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ORIGINAL RESEARCH ARTICLE

Bioinspired Gold Nanoparticle Synthesis Using *Terminalia bellerica* Fruit Parts and Exploring Their Anti-bacterial Potency In Vitro

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Abstract Gold nanoparticles with their excellent biocompatibility are extensively used in pharma and biological applications. *Terminalia bellerica* (TB) dry fruit parts mediated gold nanoparticles were synthesized using the aqueous extracts. The secondary metabolites screening of the aqueous extracts was done using phytochemical analysis. The green synthesized gold nanoparticles show vibrant colours. They were characterized using UV–Visible spectroscopy, FT-IR spectroscopy, XRD analysis and FE-SEM. The analytical characterization methods ensured the formation of nanoparticles and could predict the nanometric size of the nanoparticles. The study also lay to determine the antibacterial potential of the TB fruit parts and TB fruit parts mediated gold nanoparticles. The pathogens chosen for the study were pathogens from clinical species such as *Acinetobacter pneumonia*, *Bacillus subtilis*, and *Enterococcus faecalis* which cause common infections. The TB fruit part extracts, as well as TB fruit parts mediated gold nanoparticles were capable enough to destroy clinical pathogens.

Keywords *Terminalia bellerica* · Gold nanoparticles · Antibacterial activity · FE-SEM · Green synthesis

Introduction

Nanoparticles constitute primary building blocks for the several complex nanostructures. The agglomeration of atoms or molecules in the range of 1–100 nm is termed as Nanoparticles. The prime importance of nanotechnology is laid by nanoparticles and their intriguing application in diverse fields [1]. There are several metallic nanoparticles, of which Gold nanoparticles have paid more attention as it has numerous applications and characteristic physical and chemical properties [2]. Due to adverse effects of chemicals, synthetic chemical route for the synthesis of nano particles are not preferred by the research community. Hence plants and biological materials were chosen for the synthesis of nano materials [3]. In time, medicinal plant-mediated metal nanoparticle synthesis has been given much importance and gained extensive application in the field of pharma and medicine. There are numerous reports on nano particle synthesis methods, such as room temperature method, sonic bath method, microwave irradiation, solar irradiation and hydrothermal method [3–5]. Of these methods enlisted room temperature method is economical and it is employed in the present study for the synthesis of gold nanoparticles.

Reports say that plants are "chemical factories" as they are abundant in secondary metabolites. These are utilized in metallic and non-metallic nanoparticle synthesis as a redox mediator, capping agents, and stabilizers [6]. The nanoparticles thus synthesized are eco-friendly, biocompatible and simple [7–9]. Iron oxide yolk shell nanoparticles are reported to be more biocompatible than the

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commercial one [10, 11]. The greener approach of nanoparticle synthesis and the influence of secondary metabolites in the plants are the major reasons for utilizing plants as capping agents in metal nanoparticle synthesis. Also green chemistry approaches will helps for the rapid formation of nano structured materials [12].

Still, multi microbe-resistant broad-spectrum antibiotics with lesser side effects are a big challenge for researchers as microbes are capable enough to obtain resistance to the drugs [13]. Quorum sensing inhibitors such as plant extracts act as anti-pathogens [14]. Here comes the role of plant-mediated natural therapeutic agents. Natural therapy is always welcomed by human beings as they are harmless always. Indeed natural therapy like Ayurveda embraces in the tradition of India. Medicinal plants and the compounds derived from them have a therapeutic role in various infections [15]. This made us explore on the fruit parts of the medicinal fruit *Terminalia bellerica.* Also nano materials and nano composites possess significant biotechnological applications such as antimicrobial activities [16].

Terminalia bellerica Roxb. (Combretaceae) is universally called as Bahera or Bastard myrobalan is a large tree which is commonly seen in moist Indian sub valleys. Almost all the plant parts are of high medicinal values [17]. The present study focuses on the dry fruit and fruit parts of *Terminalia bellerica* (TB). *Terminalia bellerica* fruit is an active ingredient of the famous Ayurvedic formulation Triphala (Three fruits) which is taken as a medication for fever, cough, skin diseases, and liver disorders [17]. Fruits are used as medication in the treatment of hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, cough, hoarseness of voice, eye diseases, scorpion-sting and also used as a hair tonic [18, 19].

There are several reports on the metallic nanoparticle synthesis using the fruit of *Terminalia bellerica* and its biological activity. To our knowledge this is the first reported work on the synthesis of gold nanoparticles using the *Terminalia bellerica* aqueous extracts of fruit parts such as Epicarp, Mesocarp, and Seed (Figure S1) and its antimicrobial activity; comparing it with the whole fruit aqueous extract. The present study aims to identify the fruit part of TB responsible for the formation of gold nanoparticle and an efficient antibacterial agent.

Materials and Methods

The entire study was carried out using doubly distilled water. *Terminalia bellirica* (TB) dried fruit was purchased from the herbal shop in Palakkad district, Kerala. The dried fruit was segregated as Epicarp (TBOS), Mesocarp (TBIS), Seed (TBS) and one portion is used as whole dried fruit (TBP). All the fruit parts are pulverized using a blender and stored at room temperature. Chemicals of AR grade were used throughout the study. Gold chloride was purchased from HIMEDIA, India.

Preparation of Aqueous Extracts

Pulverized TB fruit parts (2 g) were mixed with 20 ml doubly distilled water. These solutions were kept in a steam bath for 20 min. The solution was filtered using a cotton plug. The obtained solutions from each fruit part were refrigerated and used up for further analysis within a week.

Phytochemical Analysis

All the four obtained aqueous extracts (TBOS, TBIS, TBS and TBP) were screened for presence of secondary metabolites adopting standard procedures [19, 20].

Gold Nanoparticle Synthesis

The synthesis of gold nanoparticles was carried out by following the standard procedure [21]. Synthesis method constitutes; treating equal volume of TB aqueous extracts with gold chloride (3mM) at room temperature (27 °C). The formation of the gold nanoparticles was ensured by the colour change of the extract (yellowish colour) to the vibrant colours. The time taken for the change in colour was noted.

Analytical Characterization of the Gold Nanoparticles

To explore and ensure the formation of TB aided gold nanoparticles, certain analytical characterization methods were carried out. The gold nanoparticles synthesized were purified by centrifuging (REMI AELC-1289) at 200 rpm for 10 min. The obtained centrifugate was used for the characterization. The residue was dried and used for antibacterial studies. The characterization methods include Bio spec Nano spectrophotometer (Shimadzu) which determines the Surface Plasmon Resonance (SPR) of the gold nanoparticles. The functional group in the extract responsible for the reduction of the gold chloride and therefore the formation of the gold nanoparticle was determined using the ATR-FTIR spectrophotometer (Shimadzu IR affinity). The nanoparticles were coated on a glass substrate and kept in ATR (ZnSe) of FTIR. The crystalline nature of the gold nanoparticles was explored using X-ray diffractometer (XPERT-PRO). The samples were coated on a glass substrate and subjected to XRD analysis. The microscopic nature and the surface morphology of the gold nanoparticles were identified using

Field Emission Scanning Electron Microscope (TESCAN MIRA3). The Nanoparticle coated glass substrate was gold-sputtered which possibly made it conducting.

Sample, Strain and Culture Media Preparation for Antibacterial Study

Aqueous extracts of *Terminalia bellirica* dry fruit parts (1 mg/ml), Gold nanoparticles capped using this extracts (1 mg/ml) and Standard drug (Amikacin 100 mg/2 ml) were conducted for bacterial activity against to clinical *Acinetobacter pneumonia*, *Bacillus subtilis*, and *Enterococcus faecalis*. Microbial cultures procured from Government Medical College, Tiruchirappalli, Tamil Nadu, India. Muller-Hinton agar media (Himedia Pvt. Mumbai, India) was used to feed the bacterial culture. Himedia zone reader was used to evaluate antibacterial activity.

Antibacterial Activity of Gold Nanoparticles

Antibacterial activity of formulations was studied by using a well-plate method. Clinical Pathogens from clinical samples *Acinetobacter pneumonia*, *Bacillus subtilis*, and *Enterococcus faecalis* inoculums were prepared by using nutrient broth media. Double strength sterile Mueller Hinton agar media were prepared by autoclaving 7.6 g in 100 ml. Using sterile cotton swabs, test pathogens were inoculated on the Mueller Hinton agar plates. *Terminalia bellirica* dry fruit part extracts 100 µg/µl (stock solution 1 mg/ml, from that 100 µl, was taken), synthesized gold nanoparticles 100 µg/µl (stock 1 mg/ml from that 100 µl, was taken) and Amikacin 100 µl/250 µg were placed on agar well. Plates are incubated for 30 min at the refrigerator to diffuse the formulation into the agar plate and finally, plates are incubated at 37 °C for 24 h. Zone of Inhibition (mm) was calculated to determine the antibacterial efficacy. Duplicate studies were done to ensure the reliability of the results.

Results and Discussion

Phytochemical Screening of *Terminalia bellirica* Fruit Parts

Secondary metabolite screening is carried out for the aqueous extracts prepared using the fruit parts of the *Terminalia bellirica*. The result obtained is given in Table S1.

The phytochemical screening revealed presence of flavonoids in all the fruit parts of TB. Also, TBP contains Phenols and Tannins. But only TBOS shows the presence of Phenols, whereas TBOS and TBIS showed the presence of Tannins. The decoction of the TB fruit is taken for Cough [22]. This portrays that the effect of consuming the decoction of the whole fruit of TB can be obtained even if TBOS decoction is administered. The aqueous extracts show only the presence of these mentioned secondary metabolites in the TB fruit parts. Petroleum ether, Chloroform, Ethyl acetate, Acetone, Methanol, Ethanol extracts of the TB fruit parts shows the presence of Alkaloids, Glycosides, Steroids, Cardiac glycosides, Phenolics, Terpenoids, Fatty acid ester, Free acids, Lignans, Phlobatannins and Carbohydrates [23]. This result shows that a specific solvent extraction method has to be carried out for the effective isolation of the compounds from the fruit parts.

TB Dry Fruit Mediated Gold Nanoparticle Synthesis

The reduction of Au^{3+} to Au^{0} was carried out using the aqueous extracts of *Terminalia bellirica* fruit parts. The aqueous extracts of the TB fruit parts have potential secondary metabolites. This is the reason aqueous extract utilization for the gold nanoparticle synthesis. Preliminary confirmation of gold nanoparticle formation was carried out by the colour change of the colloidal solution. Table 1 shows the colour of the gold nanoparticle and the time taken for the formation.

From the obtained results it is clear that all the fruit parts of TB show vibrant colours by the formation of the Gold Nanoparticles. Instant synthesis of gold nanoparticles from the fruit parts of TB is possible at room temperature. The difference in colour can be attributed to the size and shape of the Nanoparticles. The time taken for the formation of gold nanoparticles is more for TBS aqueous extract. This might be due to the constituents present in the seed extract being less efficient to reduce the Au ions. Whereas the time of formation is instantaneous for all other aqueous extracts. Also, each extract contains different metabolites. The activity of the metabolites present in the aqueous extracts involved in the reduction of the Au^{3+} to Au^{0}. Several potent compounds have been isolated from the fruit extracts of TB [24]. These compounds might be the reason for the reduction of gold chloride. However, studies on the isolation of the compounds from the aqueous extracts of the TB fruit parts are warranted to elucidate the possible mechanism for the formation of gold nanoparticles.

Analytical Characterization of Gold Nanoparticles

UV–Visible Spectroscopy

The SPR, one of the important parameters that defines the property of the gold nanoparticles was located at 530–550 nm (Fig. 1) for TB fruit part-mediated gold nanoparticles,

Table 1 Color and time of formation of TB fruit parts mediated gold nanoparticles

Sample code	Concentration of gold chloride: extract	Colour of gold nanoparticle	Time required for the formation of gold nanoparticle (s)
TBOSGNP	1:1	Navy blue	3
TBISGNP	1:1	Green	3
TBSGNP	1:1	Greyish blue	261
TBPGNP	1:1	Pink	2

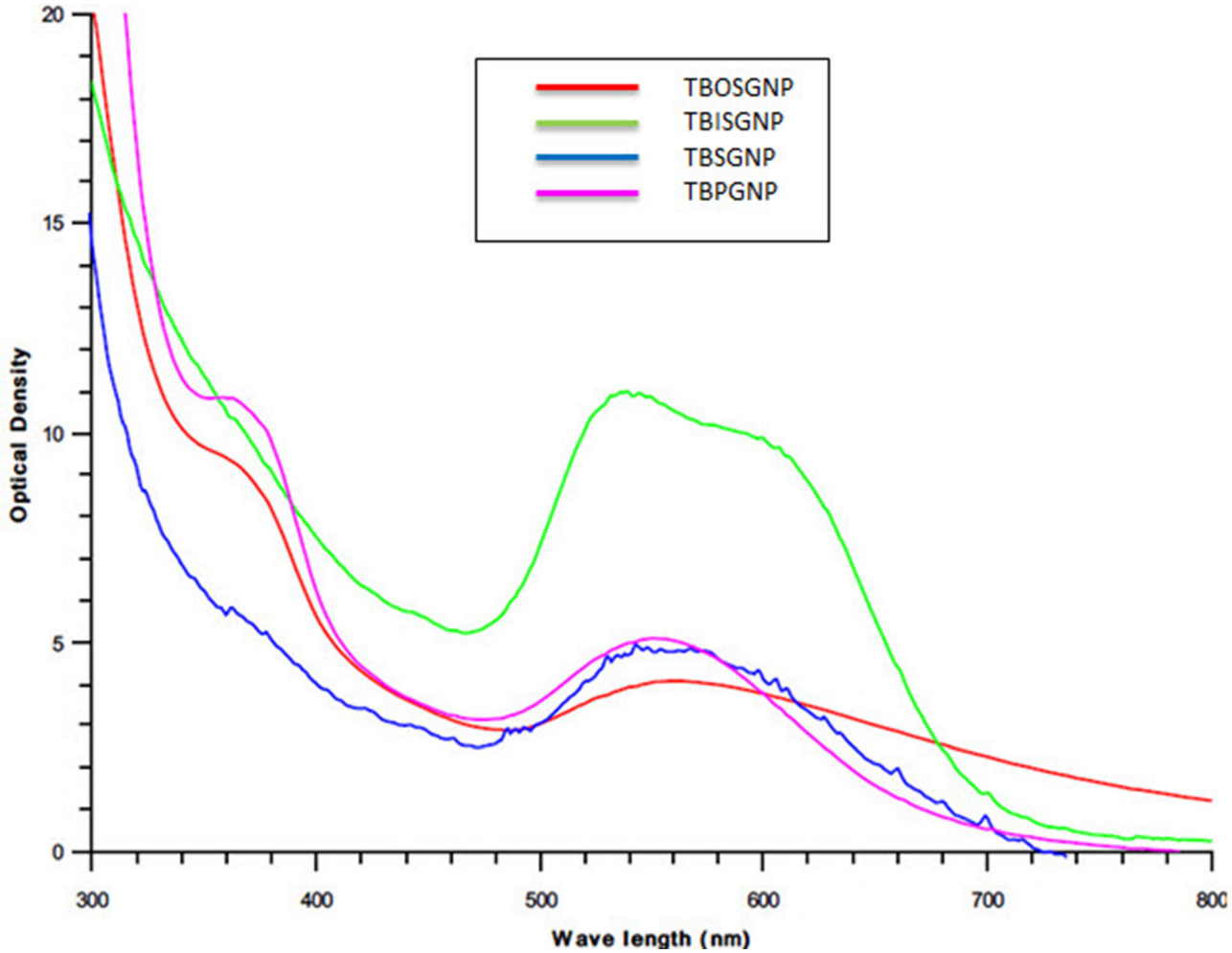

Fig. 1 UV–visible spectra of TB mediated gold nanoparticles

ensuring its formation. Cumulative oscillations of the free electrons in a material are termed as a plasmon. Redshift of SPR in the gold nanoparticles, in general, is due to the extinction cross-section. SPR is sensitive to stabilizing agents [25]. The perfect SPR obtained in this study ensures the efficacy of the TB fruits parts as an excellent stabilizing agent for the synthesis of gold nanoparticles. Also, it ensures the nanometric size of the gold nanoparticles. The wavelength vs. optical density (OD) relationship clearly defines the concentration of the nanoparticles. The higher values of OD in our results determine highly concentrated gold nanoparticles.

FT-IR Spectroscopy

The FT-IR spectra give the details about the functional group present in the materials. The spectra of gold nanoparticles give information regarding the functional groups which are responsible for the reduction of Au(III) to Au(0). Comparing the spectra of TBOS and TBOSGNP,

(Figure S2) the weak bands at 1126 cm^{-1} and 1381 cm^{-1} in TBOS corresponds to the C-O stretching which is of any primary alcohol group which would have reduced during the formation of the gold nanoparticles and these peaks are not observed in the spectra of TBOSGNP. Similarly while comparing the extract of TBIS and TBISGNP (Figure S2) strong intense peak at 1643 cm^{-1} in the spectra of TBIS corresponds to C of monosubstituted group. Whereas a new weak peak at 2129 cm^{-1} in TBISGNP corresponds to C=C=C group, which is not observed in the spectra of TBIS. The spectra of TBS and TBSGNP(Figure S2) shows the common peak at 1635 cm^{-1} and 3556 cm^{-1} corresponding to C=C (Str) and O–H (Str) respectively (Figure S2). The phenolic and flavonoid groups are the possible bioreductants of gold chloride as they are the major secondary metabolites present in the extracts. Metal peak in the range of 400–500 cm^{-1} ensures the formation of gold nanoparticles.

XRD Analysis

The crystalline nature of the green synthesized nanoparticles was determined using XRD analysis. Figure S3 shows the XRD pattern of the gold nanoparticles. The 2θ values of TBOSGNP, TBISGNP, TBSGNP and TBPGNP are 27°, 27°, 28° and 27° of face centered cubic lattice. The crystallite size of the nanoparticles is calculated from Debye-Scherrer's equation (Table 2).

FE-SEM Analysis

Surface morphology and topography of TB mediated green synthesized nanoparticles were determined using FE-SEM analysis. The nanometric size of the nanoparticles was determined from FE-SEM monograph (Fig. 2). TBOSGNP shows closely packed spherical shaped nanoparticles. The size of the nanoparticles ranges from 13nm-25nm. Also the monograph was recorded applying 30 kV which ensures the stability of the nanoparticle as it withstands the electrical potential. This implies the nanoparticles to be stable and doesn't contain any of the constituents of the extracts at higher kV (Fig. 2a). Sputtering process would have removed the impurities of the plant residue.

Similarly, TBISGNP shows spherical shaped particles with a particle size of about 25nm-40nm. Particles could withstand the potential of 20 kV. Compared to TBOSGNP, TBISGNP shows scattered spherical particles and hexagonal particles. (Fig. 2b). TBSGNP shows multi-shaped particles such as flakes, triangular and spherical nanoparticles. These particles withstand only 15 kV of electrical potential. This portrays that TBSGNP is less stable compared to other gold nanoparticles. The size of the nanoparticles ranges from 21 to 49 nm (Fig. 2c). TBPGNP monograph shows perfectly aligned nano balls (Fig. 2d). This is the first report on the nano ball structure of TB whole fruit mediated gold nanoparticles. The size of the nano balls ranges from 29 to 89 nm. The particles are stable and they could withstand the electrical potential of 25 kV. The spherical shapes of the TB fruit parts mediated gold nanoparticles shows the efficiency of gold nanoparticles as potential pharmaceutical agents.

Anti-bacterial Efficacy of *Terminalia bellerica* Mediated Gold Nanoparticles

The antibacterial potency of the aqueous extracts of TB fruit parts and the green synthesized gold nanoparticles from TB fruit parts were carried out by well diffusion methods.

TB mediated gold nanoparticles show inhibition towards the clinical pathogens (Table S2). Amikacin was employed as the standard for the study which is administered for the destruction of a broad spectrum of pathogens. Clinical *Acinetobacter pneumonia* shows resistance to both aqueous extracts of TB fruit parts and TB fruit parts mediated gold nanoparticles. No zone of inhibition (ZOI) was observed for this specific pathogen.

A ZOI 10mm has been observed for TBOS and TBP aqueous extracts against *Enterococcus faecalis* pathogen. While TBPGNP shows 12mm ZOI against *Enterococcus faecalis*, which clearly shows TBPGNP to be more effective as an antibiotic against *Enterococcus faecalis* than TBP aqueous, extract (Figure S5). Clinical *Bacillus subtilis* were resistant towards the aqueous extracts of TB fruit parts (Figure S4). Bacillus is known to compete with other microbes as it helps in its survival [26] A ZOI 10 mm and 14 mm was observed for TBOSGNP and TBPGNP respectively. This shows the efficiency of the TB aided gold nanoparticles as anti-bacterial agents towards both clinical gram-positive and gram-negative bacterias. It is

Table 2 Crystallite size of TB fruit parts mediated gold nanoparticles

S. no.	Sample	2θ (°)	Cos θ	$\beta = \pi$*FWHM/180(radians)	$D = k\lambda/\beta \cos\theta$ (nm)
1	TBOSGNP	27.8841	0.9705	0.0049	27.00
2	TBISGNP	27.6130	0.9711	0.0056	23.50
3	TBSGNP	28.2747	0.9697	0.0010	12.60
4	TBPGNP	27.8314	0.9706	0.0052	25.38

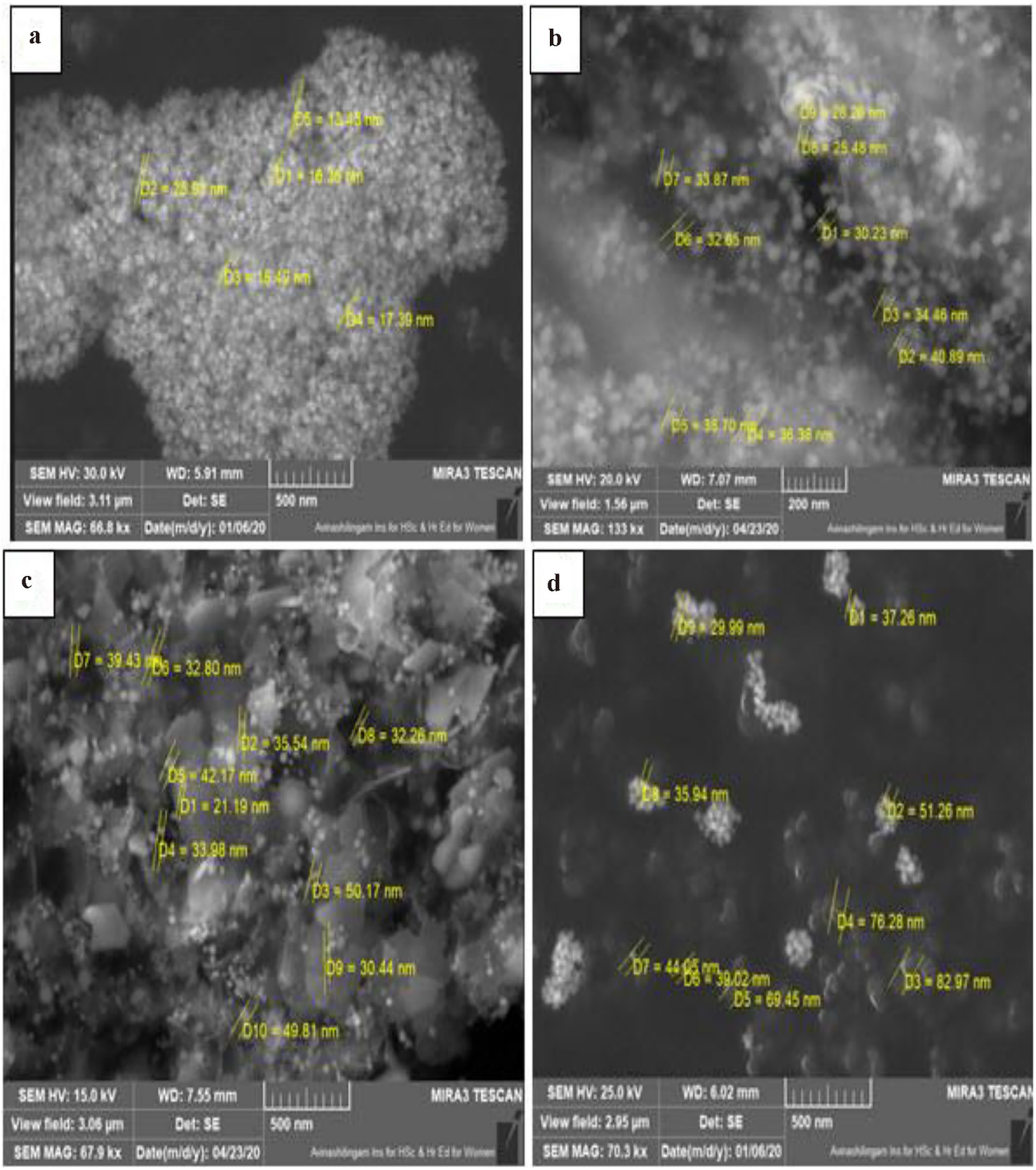

Fig. 2 FE-SEM monographs of (**a**) TBOSGNP (**b**) TBISGNP (**c**) TBSGNP and (**d**) TBPGNP

reported that nano structures enhance the bacterial cell adhesion [27].

Gold nanoparticles are very much efficient to fight against pathogens. Anti-bacterial efficiency of the gold nanoparticles increases when they attach to the cell wall of the pathogens; this is due to the surface charge. There happens to a surface modification in the bacteria and gold nanoparticles and this will gradually lead to protein, DNA and mitochondrial destruction and then cell death [28]. The synergistic effect of the plant metabolites might be the reason for the anti-bacterial efficiency of the TB fruit parts mediated gold nanoparticles.

Table S3 shows the previously reported works on the anti-microbial activity with the TB fruits and TB fruit mediated gold nanoparticles. The literature show all the solvent extracts to have antimicrobial activity against almost all gram-positive and gram-negative bacteria. TB fruit is categorized to the list of broad-spectrum antibiotics [29]. TB fruit extracts and TB fruit mediated gold nanoparticles show good antimicrobial activity [30, 31]. This is the first reported work on the anti-bacterial activity of the fruit parts of TB and TB mediated gold nanoparticles using the pathogens isolated from the clinical samples.

Pathogens obtained from the clinical samples would have obtained resistance to the drugs. Our study has proven that TBOS and TBP aqueous extracts are much efficient as anti-bacterial agents as they are showing ZOI against the chosen pathogens at 100 µg/µl. Also, the gold nanoparticles synthesized using these extracts show better antibacterial activity than the aqueous extracts. This study also reveals that aqueous extract of seed and mesocarp of the TB is not efficient antibacterial agents for the clinical pathogens such as *A.pneumonia, B.subtilis, and E. faecalis* with 100 µg/µl of the samples. The shape of the gold nanoparticles plays an important role in the prediction of the properties of the particles. In this study, the only spherical shaped particles show antibacterial efficiency against the test pathogens i.e., TBOSGNP and TBPGNP.

Conclusions

Instant green synthesis of gold nanoparticles was accomplished using the common dry fruit parts of *Terminalia bellirica*. Each fruit part such as Epicarp, Mesocarp and seeds of TB have produced different coloured gold nanoparticles instantaneously at room temperature. The phytochemical screening of the aqueous extracts of the whole fruit and the fruit parts shows different secondary metabolites. The synthesized gold nanoparticles have spherical, triangular, flakes and nano balls shapes. The analytical characterization methods such as UV–Visible spectroscopy, FT-IR, XRD and FE-SEM helped to predict the formation and nano size of the nanoparticles. The study also determines the efficiency of TB fruit parts and TB fruit part mediated nanoparticles as anti-bacterial agents using agar well diffusion method. The anti-bacterial potency was determined against pathogens isolated from the clinical samples *Acinetobacter pneumonia, B. subtilis* and *Enterococcus faecalis*. The TB fruit parts mediated gold nanoparticles are capable enough to fight against both gram-positive and gram-negative bacterias. TB fruit is known to be anti-bacterial, but the study shows that the Epicarp of the fruit is equally good as the whole fruit than the seed part and Mesocarp. However, the active constituent responsible for the reduction of Au^{3+} to Au^{0} and the anti-bacterial efficiency is yet to be determined and it is warranted.

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Declarations

Conflict of interest Authors declare there is no conflict of interest.

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