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ORIGINAL ARTICLE

One-step synthesis of highly-biocompatible spherical gold nanoparticles using *Artocarpus heterophyllus* Lam. (jackfruit) fruit extract and its effect on pathogens

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Abstract

Introduction. Novel approaches for the synthesis of gold nanoparticles (AuNPs) are of great importance due to its vast spectrum of applications in diverse fields, including medical diagnostics and therapeutics. Te presented study reports the successful AuNPs' synthesis using *Artocarpus heterophyllus* Lam. extract, and provides detailed characterization and evaluation of its antibacterial potential.

Objective. The aim was to develop a cost-effective and environmentally friendly synthesis method of gold nanoparticles using aqueous fruit extract of *Artocarpus heterophyllus* Lam. as a reducing and capping agent, which has proven activity against human pathogens, such as microbial species *E.coli* and *Streptobacillus sps.*

Materials and method. Characterizations were carried out using ultraviolet-visible (UV-Vis) spectrophotometry, scanning electron microscopy (SEM), energy dispersive X-ray and Fourier-Transform infra-red spectroscopy (FT-IR).

Results. SEM images showed the formation of gold nanoparticles with an average size of 20–25 nm. Spectra collected while infra-red analysis contained broad peaks in ranges from 4000–400 cm^{-1}.

Conclusions. It can be concluded that the fruit of *Artocarpus heterophyllus* Lam. can be good source for synthesis of gold nanoparticles which showed antimicrobial activity against investigated microbes, in particul *E. coli*, and *Streptobacillus.* An important outcome of this study will be the development of value-added products from the medicinal plant *Artocarpus heterophyllus* Lam. for the biomedical and nanotechnology-based industries.

Key words

Artocarpus heterophyllus, UV- visible spectra, X-ray spectroscopy, EDX analysis

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science and technology [1]. It provides the ability to create materials, devices and systems with fundamentally new functions and properties [2]. In the era of nanotechnology, research on nanomaterials is continuously growing with increasing demand. This is because metals in nanometer size exhibit special properties, usually different and superior in comparison with bulk metals [3]. Nanostructured metals are becoming more important in catalysis, sensors, electronics, biotechnology and biomedicine [4]. Recently, research in the synthesis of nanoparticles using microbes and plant extracts has been gaining more importance due to its ecological compliance, flexibility and, most importantly, elimination of toxic chemicals [5]. Plant-mediated synthesis is actively practiced by researchers because of its positive advantages, such as avoidance of maintaining microbial culture, which can be time-consuming and cost effective [6]. Biological routes of nanoparticles synthesis using microorganisms [7], enzymes [8] and plants or plant extracts [9, 10] have been suggested as potential eco-friendly alternatives to chemical and physical methods. Using plants for nanoparticles synthesis can be advantageous over other biological processes because of elimination of the elaborate process of maintaining cell cultures [11]. Several plants and their parts have been successfully used for the extracellular synthesis of metal nanoparticles [12].

Developing clean, non-toxic, and eco-friendly procedures for synthesis of nanoparticles is desirable [13]. Synthesis of metal nanoparticles is individually dependent on knowledge of both plants and micro-organisms, which play a crucial role. Bearing this in mind, researchers have been working extensively on extracellular and intracellular synthesis of metal nanoparticles using bacteria, fungi, yeasts and many other biological resources [14]. One of the major disadvantages of using microbes for bio-reduction is the necessity of maintaining the aseptic conditions, which is not only labour-intensive but also very expensive in terms of industrial scale production. Leaf extracts have been used for the synthesis of silver nanoparticles, which has shown the possibility of rapid synthesis and also reduction of the

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steps involved in downstream processing, thereby making the process more cost-efficient [15].

The presented study explores an inventive contribution in the synthesis of gold nanoparticles using fruit extract of Jackfruit (*Artocarpus heterophyllus* Lam.), a categorized under the Kingdom- *Plantae*, Family – Moraceae, Genus- *Artocarpus*, and Species- *heterophyllus*. It is a tree species in the *Artocarpus* genus of the mulberry family (*Moraceae*), a native of parts of South and Southeast Asia. It is believed to have originated in the southwestern rain forests of India, in present-day Kerala and coastal Karnataka; it has also been reported in Ayurveda.

The flesh of the Jackfruit is starchy and fibrous, and is a source of dietary fibre. It is an important source of compounds like morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone, betulinic acid, artocarpanone and heterophylol, which are useful in treating fever, boils, wounds, skin diseases, convulsions, constipation, as well as diuretic and ophthalmic disorders and snake bites. The bark extract of *Artocarpus heterophyllus* possesses significant anti-inflammatory activity [16].

Objective. The aim of the study is to develop a novel approach in the preparation of gold nanoparticles by a method that differs from conventional physical and chemical approaches, i.e. by using fruit extract as the reducing and capping agent, which also possesses antibacterial potential against human pathogens. The most popular chemical synthesis methods usually involve the use of toxicants, while physical methods usually require expensive equipment. Due to these obstacles, biological methods offer potential advantages because they are eco-friendly, cost effective, and offer one-step approaches to the synthesis of gold nanoparticles. Characterization is carried out using UV–visible spectrophotometer to monitor the reduction of Au ions in solution, while FT-IR spectroscopy analysis is employed to characterize and identify the biomolecules responsible for the reduction of Au ions into gold nanoparticles. Scanning electron microscopy is used to study the particles' morphology and their sizes, while Energy-Dispersive X-ray Analysis (EDAX) is used to confirm the presence of elemental gold. Finally, screening of antibacterial potential against two human pathogens was carried out using *E. coli*, and *Streptobacillus* sps.

Jackfruit was chosen because of its proven antibacterial, anti-inflammatory, antidiabetic, antioxidant and immunomodulatory properties, making it ideal for numerous biomedical applications.

MATERIALS AND METHOD

Preparation of jackfruit extract. Chloroauric acid ($HAuCl_4$) (Thomas Baker, Pvt, Ltd., Mumbai, India) was used without further purification. Fresh jackfruit was purchased from a local market and authenticated by the Department of Bioscience, PG Centre, Hassan, University of Mysore, India. In a typical preparation of Jackfruit extract, 20 g of fruit slices were ground up in a blender with 100 ml of double-distilled water in order to achieve a concentration of 10 mg/mL. The filtered extract was centrifuged twice at 10,000 rpm for 15 min at 4 °C by REMI cooling centrifuge to remove cell debris. The resulting supernatant was then filtered through a 0.2 µm filter paper and employed for the synthesis of gold nanoparticles. Double-distilled water was used to dilute the aqueous chloroauric acid stock solution and the original jackfruit extract.

Jackfruit extract-mediated synthesis of gold nanoparticles. In the presented study, jackfruit extract was used to obtain phytochemically-derived reducing agents for the production and stabilization of gold nanoparticles. The nanoparticles were examined for their consistency in Surface Plasmon Resonance (SPR). Properties and reduction rate were assessed by varying the concentration of the jackfruit extract. The same plasmon resonance band was observed at 580 nm at various concentrations, indicating uniformity in the formation of gold nanoparticles. In a typical experiment, dark conditions were required and pre-incubation at 90 °C for 5 min was applied to 0.002M $AuCl_4$ aqueous solution to dissolve metal completely, and the final total reaction mixture volume was 20 mL. Biosynthesis of gold nanoparticles (jackfruit-AuNPs) was begun by adding jackfruit extract at 50% (v/v) to a 0.002M solution of $AuCl_4$. The reacting mixture was kept in a dark place for 2 h at room temperature, and the synthesis of AuNPs indicated by colour changes from yellow to dark brown. The formation of nanoparticles was monitored by UV-Vis spectroscopy. The mixture was centrifuged at 10,000 rpm for 15 min at 4 °C. The process of centrifugation and re-dispersion was repeated three times to remove unbound jackfruit phytochemicals. Rapidly produced jackfruit -AuNPs within 20 min were collected and purified by repeated centrifugation, as described above, and then used to determine the physicochemical and biocompatibility properties.

Characterization of gold nanostructures

UV-visible spectroscopy analysis. The colour change in reaction mixture (metal ion solution + fruit extract) was recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored by periodic sampling of aliquots (1 ml) and subsequently collecting UV-vis spectra of the solution. UV-vis spectra of these aliquots were monitored as a function of time of reaction on Elico UV-vis spectrophotometer (Model SL 164, double beam) operated at a resolution of 1 nm.

SEM analysis. A scanning electron microscopy (SEM) image was obtained using JEOL JSM 7500F Field Emission Scanning Electron Microscope with a back scattered electrons (BSE) detector (marked as COMPO). K575X Turbo Sputter Coater was used for coating the part of the sample with chromium (deposited film thickness – 20 nm). The microstructure of samples was supported by chemical analysis carried out using energy dispersive X-ray spectroscope (EDX) at 20.0 kV and 15.0 mA.

FT-IR analysis. The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000–400 cm^{-1}. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy (Uvasol®, Merck, Germany). The number of scans equal to 16 and resolution of 4 cm^{-1} characterized these measurements.

Antibacterial activity

Microbial cultures:. *E.coli* and *Streptobacillus* were cultured in Nutrient broth (Hi-Media).

Antibacterial activity by agar well diffusion method. Antibacterial activity was measured using the well diffusion method. Wells were prepared in the medium using sterile gel puncture. The bacterial cultures were swabbed with a sterile cotton swab on plates containing Muller Hinton agar (MHA) medium (Hi-Media). Then 10 µl of antibiotic solution (0.1mg of antibiotic in 10 mL of methanol) and 10 µl of gold nanoparticles (0.1mg of gold nanoparticles in 10 mL of methanol) were added to the wells. Wells with antibiotic solution alone served as positive controls. The Petri plates were incubated at 37°C for 24 h for bacteria.

RESULTS

The extracellular synthesis of gold nanoparticles occurred during the exposure of jackfruit extract to 0.002M $AuCl_4$ aqueous solution. The complete reduction of gold ions was observed after 2–3 hours. The colour change in the reacting mixture was observed during the incubation period because the formation of gold nanoparticles results in a particular, brownish colour. The appearance of this dark brown colour (Fig. 1) clearly confirms formation of gold nanoparticles after the addition of the fruit extract [17, 18, 19].

UV-Vis analysis. Equivalent amounts of the suspension (0.5 ml) were diluted in a a constant volume of de-ionized water (5 ml) and subsequently analyzed at room temperature. The progress of the reaction between metal ions and the fruit extracts were monitored by UV–visible spectra of Au nanoparticles in aqueous solution. The peak in the

Figure 1. Optical photograph of (A) $AuCl_4$ solution (B) Fruit extract (C) Fruit extract + $AuCl_4$

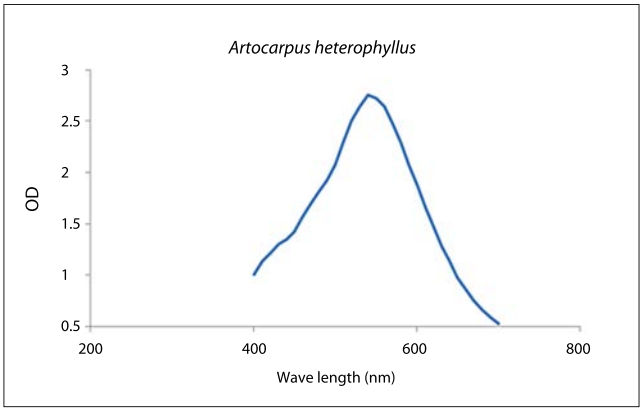

Figure 2. UV-visible absorption spectra of a representative gold nanoparticles synthesized using fruit extract of *Artocarpus heterophyllus* Lam

absorption spectrum of AuNPs was observed at ~580nm (Fig. 2). The reduction of gold ions and the formation of stable nanoparticles occurred rapidly within an hour of reaction, making it one of the fastest bio-reducing methods to produce Au nanostructures. Later, these nanoparticles sedimented at the base of the tube, leaving an almost transparent fluid at the top.

SEM analysis. Figure 3 shows typical SEM images of gold nanoparticles synthesized by use of the above synthesis

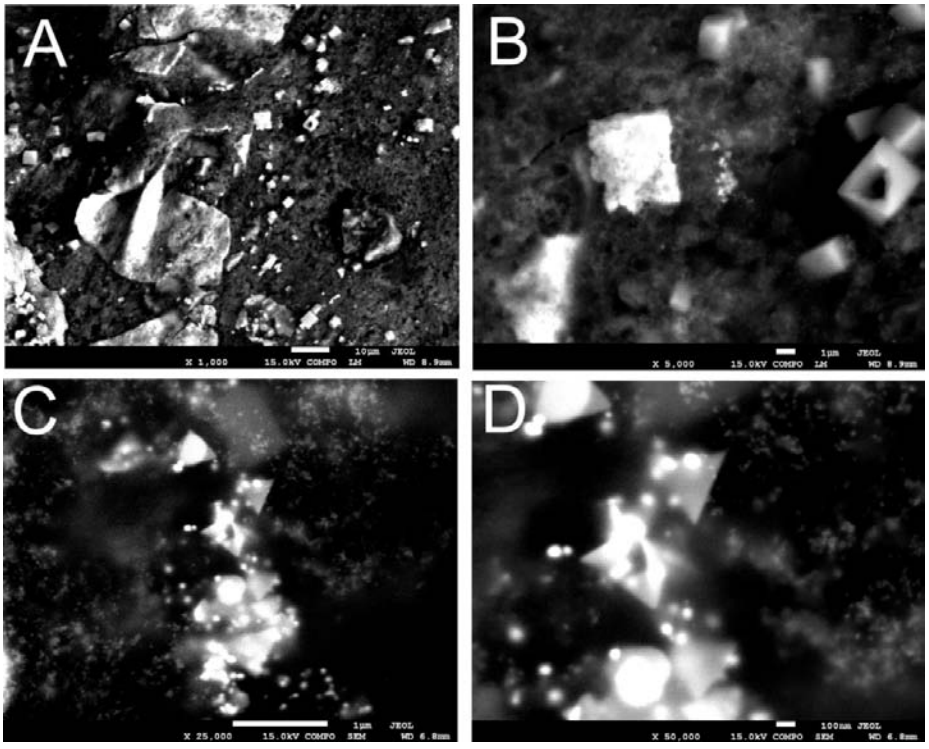

Figure 3. SEM photographs of gold nanoparticles obtained using *Artocarpus heterophyllus* Lam. (Jackfruit) fruit extract

method using *Artocarpus heterophyllus Lam.* (jackfruit) fruit extract. The obtained gold particles have polyhedron shapes which are relatively large (Fig. 3A, 3B). Fig. 3B shows the image of an individual cubic gold polyhedron. It shows that the polyhedron has very sharp edges with an average width of 5 µm. Figure 3A gives representative SEM photographs of gold nanoparticles with different shapes. Figures 3C and 3D show SEM images indicating that the obtained gold nanoparticles were a mixture of spherical nanoparticles and polyhedrons. SEM observation of small gold nanoparticles shows that their shape is mostly spherical.

Figure 4 shows the energy dispersive X-ray (EDX) spectra of synthesized Au nanoparticles. The peaks of C, O, K and Na elements are probably from the jackfruit extract; however, the chlorine comes from gold-chloroauric acid.

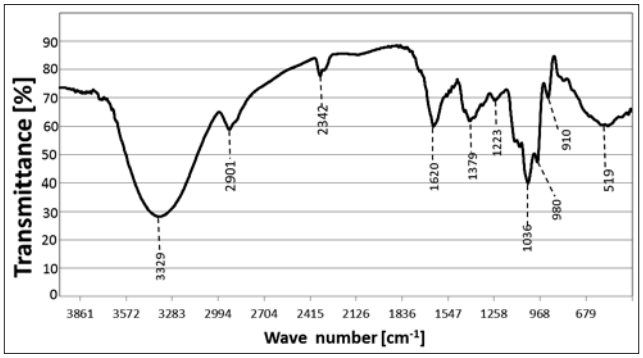

Figure 4. SEM images of Au nanoparticles with EDS spectra (S1 and S2)

FT-IR analysis. The FT-IR spectrum of gold nanoparticles obtained using jackfruit extract is shown in Figure 5.

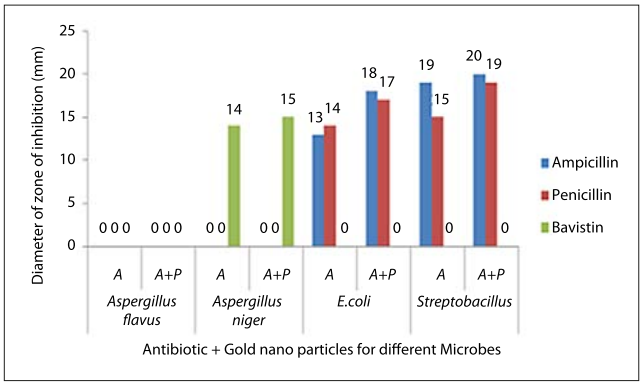

Figure 5. Wave number (cm^{-1}) of dominant peaks obtained from FT-IR transmission spectrum

In the transmission spectrum, the following bands were identified and the wave numbers (cm^{-1}) of dominant peaks recorded:

- Amines N–H stretching, N–H bending and C–N stretching bands at 3,329 (cm^{-1}), 2,342 (cm^{-1}), 1,620 (cm^{-1}) and 1,036 (cm^{-1});
- Amides N–H stretching and C–O stretching bands at 3,329 (cm^{-1}), 2,342(cm^{-1}), 1,620 (cm^{-1}) and 1,379 (cm^{-1});
- Amino acids N–H stretching, N–H bending and C–O stretching bands at 3,329 (cm^{-1}), 2,342 (cm^{-1}),1,620(cm^{-1}), 1,379 (cm^{-1}) and 1,223 (cm^{-1});
- Carboxylic Acids O–H stretching and C–O stretching bands at 1,620 (cm^{-1}) and 1379(cm^{-1});
- Carbohydrates C–O–C stretching bands at 1,223 (cm^{-1}) and 1,036 (cm^{-1});
- Lipids/Alkanes C–H stretching band at 2,901 (cm^{-1});
- Alkanes are C=C stretching C-H out-of plane bending bands at 1,620 (cm^{-1}), 980 (cm^{-1}) and 910 (cm^{-1});
- Aromatics C-H out-of plane bending bands at 980 (cm^{-1}) and 910 (cm^{-1});
- Nitrates N–H bending bands at 1620 (cm^{-1}) and 1,223(cm^{-1});
- Nitro N=O band at 1,379 (cm^{-1});
- Chlorate C–H stretching band at 2,901 (cm^{-1});
- Fluoride C-F band at 1,379 (cm^{-1});
- Bromide, Iodide C-Br, C-I band at 519 (cm^{-1}).

The observed bands corresponding to amines, amides and amino acids indicate the presence of proteins. Several other transmittance bands indicate the presence of biomolecules, such as carbohydrates, polysaccharides and lipids.

Antibacterial activity. Study of the antibacterial properties indicated that antibiotics with gold nanoparticles extracted from jackfruit (A+J) exhibited a greater zone of inhibition when compared to standard antibiotics (A) alone (Tab. 1; Figure 6), and the effects of the antibiotics were analysed, based on the zone of inhibition around the microbial colonies. Ampicillin, Penicillin and Bavistin were used for studies of antibacterial activity against two organisms: *E.coli* and *Streptobacillus sps.* Ampicillin and Penicillin inhibited the growth of *E. coli* and *Streptobacillus* sp.

Table 1. Antibacterial activity of gold nanoparticles synthesized from Jackfruit

Antibiotics	Diameter of zone of inhibition in mm			
	Organisms			
	E.coli		*Streptobacillus*	
	A	A+J	A	A+J
Amphicilin	15	17	13	15
Penicillin	14	18	14	16
Bavistin	0	0	0	0

A – Antibiotic; A+J – Antibiotic coated with nanoparticles synthesized from Jackfruit

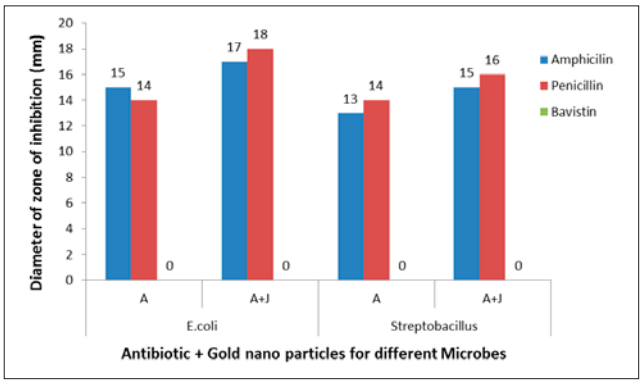

Figure 6. Antibacterial activity of gold nanoparticles synthesized from Jackfruit

Ampicillin effect was stronger against *E.coli* and weaker against *Streptobacillus sp.*, compared to Pencillin (15 and 13 mm, respectively). However, the activity of both Pencillin and Ampicillin against *E.coli* and *Streptobacillus* was greatly increased due to the presence of gold nanoparticles; the observed change was greater for Penicillin than Ampicillin.

Along with the gold nanoparticles, Penicillin showed inhibition against both bacteria stronger than Ampicillin. *E.coli* was inhibited more by Penicillin than *Streptobacillus.* Bavistin did not show any effect on either bacteria.

DISCUSSION

Reduction of gold ions into gold particles during exposure to the fruit extracts can be observed by change in colour. Gold nanoparticles exhibit a dark brown colour in aqueous solution due to the surface plasmon resonance phenomenon. Currently used methods, namely physical and chemical, carry several disadvantages and thus biosynthesis of nanoparticles from plant extracts is under investigation, as more eco-friendly and sustainable processes are sought for. Therefore, the development of biologically inspired preparation processes of nanoparticles has evolved into an important branch of nanotechnology. The presented study focuses on the use of plants with medicinal properties for synthesis gold nanoparticles [20].

The nanoparticles were primarily characterized by UV-Vis spectroscopy, allowing observation of the progress of the reaction. The results showed the reduction of Au ions in the aqueous solution of gold complex with the ingredients present in the plant leaf extracts, allowing correlation of the UV-Vis spectra with gold nanoparticles' formation. As the fruit extracts were mixed with the aqueous solution of the gold ion complex, the colour gradually changed to dark brown due to excitation of surface plasmon vibrations, which indicated the formation of gold nanoparticles [21]. UV-Vis spectra of the colloid suspensions of gold nanoparticles have been recorded as a function of time by using a quartz cuvette with gold chloroauric acid as the reference. In the UV-Vis spectrum, the broadening of peak indicated polydispersity of the particles' suspension. The reduction of gold ions and the formation of stable nanoparticles occurred within 2–3 hours of the reaction, making it one of the fastest bio-reducing methods to produce gold nanoparticles [22].

The surface Plasmon band in the gold nanoparticles solution remained close to 580 nm throughout the reaction, indicating that the particles were dispersed in the aqueous solution, with no evidence of aggregation. The results of SEM imaging proved that the gold nanoparticles were predominantly cubic with polyhedron shape. It is known that the shape of metal nanoparticles can considerably change their optical and electronic properties [23]. The SEM images also showed smaller, relatively spherical-shaped nanoparticles formed with diameter in the range of 20–25 nm. Energy dispersive spectrometry (EDS) micro-analysis was performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a sample. From the recorded EDS spectra, it was clear that gold nanoparticles were reduced by the *Artocarpus heterophyllus,* and the peaks of C, O, K and Na elements are most probably from the jackfruit extract. Chlorine peak, which was also observed, most certainly originates from the source of gold-chloroauric acid.

FT-IR peaks in the range from 4,000–400cm^{-1} confirmed the presence of main groups in natural plant extracts from *Artocarpus heterophyllus* Lam. (jackfruit). The observed bands corresponded to amines, amides, amino acids, indicating the presence of protein. Several other transmittance bands indicated the presence of biomolecules, e.g. carbohydrates, polysaccharides and lipids. Analysis of these spectra confirmed the formation of gold nanoparticles.

Food poisoning and rat bite fever are problems frequently occurring in developing countries and very often antibiotics are the only effective treatment for these diseases, as these infections do not elicit pronounced immune response, and hence effective vaccination may not be possible. Meanwhile, the traditional medicines, such as jackfruit extracts, have been used to treat these diseases for thousands of years. Moreover, the extract of this plant is also a very good bio-reductant which may be used in the synthesis of gold nanoparticles. In the presented study, the antibacterial activity of AuNPs against gram negative *E. coli* and *Streptobacillus* using agar well diffusion method was evaluated. The AuNPs exhibit significant inhibition against *E. coli* and *Streptobacillus* in comparison with standard antibiotics (Tab. 1). In addition to antimicrobial activity, the leaf extract of *Artocarpus heterophyllus* shows significant antioxidant [24], anticonvulsant, as well as wound healing properties [25].

CONCLUSION

This novel and feasible method of biosynthesis of gold nanoparticles offers a valuable contribution in the area of green synthesis and nanotechnology, without any additional physical and chemical procedures. *Artocarpus heterophyllus* fruit extract was prepared and successfully employed for the reduction of gold ions into gold nanoparticles of both spherical and polyherdon shapes for small and larger structures, respectively. FT-IR study showed absorption bands corresponding to the main functional groups present in natural plant extracts. The average particle size of AuNPs was found to be between 20 nm – 50 nm. The significant reduction in reaction time with fruit extract is an important result, enabling nanoparticle biosynthesis methods to compete with other routes for the formation of nanoparticles, which currently are much more rapid and reproducible.

The results of the presented study show that the combination of gold nanoparticles and antibiotics had synergistic antibacterial efficiency against the test microbes, in particular *E. coli*, and *Streptobacillus.*

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