently, there has been a growing demand for safe, eco-friendly and non-toxic approaches to synthesise AgNP of various sizes and morphology while minimising toxicity (Abdullah *et al.*, 2021).

In the green synthesis method of AgNP, biological sources such as bacteria, fungi, algae and plants have previously been reported as advantageous to mediate the reduction of silver ions (Ag^+) to AgNP. The use of bacteria, fungi, and algae has been reported previously by Aziz *et al.* (2015), Murugesan *et al.* (2017) and Uzair *et al.* (2020) but has encountered challenges in achieving good control over the size distribution, shape and crystallinity of the nanoparticles (Mukherji *et al.*, 2019). Moreover, the steps are time-consuming, and require substantial aseptic conditions and maintenance, limiting their suitability for industrial applications (Yahya & Alharbi, 2023).

In contrast, plant-mediated synthesis of AgNP is comparatively simpler and more cost-efficient. Various phytochemicals from various plant species and plant parts including leaves, vegetables, oilseed, barks, roots and fruits aid the reduction process of metal ions. Notably, fruit peels such as banana (Cheong *et al.*, 2022; Teo *et al.*, 2023), citrus (Khane *et al.*, 2022; Nahar *et al.*, 2021) and dragon fruit (Phongtongpasuk & Poadang, 2016) have demonstrated the ability to produce biocompatible AgNP. These plants produce biomolecules such as steroids, sapogenins, tannins, terpenoids, polyols, alkaloids, polysaccharides, flavonoids, phenolics, proteins, amino acids, enzymes and vitamins that function as strong chelating, reducing and stabilising agents for the AgNP synthesis (Asmat-Campos *et al.*, 2020; Githala & Trivedi, 2023).

Recently, there has been a pressing need to exploit agro-industrial biowaste for materials synthesis to mitigate environmental pollution and promote sustainability. Exploiting agro-industrial waste to produce profitable high-value products facilitates the implementation of a circular economy, which in turn helps reduce the amount of waste generated. Among such agro-industrial waste is palm oil mill effluent (POME), which is a by-product generated during oil extraction from fresh fruit bunches (FFB) in palm oil mill industries. It is estimated that about 3 t of POME are produced for every tonnes of crude palm oil, with Malaysia alone processing over 90 million tonnes of FFB annually (Mahmod *et al.*, 2023; Tang *et al.*, 2024). Managing POME poses a significant challenge for the palm oil milling industry due to its high concentration of pollutants and other harmful substances, which can adversely affect aquatic life. The brown colour of POME was known to have high levels of phenolic compounds, fatty acids, polyphenols and proteins (Chantho *et al.*, 2016).

To the best of our knowledge, the utilisation of POME in mediating AgNP formation has been relatively unexplored until now, unlike its role in forming other metallic nanoparticles such as gold nanoparticles (AuNP), in which hydroxyl and carbonyl functional groups served as reducing and capping agents. This study aims to identify

compounds containing hydroxyl and carboxyl functional groups present in POME extract that are involved in the biosynthesis of AgNP without the addition of external surfactant or capping agent. Synthesis parameters were optimised using one factor at a time (OFAT) approach. The successful synthesis was confirmed using various analytical techniques including vis-spectroscopy, Fourier transform infrared (FTIR) spectroscopy and dynamic light scattering (DLS).

MATERIALS AND METHODS

POME Collection and Extraction

Raw POME was obtained from the Maokil Palm Oil Mill in Labis, Johor, Malaysia, and was dried at 60°C for 24 hr using a glass container. Subsequently, the raw material was scraped, ground to powder and sieved (Gan *et al.*, 2012). The 2% POME powder was dissolved in 100 mL of deionised (DI) water, stirred for 15 min at 90°C and filtered using Whatman filter paper to obtain pure POME extract. The extracted POME was kept in a refrigerator at 4°C for further analysis. The schematic process of extraction is shown in *Figure 1.* The extract was used to quantify total phenolic, flavonoid and tannin content and synthesise AgNP. For liquid chromatography-mass spectroscopy (LC-MS) analysis, 2 g of POME powder was added to 100 mL of methanol and stirred overnight and the prepared sample was submitted for analysis.

Profiling of Organic Compounds in POME Extract using LC-MS

Water extract of POME was characterised using an LC-MS instrument from Hewlett Packard (Agilent Technologies, Waldbronn, Germany) to examine the chromatographic profiles of organic compounds in POME extract. The chromatographic system consists of a normal phase. The mobile phase comprises 0.1% formic acid (v/v) (A), while the aqueous organic phase contains 0.1% formic acid (v/v) (B). The analysis was conducted using C18 column (150.0 × 2.1 mm), with a vacuum pump flow velocity of 1 mL/min and temperature of 40°C. The eluent gradient was set from 10.0%-30.0% B in 20 min, followed by 30.0% B in 20-30 min, with a 0.8 mL/min flow rate. Positive ion mode of electrospray ionisation-mass spectrometry (ESI-MS) was used to monitor the eluent, scanning from m/z 110-810. An electrospray ionisation (ESI) was performed at 3.5 kV with an optimum collision energy of 60.0%. At a flow rate of 12 L/min and a capillary temperature of 350°C, high-purity nitrogen was used as the dry gas, while nitrogen was used as the nebuliser at 40/inch^2. The compounds from POME extract that could potentially act as reducing and stabilising agents in the biosynthesis of AgNP were chosen using the available reference library (Metlin_Metabolites_AM_PCDL.cdb). The compounds exhibiting the best match percentage with a database score above 70.0%, along with hydroxyl and carboxyl functional were recorded.

Total Phenolic Compound (TPC)

The TPC of aqueous POME is to assess the concentration of phenolic compounds in a sample (Baba & Malik, 2015). A 1.00 mL of POME extract sample, standard calibration solutions containing different concentrations of gallic acid (0.00, 0.02, 0.04, 0.06, 0.08, 1.00 and 1.20 mg/mL), 0.50 mL of Folin-Ciocalteu (FC) reagent and 5.00 mL of DI water were mixed and centrifuged in a 15.00 mL tube for 5 min. Subsequently, 1.50 mL of 20% Na_2CO_3 was added, and the final volume was adjusted to 10 mL with DI water. The absorbance was recorded after 30 min of incubation at 40°C using a visible spectrophotometer at 750 nm. These analyses were conducted in triplicate and

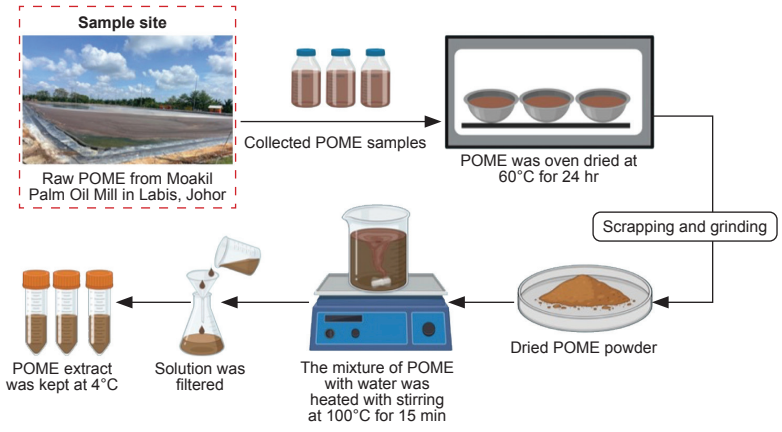


Figure 1. Schematic process of extraction using palm oil mill effluent (POME).

linear regression depicts the correlation coefficient, r^2>0.95. The total phenolic contents were estimated as gallic acid equivalents (mg GAE/g).

Total Flavonoid Content (TFC)

The assessment of the TFC of aqueous POME was conducted as described by Chantiratikul *et al.* (2009). Quercetin standard concentrations (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/mL) were used to make a standard calibration curve. The sample (1.0 mL) was placed in a 15.0 mL centrifuge tube, followed by 4.0 mL DI water. Subsequently, 0.3 mL of 5% sodium nitrate was added to the solution and allowed to stand for 5 min before adding 0.3 mL of 10% aluminium chloride solution and 2.0 mL of 1 M sodium hydroxide. Subsequently, the mixture was adjusted to 10.0 mL with DI water. Finally, the absorbance was measured using a Jenway 7200 visible spectrophotometer at 510 nm. TFC was measured as quercetin equivalent (mg QE/g). All measurements were performed at least three times.

Total Tannin Content (TTC)

The TTC of POME was spectrophotometrically determined by FC phenol reagent assay to determine the total tannin contents according to (Haile & Kang, 2019). POME (1.0 mL) extract was mixed with 7.5 mL DI water in a falcon tube, followed by the addition of 0.5 mL of FC phenol reagent and 1.0 mL of Na_2CO_3. DI (1.0 mL) water was added to achieve a total volume of 10.0 mL. The mixture was vortexed and shaken vigorously using a vortex mixer, and the homogeneous mixture was wrapped with aluminium foil and kept at room temperature for 30 min. Standard solutions of tannic acid (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg/mL) were prepared, and the absorbance was measured at 760 nm using a visible spectrophotometer. TTC in POME was expressed as mg of tannic acid equivalents (mg TAE/g).

Optimising AgNP Synthesis Parameters using OFAT

POME-AgNP were synthesised by incubating POME extract with silver nitrate ($AgNO_3$) solutions at different temperatures. The results will provide an overview of the ideal conditions for synthesising AgNP under optimised conditions. OFAT was then employed to explore optimal levels of operating conditions that positively influenced AgNP formation. The study focuses on the effect of four variables, such as the volume of POME extract, $AgNO_3$, temperature and pH of the extract. For each 15 mL test tube, 10 mL of $AgNO_3$ at 1.0 mM concentration was used. The tubes were incubated for 24 hr at specific temperatures (30°C, 40°C, 50°C, 60°C, 70°C and 80°C) and contained different volumes (0.2, 0.4, 0.6, 0.8 and 10.0 mL) of plant extract at specific pH levels (4, 5, 6, 7, 8, 9 and 10). The pH of the experiment was adjusted to 4, 5, 6, 8, 9 and 10 using 0.1 M HCl and 0.1 M NaOH. The absorbance, indicating surface plasmon resonance (SPR), was measured using a vis-spectrophotometer across a wavelength range of 350-750 nm. Detailed information on the experimental setup is provided in *Table 1*.

Characterisation of POME-AgNP

The confirmation of biosynthesised POME-AgNP formation was determined using vis-spectroscopy (Jenway 7200). The SPR was examined from 350-750 wavelength (nm). The surface functional group of biomolecules was investigated using FTIR (Nicolet IS5 IR, Thermo Scientific, US) spectroscopy in the range of 400-4,000 cm^{-1}. The size distribution and zeta potential of POME-AgNP were analysed using a Zetasizer Ver. 7.11 (Malvern DLS, UK) through DLS. To prevent aggregation, the samples were diluted before analysing particle size distribution and zeta potential at 25°C.

RESULTS AND DISCUSSION

Organic Compounds Identification in POME Extract

LC-MS analysis revealed that major organic compounds of POME extract contain alkaloids, phospholipids, fatty acids, sphingolipids, saponins, 1,2-aminoalcohols and ketones. Among the organic compounds identified include

TABLE 1. THE DESIGN STUDY OF DIFFERENT SYNTHESIS PARAMETERS OF AgNP USING POME

Parameter	Volume of POME (mL)	$AgNO_3$ (mM)	Temperature (°C)	Extract pH
Volume of extract	0.2, 0.4, 0.6, 0.8, 10.0	1.0	30	7
Extract pH	0.6	1.0	30	4, 5, 6, 7,8, 9, 10
$AgNO_3$	0.6	0.5, 1.0, 1.5, 2.0, 2.5, 3.0	30	7
Temperature	0.6	1.0	30, 40, 50, 60, 70, 80	7

Note: POME - palm oil mill effluent; $AgNO_3$ - silver nitrate.

anhalamine, hebevinoside VII, aminopentol, camptothecin, safingol, stearic acid, palmitic acid and myristone as shown in *Table 2*. These constituents could potentially act as reducing agents in the biosynthesis of AgNP. It is deduced that the AgNP may be capped and stabilised by hydroxyl (OH), carboxyl (-COOH) and amine (-NH_2) functional groups from POME, such as alkaloid through the interaction of amine group, or fatty acids and phospholipids through OH and carboxy (C=O), or amine (N-H). Gan *et al.* (2012) reported similar findings and deduced the capping and stabilisation involvement of OH, C=O, and H-H functional groups from proteins and polyphenols.

The classes of organic compounds portrayed in this study was also found present in numerous plant extracts as depicted in *Table 3*. Their potential to serve as reducing and stabilising agents in AgNP synthesis not only relate with OH, N-H_2, and C=O but also possible interaction with C=C, C-H, -COOH, C-O-C, -$COCH_3$, -COO and NH_2 (*Table 3*). Stearic acid, one of the compounds identified in POME extract belongs to the saturated long-chain fatty acids group. It plays a crucial role in the biosynthesis of AgNP by forming a long hydrocarbon chain that attached to the surface of AgNP, creating a protective layer. This layer could effectively prevent aggregation and reaction with other substances. Grizzo *et al.* (2023) have demonstrated that stearic acid enhances the formation of AgNP on graphene and improves the surface wetting of the nanoparticles when combined in a composite with polylactic acid (PLA). Similarly, it also increased the atomic concentration of AgNP within the composite, emphasising its role in promoting nanoparticle formation (Grizzo *et al.*, 2023). On the other hand camptothecin, an alkaloid identified in POME is not commonly found in the effluent, which primarily consists of degradable organic matter such as water, unrecovered oil, free fatty acids (FFA), starches, proteins and non-toxic plant tissues. This compound likely originates from microorganisms that are present in POME aeration ponds during the aerobic digestion process (Mohammad *et al.*, 2021).

Total Phenolic, Flavonoids and Tannin Contents

In this study, total phenolic, flavonoids and tannin contents of POME were quantified using three different assays, namely, TPC, TFC and TTC. Results in *Figure 2* showed that flavonoids content was the highest compared to tannins and phenolics. Quantitatively, the concentrations of phenols, flavonoids and tannins in 2 g of dried POME were 75 ± 4.10, 102 ± 3.60 and 83 ± 1.89 mg/g as depicted in *Figure 2*.

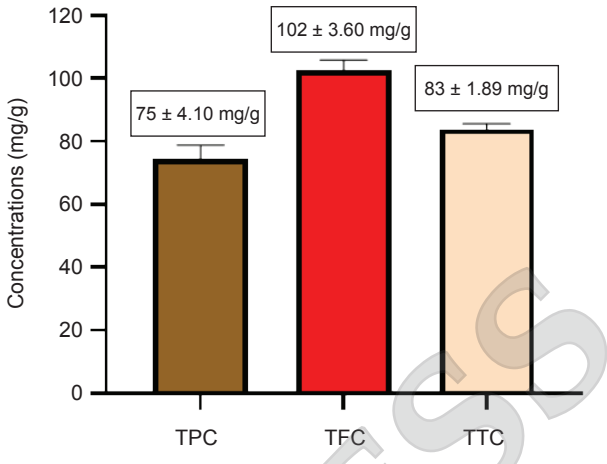


Figure 2. Total phenolic (TPC), flavonoid (TFC) and tannin content (TTC) of aqueous palm oil mill effluent (POME).

The increased flavonoid content is likely due to a higher concentration of organic compounds belonging to the flavonoid groups in POME. Among the identified organic compounds in POME are palmitic acid, stearic acid, anhalamine, hebevinoside VII and aminopentol. These compounds are potential agents that play a role in both reducing and stabilising the nanoparticles during the green synthesis of AgNP (Asmat-Campos *et al.*, 2020).

Screening Process of Synthesis Parameters using OFAT Approach

The production of AgNP is affected by several factors, such as the volume of extract, $AgNO_3$ concentration, temperature and pH, which can be adjusted to control the size, shape, morphology, efficacy and applicability of the nanoparticles. The absorption peak spectrum for AgNP formation ranges from 410-500 nm (Dhaka *et al.*, 2023). In this study, the visible spectrum between 400 and 500 nm was used to verify the formation of AgNP and assess the effects of different factors such as volume of extract, temperature, pH and $AgNO_3$ concentration on the POME-AgNP synthesis.

Effect of Extract Volume

The organic compounds within the extract are crucial for the synthesis of AgNP. Therefore, varying volumes of POME extract were investigated to achieve a stable synthesis of POME-AgNP. It was observed that increasing the extract volume from 0.2-0.6 mL corresponded to an increase in the absorption band intensity, as depicted in *Figure 3a*. This enhances the participation of organic compounds in the reduction and stabilisation of the synthesised AgNP. However, the increase of the extract volume above 0.6 mL led to shifts in the absorption intensity and increased noise in the spectrum, which could

TABLE 2. SELECTED ORGANIC COMPOUNDS IDENTIFIED USING LC-MS ANALYSIS

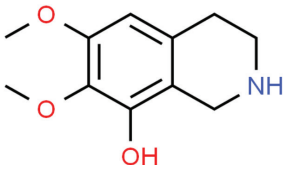
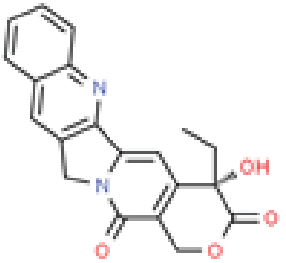
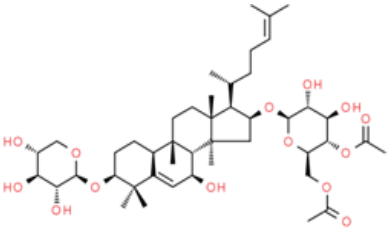
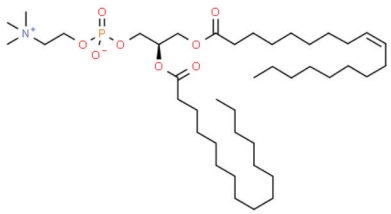
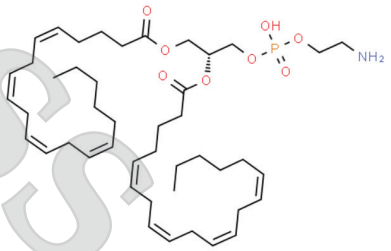
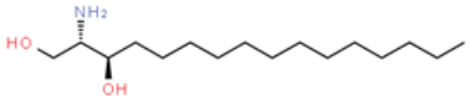
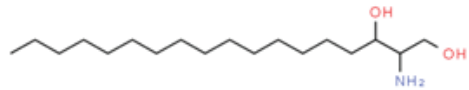
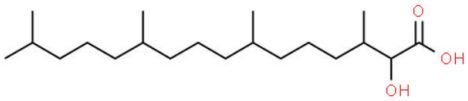
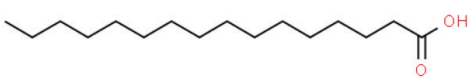
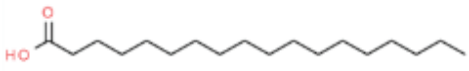
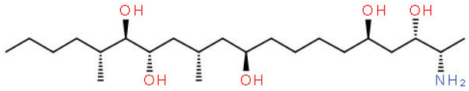
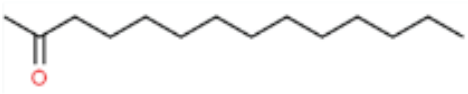
Compound	Rt	Molecular formula	Mass	Class	Functional groups	Structural molecules
Anhalamine	6.649	$C_{11}H_{15}N_{03}$	209.1050	Alkaloid	OH and NH	
Camptothecin	14.153	$C_{20}H_{16}N_2O_4$	348.1098	Alkaloid	-COO and OH	
Hebevinoside VII	8.780	$C_{45}H_{72}O_{14}$	836.4956	Saponins	-OH, C-O-C and -$COCH_3$	
1-Oleoyl-2-palmitoyl-sn-glycero-3-PC	7.142	$C_{45}H_{74}NO_8P$	787.5150	Phospholipids	-COOH, -COOR and -$N^+(CH_3)_3$	
Phosphatidylethanolamine (40:8)	8.643	$C_{45}H_{74}O_{14}$	787.5146	Phospholipids	-PO_4, OH, NH_2 and -COOH	

TABLE 2. SELECTED ORGANIC COMPOUNDS IDENTIFIED USING LC-MS ANALYSIS (continued)

Compound	Rt	Molecular formula	Mass	Class	Functional groups	Structural molecules
C_{16} Sphinganine	12.037	$C_{16}H_{35}NO_2$	273.2661	Sphingolipids	-OH and -NH_2	
Safingol	12.633	$C_{18}H_{39}NO_2$	301.2970	Sphingolipids	-OH and -NH_2	
2-hydroxyphytanic acid	11.635	$C_{20}H_{40}O_3$	328.2968	Fatty Acids	-COOH and -OH	
Palmitic acid	12.037	$C_{16}H_{32}O_2$	256.2396	Fatty Acids	-COOH	
Stearic acid	12.633	$C_{18}H_{36}O_2$	284.2705	Fatty acids	-COOH	
Aminopentol	12.153	$C_{22}H_{12}O_3$	192.0791	1,2-aminoalcohols	-OH and -NH_2	
Myristone	12.124	$C_{14}H_{28}O$	212.2138	Ketone	-CHO and C=O	

Note: OH - hydroxy; -OH - hydroxyl; NH - secondary amine; -COO - carboxylate; C-O-C - ether; -COOH - carboxylic acid; -CHO - aldehyde; -NH_2 - primary amine; -PO_4 - phosphate; -$COCH_3$ - acetyl; COOR - ester; $N^+(CH_3)_3$ - quaternary ammonium.

TABLE 3. PLANTS SOURCE AND PHYTOCHEMICALS INVOLVED IN GREEN SYNTHESIS OF AgNPS WITH DIFFERENT FUNCTIONAL GROUPS

Source of extract	AgNPs-based	Plant parts	Functional groups	Reference
Mussaenda frondose leaf extract	Chitosan/Gelatin@Ag nanocomposites film	Leaves	-CO-NH_2	Ediyilyam *et al.* (2021)
Dioscorea altissima (dunguey)	AgNPs incorporated nanofilm	Tuber	OH-group of starch	Silva *et al.* (2021)
Pomegranate and citrus fruit peel	AgNPs on cellulose wrapper	Fruit peel	OH in phenols and flavonoids groups	Gopalakrishnan *et al.* (2023)
Azadirachta indica leaf extract	AgNPs@microcrystalline cellulose	Leaves	OH functionality	Pandian *et al.* (2023)
Ficus carica extract	AgNPs into Chitosan	Leaves	OH group	Goh *et al.* (2023)
Banana waste peduncles	AgNPs/Degussa	Fruit peel	Phenolic OH	El-Desouky *et al.* (2021)
PVA starch	Cassava starch/polyvinyl alcohol@ AgNPs film	Vegetable	OH group	Srikhao *et al.* (2021)
Pea hull waste	Carboxymethyl cellulose nanocrystals@AgNPs	Vegetable waste	OH and SO_3H groups	He *et al.* (2021)
Hardwood bleached kraft pulp	Cellulose-AgNP composite sheets	Wood pulp	OH group of cellulose chain	Li *et al.* (2023)
Bagasse raw material	Alginate/oxidised nanocellulose-AgNPs	Plant waste material	H-bonding	Abutalib and Rajeh (2021)
Fresh leaves of tulasi, mint and aloe vera	AgNPs@cellulosic network	Leaves	NH_2 and OH groups	Kumari *et al.* (2021b)
Corn cobs	AgNPs with poly (lactic acid)/ nanocellulose nano-films	Corn cobs	Polyphenols, OH and NH groups	Ng *et al.* (2021)

Note: CO - carbonyl; -NH_2 - primary amine; OH - hydroxyl; PVA - polyviyl alcohol; SO_3H - sulfonic acid; NH - secondary amine.

be due to a high concentration of reducing agent leading to agglomeration. Therefore, the optimal volume of POME extract for AgNP synthesis is 0.6 mL. Vadakkan *et al.* (2024) reported that an increase in concentration enhances the synthesis of AgNP until the synthesis reaches the threshold level, however, beyond which particle accumulation occurs. When nanoparticles aggregate, they may lose their essential features and economic value. Hence, it is crucial to maintain an optimal concentration.

Effect of Temperature

Figure 3b illustrates the variation in the absorption spectrum of AgNP synthesised using POME extract at different reaction temperatures. The spectrum shows that the optimal temperature is 50°C. Moreover, at 60°C, 70°C and 80°C, there is an increase in activation energy, which results in AgNP agglomeration. Chemical reactions are facilitated by temperature, as it provides the activation energy required to initiate the fastest reaction. Additionally, temperature also increases the likelihood of reactants colliding, thus increasing the chances of them converting into the desired product (Lotfy *et al.*, 2021).

Effect of pH

AgNP synthesis is significantly influenced by the pH range of the solution as it enhances the ability of phytochemicals to bind with Ag^+, this facilitates their reducing and capping potentials (Azarbani & Shiravand, 2020). In this study, the synthesis of AgNP using POME at its natural pH of 4.5 showed no absorbance peak, indicating the absence of AgNP formation. To achieve optimal synthesis conditions, the pH of the POME extract was adjusted. As shown in *Figure 3c*, the optimal pH for synthesising AgNP is pH 7. At this pH, the reduced concentration of H^+ ions increases the surface charge of the particles, facilitating AgNP formation. In contrast, synthesis at pH levels of 4 and 5 showed no SPR absorbance peak, suggesting that acidic conditions are unfavourable for AgNP synthesis. Supporting these findings, Hamouda *et al.* (2019) reported that the optimal pH for AgNP formation using *Oscillatoria limnetica* was 6.7, with synthesis being completely inhibited at pH 4.7 and 5.7.

Effect of $AgNO_3$ Concentration

In this study, various concentrations of $AgNO_3$ (ranging from 0.5-3.0 mM) were examined

to determine the optimal concentration for AgNP formation with POME extract at ambient temperature. The absorption spectrum of the POME-AgNP showed that increasing the $AgNO_3$ concentration up to 1.5 mM led to higher absorption band intensity. However, at concentrations between 1.5 and 3.0 mM as shown in *Figure 3d*, there was a decrease in the absorption spectrum, likely due to nanoparticle aggregation. Therefore, a concentration of 1.5 mM $AgNO_3$ was identified as the optimal condition for the green synthesis of AgNP.

Characterisation of POME-AgNP

FTIR spectroscopy. The involvement of surface functional groups in the biosynthesis of POME-AgNP was examined using FTIR analysis. *Figure 4* displays a comparative analysis of the FTIR spectrum for raw POME powder and POME-AgNP. The absorption peaks in the FTIR spectrum of POME-AgNP showed reduced intensity at 3,761 and 1,744 cm^{-1}, which indicates C=O stretching vibrations of the carbonyl group present in the raw POME. This can be attributed to the involvement of compounds with carbonyl functional groups in the synthesis of POME-AgNP, which aligns with previous studies on gold nanoparticles synthesised using POME (Gan *et al.*, 2012). The significant absorption bands observed in the range of 3,400-2,900 cm^{-1} are associated with phenolic compounds in the biosynthesised AgNP. The presence of carbonyl groups, indicated by the C=O stretching vibration at 1,700 cm^{-1}, suggests the presence of aromatic structural vibrations in flavonoids and glucosides. Thus, the FTIR results imply that the organic compounds in POME contain functional groups responsible for the reduction and stabilisation of POME-AgNP.

The differences in band and peak intensity showed the structural changes and the change in the extent of hydrogen bonding. These might be due to the oxidisation of phytochemicals in POME. In Indian propolis extract, flavonoids contribute to AgNP synthesis by reducing Ag^+ to Ag^0 (Rashid *et al.*, 2019). In the process, flavonoids undergo oxidation, which results in the formation of diketone and unsaturated carbonyl groups. It is possible that the carbonyl and hydroxyl functional groups found in POME can serve not only as reducing agents by giving electrons to convert Ag^+ to Ag^0 during AgNP synthesis but also as capping agents through electrostatic interactions to keep the synthesised AgNP stable. This suggests that these active functional groups play a dual role in the synthesis and stabilisation of AgNP (Chi *et al.*, 2022).

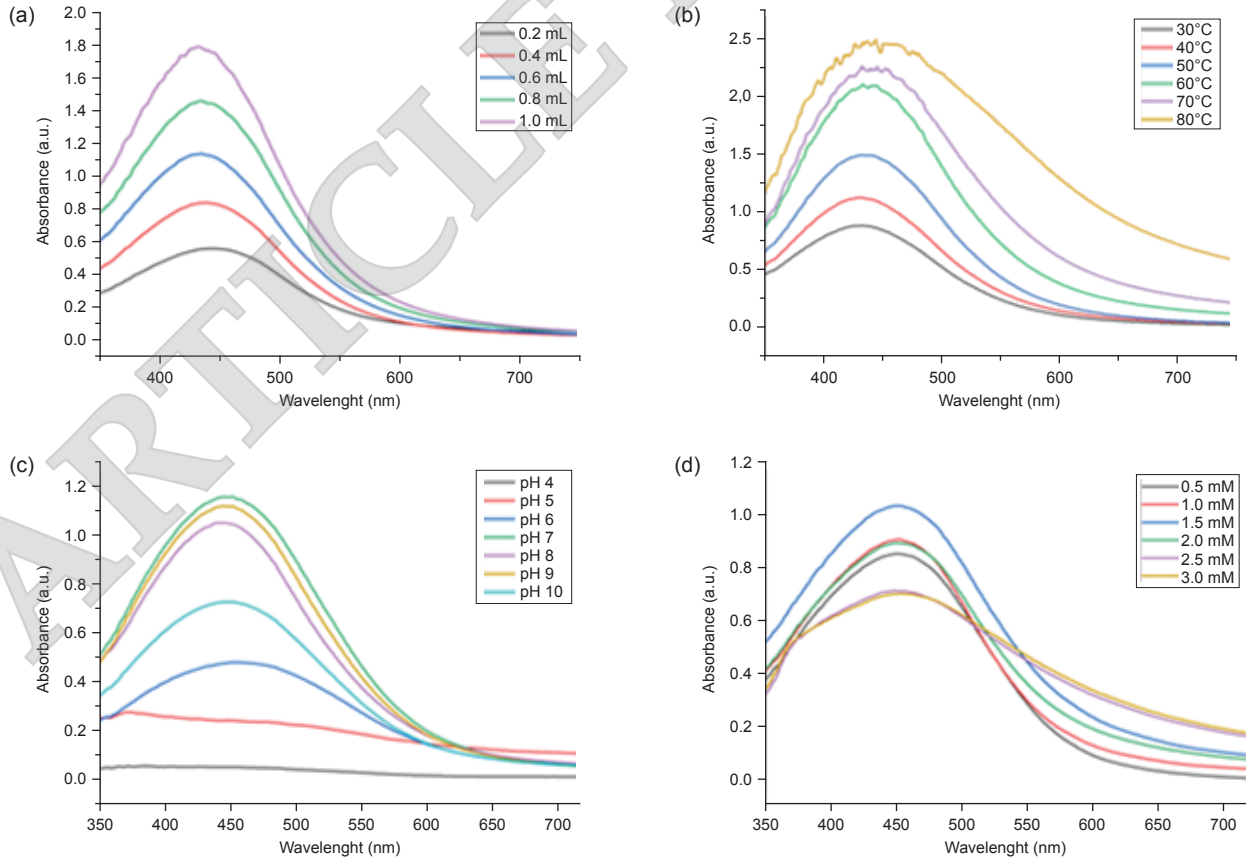


Figure 3. Visible spectra of POME-AgNP: (a) Volume, (b) temperature, (c) pH values and (d) $AgNO_3$ concentrations of palm oil mill effluent (POME) extract.

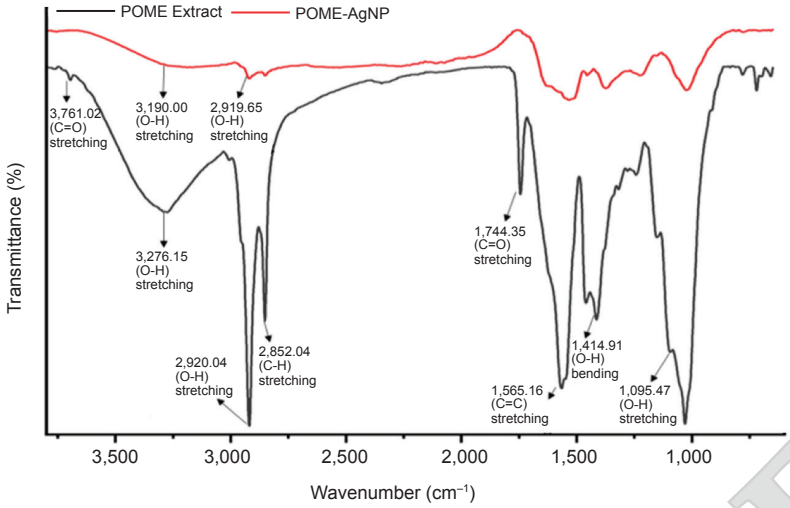


Figure 4. Fourier Transform Infrared (FTIR) spectra of raw palm oil mill effluent (POME) extract and POME-AgNP.

DLS analysis. DLS determines the time-dependent light intensity variations caused by particle Brownian motion and measures the hydrodynamic diameter of the nanoparticles (Yusof *et al.*, 2020). The DLS allows the quantitative measurement of size distribution as well as the dispersion (polydispersion or monodispersion) of nanomaterials in a colloidal system (John *et al.*, 2020). *Figure 5a* shows the average hydrodynamic particle sizes of 21.18 nm with a polydisperse index (PdI) of 0.259. A value of PdI less than 0.5 indicates the mono dispersion of nanoparticles (Agnihotri *et al.*, 2014). In addition, the AgNP stability was evaluated by measuring the zeta potential magnitude. *Figure 5b* shows the ζ-potential value of -19.3 mV for the POME-AgNP colloidal solution. The charge surface (-) obtained is due to the macromolecules in the raw POME adhering to the AgNP surface which indicates repulsion between the particles, preventing their aggregation. According to Tavakol *et al.* (2017), suspended particles with a negative surface charge are likely to repel each other and less likely to aggregate (electrostatically stable). In this work, a zeta potential of -19.3 mV indicates that the POME-AgNP are considerably stable. Thus, AgNP with zetapotential of < -25 mV or > +25 mV have significant stability (Vadakkan *et al.*, 2024).

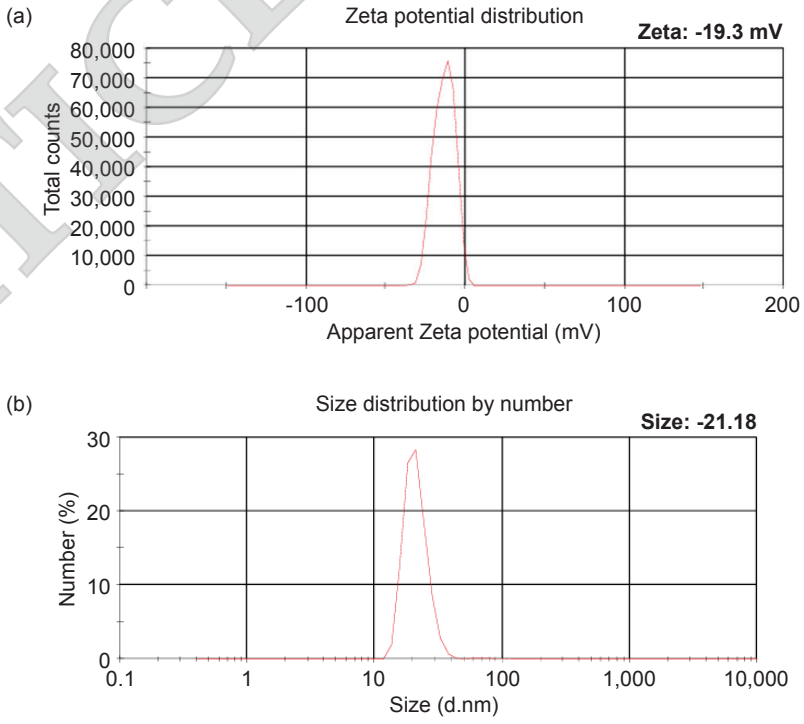


Figure 5. DLS analysis showing the (a)zeta potential and (b)size distribution.

CONCLUSION

This study presents the novel approach to the biosynthesis of AgNP using POME. Utilising agro-industrial waste such as POME for AgNP synthesis is both sustainable and advantageous, as it helps reduce environmental pollution while providing antimicrobial properties. The LC-MS analysis identified several compounds, including stearic acid, palmitic acid, anhalamine, phosphatidylethanolamine (40:8), hebevinocide VII, aminopentol, safingol, camptothecin and myristone, which likely play roles in the reduction, capping and stabilisation of Ag^+ ions during AgNP formation. The optimal conditions for synthesising POME-AgNP synthesis were established using the OFAT approach and the physicochemical properties of the resulting POME-AgNP were comprehensively evaluated. Future research should focus on identifying and isolating individual compounds and assess their effectiveness to elucidate their roles, thereby improving our understanding of the biosynthetic process, especially considering the increasing demands for solutions in various health applications.

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