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Green synthesis and characterization of silver nanoparticles (AgNP) using *Acacia nilotica* plant extract and their anti-bacterial activity

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ABSTRACT

Green-synthesised silver nanoparticles (AgNP) used to treat antibiotic-resistant bacteria. This article addresses the synthesis and characterization of silver nanoparticles (AgNP) based on Acacia nilotica plant extract, as well as their antibacterial efficacy against human illnesses. The information of homogeneous, spherical shaped AgNP with an average size of 23.65 nm was confirmed by XRD, SEM, EDAX, TEM, UV-Vis spectroscopy, and FTIR analysis. The effectiveness of the produced AgNP against various bacteria was tested using MIC and zone of inhibition experiments, showing impressive inhibitory effects. These findings imply that the environmentally friendly production method and potent antibacterial properties of the biosynthesized AgNP hold promise for upcoming bio-related uses.

1. Introduction

The development of a sustainable method for producing silver nanoparticles (AgNP) is a key aspect of current nanotechnology studies. Various nanomaterials like Ag, Au, Pt, and Pd have been created through different methods, such as hard templates (Sastry et al., 2003; Husseiny et al., 2007; Sharma et al., 2007; Bhattacharjee et al., 2022). Silver nanoparticles are highly important in the fields of biology and medicine because of their attractive physical and chemical characteristics. The process of producing AgNP with the use of plants is well documented (Haverkamp et al., 2009).Medical plants and their derivatives are most abundant and affordable in Indian greeneries. For millennia, these medicinal plants have been employed in Ayurveda. Many of these plants are currently gaining prominence due to their unique characteristics and broad applications in a wide range of emerging research and development fields. The manufacture of nanoparticles using plants and their products is a major focus of nanobiotechnology research in current material science. They are also frequently used in medical and pharmaceutical products, putting them in close touch with human systems (Bhattacharya *et al.* 2008). Previously, the antifungal effect of silver and silver nitrate were extensively exploited in medical science. Furthermore, the medicinal usefulness of various plants and plant components has been recognized. However, the idea of using plants to produce silver nanoparticles is very new.

Nanotechnology and its derivative products differ not only in the treatment procedure, but also in particle size, physical, chemical, and biological properties, as well as a wide range of applications. This emerging field of nanobiotechnology is still in its early phases of growth due to a lack of widespread adoption of novel ideas on a large scale, and it must be upgraded using recent technologies. As a result, there is a need to create an affordable, economically feasible, and environmentally benign method to AgNP synthesis to meet expanding demand in a wide range of applications. Researchers are currently interested in the preparation of AgNP due to their diverse properties and applications in various sectors, such as optical polarizability, magnetic, electrical conductivity, antimicrobial, antibacterial and catalysis activities (Shahverdi

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et al., 2007; Anand et al., 2022), surface-enhanced Raman scattering (SERS) (Matejka et al., 1992) and sequencing of DNA (Cao *et al.*, 2001). These have bactericidal effect against gram positive and gram negative microorganisms. However, multidrug resistant gram negative bacteria are becoming more frequent because they create metallo-β-lactamases, carbapenemases, and β-lactamases, making them hard to treat (Kagithoju et al., 2012), despite the fact that they can be treated with AgNPs. *A. nilotica* is a widely widespread plant in India, the Pacific, and the southern United States. *A. nilotica* is often known as *A. nilotica* in India. *A. nilotica*, an alkaloid found in this plant, shows anticancer, anti-estrogenic, immune-modulatory and anti-amoebic properties in vitro (Selvaraj *et al.*, 2012). *A. nilotica* (L.) Willd. belongs to the Mimosaceae family. Bark decoction is used as a gargle, and pods are used to treat urogenital diseases. *A. nilotica*. Lam (Mimosaceae), sometimes known as 'Babul' or 'Kikar', is a medium-sized tree found in tropical and subtropical countries. It has a variety of medical applications, including putative antioxidant qualities. This plant produces a wide range of chemicals, including alkaloids, volatile essential oils, phenols and lots of phytochemical componentsits available. A. nilotica is a medicinal plant with a high phenolic content, including condensed tannin and protocatechuic acid, phlobatannins, gallic acid, (+)-catechin, pyrocatechol, (-) epigallocatechin-5,7-digallate and (-) epi-gallocatechin-7-gallate. Various parts of this plant, such as the seeds, roots, leaves, flowers, fruits, and immature pods and gum, act as, spasmogenic, antimutagenic, vasoconstrictor, anticancer, anti-asthmatic, antipyretic, anti-diabetic, cytotoxic, anti-plasmodial, anti-platelet aggregation, anti-fungal, molluscicidal, antioxidant activities, inhibitory activity against Hepatitis C virus (HCV) and HIV-1, anti-bacterial, anti-spasmodic and antihypertensive activities, and are also engaged. This research focuses on the precise phytochemical composition, medicinal applications, and pharmacological properties of many components of this adaptable plant. For the first time, we present (i) the biosynthesis of AgNP via a single-step reduction of silver ions utilizing A. nilotica plant extract, and (ii) the antibacterial efficacy of these biosynthesized AgNPs against three human bacterial pathogens.

Green-synthesised AgNPs are used in a wide range of therapeutic applications, including antibacterial effects. AgNP and antibiotics are now being used together to effectively combat multi-resistant gram-positive and gram-negative bacterial infections. By combining AgNP with antibiotics, the necessary dosage can be reduced and fewer side effects are produced. Using AgNP as carriers for drugs enhances the effectiveness of antibiotics, leading to improved release and targeting of resistant organisms. Green-synthesized AgNP are considered effective antibacterial agents because of their specific size and shape characteristics (Gherasim et al., 2020; Kong et al., 2020). Although the exact mechanism of antibacterial action is not fully understood, the cations produced by AgNP can attach to DNA, interfere with proteins, and damage the cell walls of bacteria. This results in increased levels of reactive oxygen species (ROS) within the bacterial cells (Rinna et al., 2015, Wang et al., 2017). The biological activity of green-synthesized AgNPs, including their anticancer and antibacterial properties, is influenced by their size and shape (Rizwana et al., 2021; Althomali et al., 2022). A. nilotica, found in regions spanning Egypt, India, South Africa, Central America, and Australia, is a beneficial plant known for its medicinal properties and potential high antioxidant content (Osama et al., 2015). Throughout the plant, various biomolecules like amino acids, proteins, carbohydrates, and antioxidants can be found. It also contains eleagic acid, gallic acid, leucocianidolum, glucopyranoside, and isoquercetin, contributing to its antibacterial, antiplasmodial, and antioxidant properties that make it valuable in treating various disorders. The phenolic compounds in A. nilotica can help prevent or inhibit carcinogens and notably reduce tumor growth (Sadiq et al., 2017). According to Ali and Qaiser (2008), A. nilotica can precipitate bacterial proteins, hindering their growth by depriving them of essential proteins. The plant can form irreversible compounds with proline-rech protein in bacterial cells, disrupting protein synthesis. The leaves of A. nilotica are rich in protein and essential amino acids, as well as phytochemicals like phenols, alkaloids, steroids, resins, phenolics, oleosins, tannins, glycosides, saponins, volatile oils, and flavonoids, contributing to a range of physiological and pharmacological effects (Revathi et al., 2017).A few studies on A. nilotica antibacterial and anticancer properties have been published in the literature. Desert climatic conditions promote a variety of physiological and biochemical activities that influence development, plant growth and metabolite production, particularly the abundance of natural antioxidants (primarily phenolic compounds), hydrocarbons, and terpenoids (Yeshi et al., 2022; JamLoki et al., 2021). In the current study, AgNP were synthesized from an extract of A. nilotica leaves from India and tested for antibacterial activity.Overall, this study addresses the growing demand for environmentally acceptable methods of synthesizing AgNP and analyzes the efficacy of A. nilotica-mediated AgNP as antibiotics. This work is based on the unique features and applications of biosynthesized AgNP, as well as the extensive medicinal qualities of A. nilotica.

2. Materials and methods

2.1. Materials

All chemicals utilized during this investigation were procured from Sigma (Bangalore, India) and Merck (Mumbai, India). A. nilotica plant leaves were obtained near the Periyar University campus and validated by Dr. N. Kayalvizhi, Zoology, Periyar University, Salem, India. They were deposited to Periyar University's Department of Zoology in Salem, India.

2.2. Preparation of plant leaf extract

Freshly cleaned A. nilotica leaves (Fig. 1) were obtained from the Foundation for Revitalization of Local Health Traditions in Bangalore, India and then shade-dried at 25 ± 2 °C for 6-7 days until firm, then transformed into powder. In a 250 mL conical flask at 60 °C for 20 minutes, 10 g powder was heated with 100 mL double-distilled water. The crude aqueous extract was filtered using Whatman filter paper 1 and kept at 4 °C.

2.3. Silver nanoparticles (AgNP) biosynthesis

Leaf aqueous extract of A. nilotica (10 mL) was combined with 90 mL of 1 M silver nitrate (AgNO3) solution (Fig. 2a) and stirred at 60 °C for 30 minutes. Initially, the pale yellow tint turned dark brown, indicating the formation of silver nanoparticles (AgNP) (Fig. 2b), as silver ions

Fig. 1. External view of A. nilotica leaves.

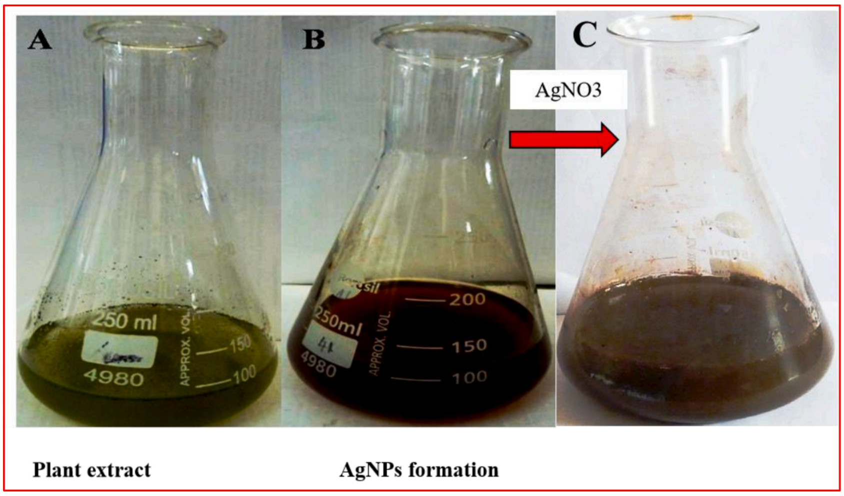

Fig. 2. Showing the biosynthesis of Silver Nanoparticles.

were reduced by the A. nilotica extract, resulting in stable AgNP in water. Then reactant solution was subjected to centrifugation for 15 minutes at 5000 rpm, after which the pellet was collected and dehydrated overnight in a hot air oven set to 60 °C. Pellets were saved and used for analysis. and bioassays.

2.4. UV-visible spectrophotometry

The early change in the color, from pale yellow to dark brown, suggested silver ion bioreduction. It was also confirmed by comparing the solution spectra with a UV-Vis Spectrophotometer (Lambda 35, Perkin Elmer, Llantrisant, United Kingdom). The wavelengths varied from 300 to 700 nm.

2.5. Fourier transform infrared spectroscopy

The presence of functional groups in biosynthesized AgNP was examined using Fourier transform infrared spectroscopy (FTIR). The KBr pellet approach was used to scan a spectrum spanning 500 to 4000 cm^{-1} (IRAffinity-1, Shimadzu Corporation, Japan).

2.6. SEM

A scanning electron microscope (SEM) was utilized to perform morphological and structural analysis. To evaluate the form and bonding arrangement of biosynthesized AgNP, dried samples were produced on a copper-coated carbon grid and inspected under a scanning electron microscope (Hitachi-S-4500).

2.7. Bacterial cultures

Gram-positive bacteria used in this stue were *Bacillus subtilis* (MTCC 441), *Staphylococcus* aureus (MTCC 737), *Bacillus cereus* (MTCC 6840), *Bacillus aryabhattai* (MTCC 14579) and *Bacillus megaterium* (MTCC 441). Also, Gram-negative bacteria were *Escherichia coli* (MTCC 1302), *Pseudomonas putida* (MTCC 1194), *Serratia marcescens* (MTCC 4822) and *Klebsiella pneumonia* (MTCC 4727). All micobial strains were grown in a nutrient broth at 37 °C.

2.8. Antibcaterial activity

The antibacterial activity of *A. nilotica*-mediated AgNP treatment against 9 bacterial strains was determined using the well-diffusion method. The test bacteria were purified at IMTECH in Chandigarh, India. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737), *Bacillus cereus* (MTCC 6840), *Bacillus megaterium* (MTCC 441), *Bacillus aryabhattai* (MTCC 14579), and Gram-negative bacteria such as, *Escherichia coli* (MTCC 1302), *Pseudomonas putida* (MTCC 1194), *Serratia marcescens* (MTCC 4822) and *Klebsiella pneumonia* (MTCC 4727) are among the test strains. Aseptically dispersing the day-old bacterial suspensions on nutrient agar plates with a sterile cotton swab. After drying for a few minutes, a 6 mm well was put into the plate using a sterile glass rod. The antibacterial activity involved loading 100 μL of AgNP into each well. The incubation was at 37 °C for 24 hours. The maximal zone of inhibition was measured in millimeters for each well. For each pathogen, three replicates were used, and the mean diameter was indicated in millimeters.

3. Result and discussion

3.1. Characterization of AgNP

The primary change in color from pale yellow to dark brown indicated the synthesis of AgNP using *A. nilotica's* aqueous leaf extract and silver nitrate. The reduction of $Ag+$ ions causes surface plasmon resonance vibration, which is necessary for the formation of AgNP. Measurements of the absorbance peak at 428 nm indicated AgNP biogenesis using A. nilotica leaf extract. The XRD pattern of AgNP shows strong peaks at 2^{θ}-38.31, 43.54, 64.62, and 77.46, corresponding to the (111), (2 0 0), (2 2 0), (3 1 1), and (2 2 2) Bragg's reflections of the face-centered cubic structure of silver (Fig. 3). The AgNP size was calculated from X-ray line broadening using the Debye-Scherrer formula given as D- 0.9lambda/$\beta\cos^{\theta}$, where D is the average crystallographic size (J), λ is the X-ray wavelength employed (nm), β is the angular line width at half maximum intensity (radians), and _ is the Braggs angle (degrees). For (1 0 1) reflection at $2^{\theta} = 38.13$, for β - 0.367200 radians, λ-1.54 J, and $^{\theta} \approx 19.06$, the average size of the AgNP is predicted to be roughly 25 nm. Parts of plant extract-assisted NP biosynthesis have gained prominence due to the existence of numerous secondary metabolites (terpenoids, alkaloids, phenols, and tannins), which act as reducing and stabilizing agents for bioreduction and the creation of unique metallic nanoparticles. In a suspension of plant extract, NP synthesis occurs in three stages: activation, growth, and termination. The synthesis starts with the reduction of metal salts/oxides, then the growth of smaller nanoparticles into larger NP, and lastly the manufacture of required NP. The presence of these important chemicals improves the stability of plant-derived NP. These chemicals can serve as a capping agent, which is essential for aggregation, inhibition, and growth

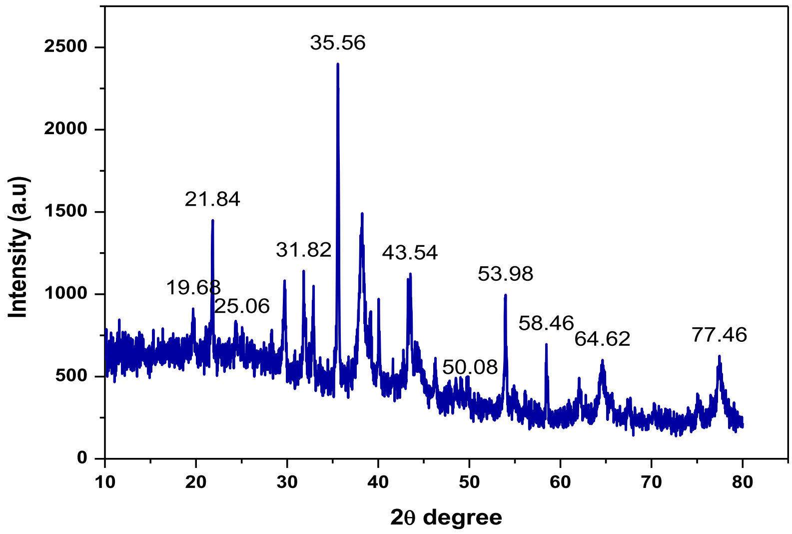

Fig. 3. graph of XRD specifies the AgNP crystalline size synthesized using A. nilotica.

termination. Furthermore, they influence the size and shape of NP due to differences in reduction ability between plant-based synthesis NPs. In terms of advantages, the plant-based green synthesis process is more commonly used than others.

SEM pictures (Fig. 4) of samples taken from colloidal Ag solutions generated at 80 °C confirm the presence of extremely small and regularly manufactured NP with irregular forms that mimic colloidal particles and can be uniformly disseminated within a solution. Green AgNP synthesized by *A. nilotica* varied in size and form, but they demonstrated strong antibacterial and anticancer activity (Revathi et al., 2017). The SEM images reveal that larger particles of AgNP are formed as a result of nanoparticle aggregation, which could be produced by solvent evaporation during sample preparation. This could explain the different particle sizes. The elemental composition of powdered materials was evaluated using SEM (JEOL-JSM 6610 LV).

Fig. 3 shows the energy dispersive X-ray analysis (EDAX) results, which indicated a high signal in the silver area and validated the synthesis of AgNP. Metallic silver nanocrystals have a typical optical absorption peak about 2.983 keV due to surface plasmon resonance. A transmission electron microscope was used to examine the size and shape of the produced nanoparticles. Fig. 5 shows that AgNP were essentially uniform, with a limited size distribution. The colorless silver nitrate solution turned brown or dark crimson, suggesting the production of AgNP. The brown color was a result of the excitation of surface Plasmon oscillations, which are specific to AgNP and have maximum values in the visible spectrum between 400 and 500 nm (Sastry *et al*., 1997). The SPR absorbance was highly sensitive to the size, shape and nature of the particles produced, as well as the inter-particle distance and surrounding material. (a) UV-Vis spectra of AgNP reveal a plasmon resonance band (at 410 nm). FTIR analysis (b) shows peaks at 850 and 2528 cm^{-1} for O H bond vibration and NO3− group, respectively.

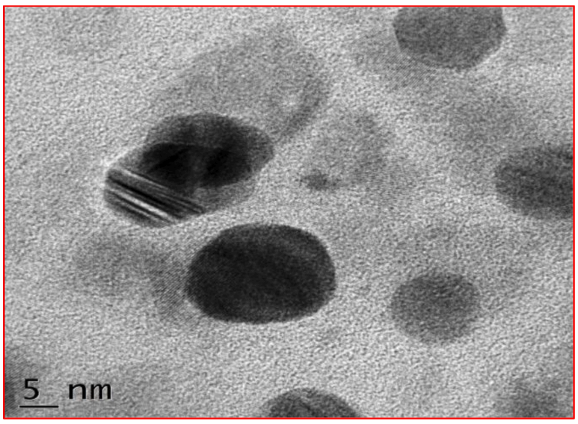

Fig. 4. Scanning electron microscope image of synthesized AgNP, shows that have tremendous capability to synthesize silver nanoparticles which were roughly spherical in shape.

3.2. Antibacterial potential of A. nilotica-AgNP against human pathogens

The rapid development of toxin and antibiotic resistance in pathogenic bacteria is a critical concern and makes infectious disease treatment challenging. To address this, numerous ways have been proposed for the production of effective antimicrobial effects, with silver nanoparticles showing the most promise due to their broad-spectrum and powerful antibacterial properties. We tested *A. nilotica*-AgNP's antibacterial efficacy against nine bacterial infections. *A. nilotica*-AgNP has demonstrated excellent antibacterial activity and a larger zone of inhibition (ZOI) for human infections. *Serratia marcescens*, a gram-negative bacterium, was found to have the strongest inhibitory zones. Extracts of *A. nilotica* that generated AgNP showed various degrees of antibacterial activity. Its inhibition zones for *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas putida* were 30 mm, 25 mm, and 15 mm, respectively (Table 1).AgNP synthesized by *A. nilotica* had the strongest inhibitory effect against gram-positive bacteria *Bacillus ariyabattai, Serettia marsences, Bacillus megaterium, Staphylococcus aureus*, and *Bacillus subtilis*, with inhibition zone diameters of 18 mm, 15 mm, 14 mm, 12 mm, and 11 mm, respectively (Table 1). Table 1 demonstrates that biologically produced AgNP has the highest anti-*Serratia marcescens* activity of any pathogenic bacteria. The efficacy of ANL-AgNP against numerous human diseases was found to be comparable to AgNP derived from *Bryophyllum pinnatum* leaf extract. The extremely low achieved concentration could be exploited to develop future antibacterial treatments based on synthesized ANL-AgNP. AgNP is thought to interact with a wide range of essential biomolecules, including cell membranes, respiratory enzymes, proteins, and DNA, causing cell death and lysis. Saratale et al. (2017) recently offered a full explanation of AgNP's probable interaction with bacterial cells. According to Dibrov et al. (2002), interaction with implanted SNP produced changes in bacterial cell wall membrane structure due to AgNP activity, resulting in enhanced membrane permeability and bacterial death. The A. nilotica plant extract can

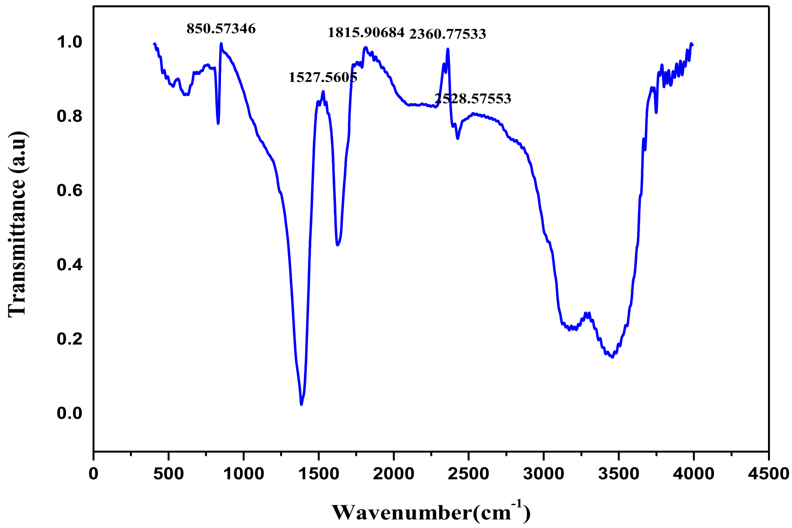

Fig. 5. FT-IR spectroscopy analysis of Ag Nanoparticals.

Table 1
Plant extract-derived synthesised zone of inhibition (mm) against gram positive and gram negative bacteria.

	Strains	Strains: Inhibition Zone (mm)
Gram positive	*Serettia marsences*	15
	Staphylococcus aureus	12
	Bacillus megaterium	14
	Bacillus ariyabattai	18
	Bacillus subtilis	11
Gram negative	Escherichia coli	25
	Klebsiella pneumoniae	20
	Pseudomonas putida Serratia	15
	marcescens	30

serve as a green and eco-friendly means to produce AgNP, presenting a straightforward, effective, and cost-efficient approach. Analysis of the manufactured AgNP through SEM showed that they are spherical in shape with an average size of 23.65 nanometers. These biosynthesized AgNP exhibit potent antibacterial properties, positioning them as promising contenders for numerous biomedical applications.

4. Conclusion

The study suggests that producing silver nanoparticles (AgNP) using A. nilotica plant extract is a straightforward, cost-efficient, and effective process. Analysis techniques like XRD, SEM, EDAX, TEM, UV-Vis spectroscopy, and FTIR confirm the creation of uniform spherical AgNPs with an average size of 23.65 nm. The antibacterial properties of the synthesized AgNP have been proven through MIC testing and zone of inhibition assays against various bacterial strains, indicating its potential for medical applications. The environmentally friendly nature of this synthesis approach, combined with its significant antibacterial efficacy, suggests that biosynthesized AgNP holds great promise for future use in healthcare and medical settings.

Institutional review board statement

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Informed consent statement

Not applicable.

CRediT authorship contribution statement

S. Revathi: Writing – original draft, Software, Methodology, Investigation. **S. Sutikno:** Software, Methodology, Investigation. **Abdulbasit F. Hasan:** Conceptualization, Software, Validation, Writing – original draft. **Ammar B. Altemimi:** Writing – original draft, Validation, Methodology, Conceptualization. **Qausar Hamed ALKaisy:** Writing – original draft, Software, Methodology, Investigation. **Ankur J Phillips:** Writing – original draft, Software, Methodology, Investigation. **Mohammad Ali Hesarinejad:** Writing – review & editing, Writing – original draft, Software, Conceptualization. **Tarek Gamal Abedelmaksoud:** Writing – review & editing, Writing – original draft, Software, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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