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UV–Vis detection of *E. coli* 0157:H7 using *Vitis vinifera* and *Musa paradaisica* modified Au-NPs

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ABSTRACT

Herein, we demonstrate the simple one-pot novel green synthesis of gold nanoparticles (Au-NPs) functionalised with a combination of banana peel (*Musa paradaisica*) and grape (*Vitis vinifera*) fruit extracts. The reaction mixture of aqueous gold chloride, banana peel and grape extracts revealed a purple colour after a reaction time of one hour, an indication of the presence and the successful synthesis of gold nanoparticles. The optical and structural properties of the green synthesized nanoparticles were analysed using Ultraviolet-Visible spectroscopy (UV–Vis) and Fourier Transform Infrared Spectroscopy (FTIR) while their surface morphology was determined using X-Ray Diffraction (XRD), High-Resolution Transmission Microscopy (HRTEM) and Small Angle X-Ray (SAX). Furthermore, a quick and simple surface plasmon resonance (SPR) study in the form of an optical sensor for the detection of *Escherichia coli* 0157:H7 strain was also achieved using UV–Vis. The obtained limit of detection (LOD) value for SPR for the GBPE|Au-NPs|GCE-based system was found to be 1×10^2 CFU/mL, a value well in the range for detection in seawater.

- Green synthesis of gold nanoparticles (Au-NPs) was functionalised using banana peel (*Musa paradaisica*) and grape (*Vitis vinifera*) fruit extracts as capping and stabilizing agents.
- Structural characterization of the Au-NPs was achieved using Ultraviolet-Visible spectroscopy (UV–Vis) and Fourier Transform Infrared Spectroscopy (FTIR) while their surface morphology was determined using X-Ray Diffraction (XRD), High-Resolution Transmission Microscopy (HRTEM) and Small Angle X-Ray (SAX).
- The green synthesized Au-NPs were used to detect *Escherichia coli* 0157:H7 (*E. coli* 0157:H7) strain using Ultraviolet-Visible spectroscopy (UV–Vis) where the surface plasmon resonance (SPR) was studied.

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Materials and Methods

Reagents and materials

Chemicals including gold auric chloride ($AuCl_3$, 99.9 %), sodium phosphate monobasic dihydrate (NaH_2PO_4•$2H_2O$: ≥99 %) and sodium phosphate dibasic dihydrate (Na_2HPO_4·$2H_2O$: ≥99.5 %) were purchased from Sigma Aldrich, Kempton Park, Johannesburg, South Africa. Fresh bananas and grapes used as capping and stabilizing agents in the synthesis of the nanoparticles were purchased from Parow Checkers Hypermarket, Cape Town, South Africa. The electrolyte and stock solutions used throughout this study was pH 7.4, 0.2 M phosphate buffer (PBS). PBS was prepared using (NaH_2PO_4•$2H_2O$) and (Na_2HPO_4•$2H_2O$). De-ionised ultra-purified water prepared with a Milli-Q water purification system procured from Merck KGaA, Darmstadt, Germany.

Preparation of the banana peel and grape extract

1. Separate banana peels from bananas and finely cut them.
2. Wash the cut banana peels repeatedly using ultra-purified water to remove any dust and organic impurities.
3. Weight 100 g of the cut banana peels and place them into a beaker.
4. Separately wash seedless grapes using ultra-purified water to remove any dust and organic impurities.
5. Weigh 50 g the seedless grapes and chop them finely and place them in a separate beaker from the cut banana peels.
6. Add 300 mL ultr-purified water to each fruit-containing beaker and heat each beaker at 80 °C and stir at 1000 rpm for 30 min.
7. After boiling, place the extracts room temperature and allow each to cool and then centrifuge each at 1000 rpm for 30 min.
8. Filter the contents of each beaker twice to remove any insoluble fractions and macromolecules.
9. Create a banana peel and grape extract mixture using the ratio of 1 (banana peel extract):3 (grape extract) known as (GBPE).
10. Heat the mixture at 60 °C and stir at 1000 rpm for 30 min.
11. Store the GBPE extract in a secure container and place it in a refrigerator at 4 °C if the mixture is not used immediately.

Synthesis of Gold nanoparticles (Au-NPs).

1. Prepare a stock solution of 2.0×10^{-5} M $HAuCl_4$ in 100 mL ethanol.
2. Add 50 mL of the $HAuCl_4$ stock solution with 50 mL of the GBPE.
3. Boil mixture for 1 h at 60 °C and stir at 1000 rpm. This is considered the synthesis of the nanoparticles.
4. The whole duration of the synthesis should be conducted on a sand bath.
5. One the mixture turns a purple-colour, stop the reaction as this is a signal of the successful synthesis of gold nanoparticles.
6. Store the Au-NPs in a secure container and place it in a refrigerator at 4 °C if the mixture is not used immediately.

Bacterial preparation

1. *E. coli* 0157:H7 was chosen as the target organism.
2. The bacterial stocks of *E.coli* 0157:H7 are available at the University of the Western Cape's Biotechnology Department.
3. Prepare a bacterial suspension by inoculating a single colony of *E. coli* glycerol stock into 20 mL of Luria broth (LB) media.
4. Place this in a shaking incubator set at 37 °C and allow the bacteria to grow for 24 h.
5. Thereafter, perform serially diluted of the bacteria between 10° and 10^{16} CFU/mL using LB media in placing each concentration in a different test tube.
6. Then plate 100 µL pf each diluting on Luria agar (LA) media plates.
7. Incubated the plates at 37 °C overnight and observe microbial clouds of growths on each plate.
8. Count the number on each plate to determine the colony-forming units per millilitre (Eq. (1)) [1,2].

$$E.coli/100\ \text{mL} = \frac{\text{Number of E.coli colonies}}{\text{Volume of sample (mL)}} \times 100 \quad (1)$$

9. Add the different bacteria volumes (0–100 µl) each to 2000 µl drinking water that each contain 100 µl of Au-NPs.
10. Detect each set of bacteria volumes (calculated as concentrations) optically using UV–vis in the wavelength range of 400 nm–800 nm.

Method validation

The optical properties of the synthesized Au-NPs were studied using Ultraviolet-Visible (UV–Vis) Spectroscopy (with a Nicolett Evolution 100) from Thermo Electron Corporation, Johannesburg, South Africa) at wavelengths between 200 nm and 650 nm). Prior to analysis, approximately 2 mL of the synthesized Au-NPs were placed in a 3.5 mL visible cuvette cell 3.5 ml and place into the spectrometer for analysis. UV–Vis was used to follow the reduction of aqueous $HAuCl_4$ ions during the reaction with GBPE. A strong absorption peak at 535 nm was observed validating the presence of Au-NPs which was also seen to gradually increase during the synthesis of the nanoparticles with increased reaction time as shown in Fig. 1. The UV–vis spectra show the reduction of Au^{3+} to Au^0 specifying the formation of Au-NPs starting from the completion time of 10 min [3,4]. Due to the interactions of charge transfer between the chloroligands of $HAuCl_4$ and the metal Au, a shoulder at 279 nm was observed as the reaction time increased from 10 min to 60 min coupled with an intense purple colour change in the reaction media over time. Consequently, the shoulder at 279 nm disappeared due to binding of amine groups from the extract to the nanoparticles. The observed maximum peak after each time interval coupled to the gradual increase of λ_{max} indicated a blueshift [5–6]. The results indicate that the time needed for the formation of Au-NPs using grape banana extract is very fast. These findings are in good agreement with a study conducted by Aljabali et al., 2018 who achieved the formation of gold nanoparticles within 3 min using leaf extract of *Ziziphus zizyphus* [7].

In this study, the physical properties of Au-NPs were studied using Fourier Transform Infrared Spectroscopy (FTIR) using a PerkinElmer Spectrum 100-FT-IR Spectrometer from PerkinElmer (Pty) Ltd, Midrand, South Africa. A small drop of dried Au-NPs was mixed with a small quantity of KBr and placed on a FTIR plate followed by placing a second on top in order to obtain an even film. The plate was then placed into the FTIR sample holder and the measurements were performed in the FTIR transmission mode. FTIR was used to identify the type of functional groups that were responsible for the reduction of Au^+ ions and to also identify the type of functional groups that are coated or capped on Au-NPs (Fig. 2). From the FTIR spectra, the presence of the sharp O-H peak at 3323 cm^{-1} represents O-H stretching vibrations from alcohols and phenols present in the GBPE. The sharp peak at 2927 cm^{-1} in GBPE represents the O-H stretching vibration bands characteristic of carboxylic acids and C-H stretches from alkanes. Thus, the

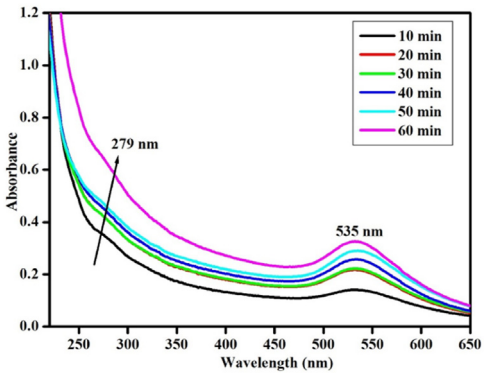

Fig. 1. UV–Vis spectra of GBPE|AuNPs at different time intervals.

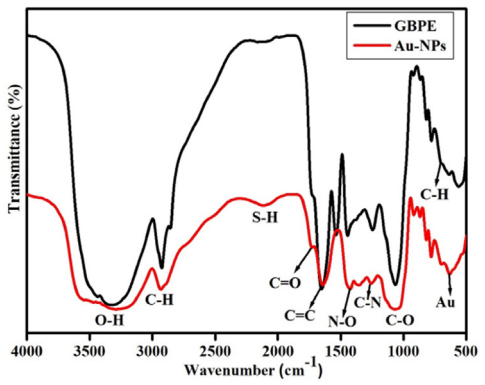

Fig. 2. FT-IR spectra of the green synthesised GBPE|AuNPs and GBPE extract.

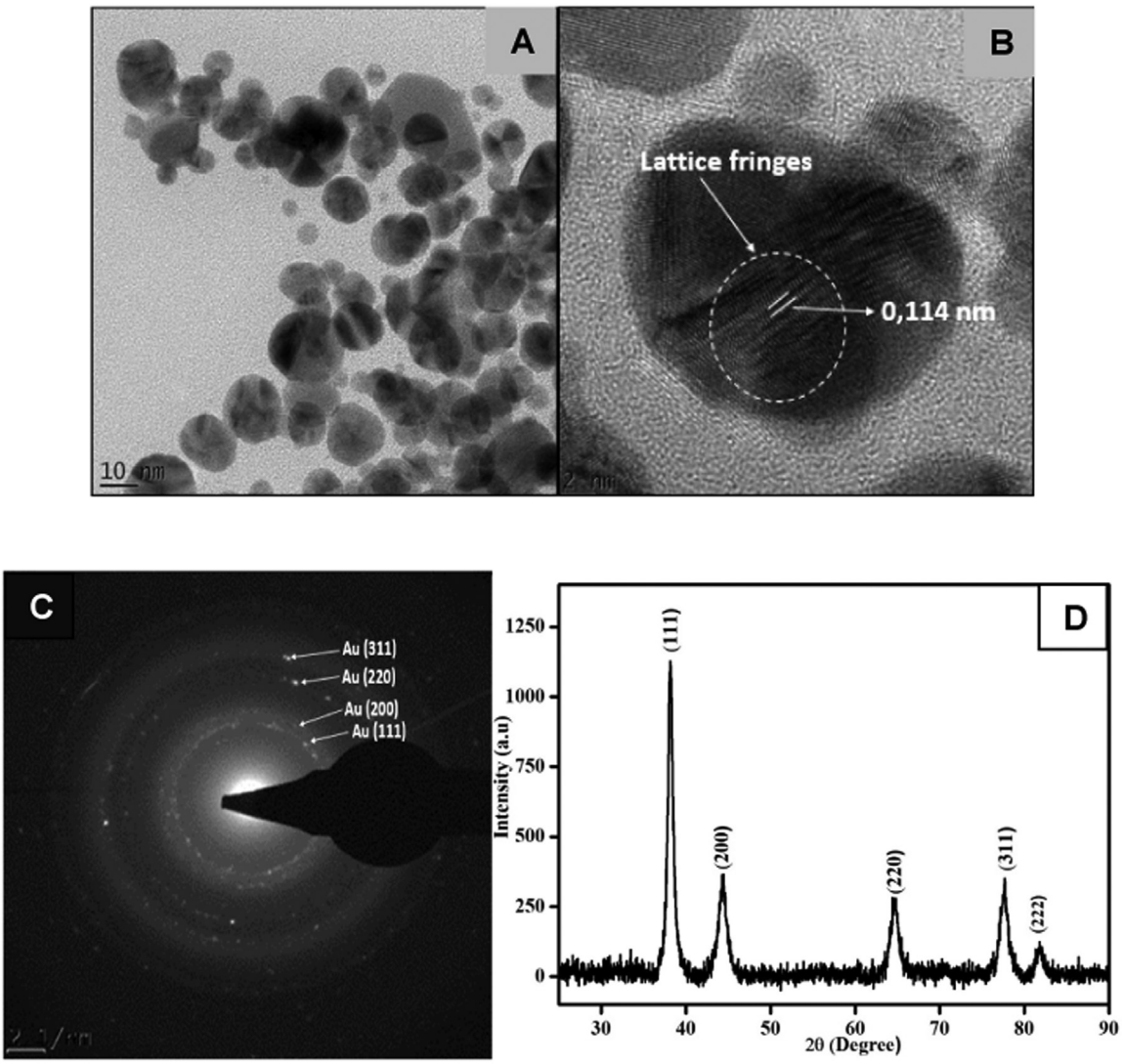

Fig. 3. (a) HRTEM (b) Lattice fringes, (c) SAED pattern and (d) XRD pattern of GBPE|AuNPs.

synthesised Au-NPs are stabilized and coated by functional groups that are present in the GBPE. [8,9]. The FTIR spectrum of Au-NPs showed a band at 3270 cm^{-1} for hydrogen-bonded stretching vibrations attributed by phenol and alcohol groups. The weak peak at 2093 cm^{-1} is due to thiols S-H stretching vibrations [10] indicating the presence of the banana peel in the GBPE extract. The peak observed at 1725 cm^{-1} was assigned as the stretching vibration of C=O from carboxylic acids (found in bananas), ketones (found in grapes) and aldehydes (found in grapes). The peak at 1633 cm^{-1} is characteristic of C=C stretching vibrations attributed to alkene groups (found in grapes) [10,11]. The weak peak at 636 cm^{-1} corresponds to asymmetric stretching vibrations of CH groups in the GBPE as shown in the spectrum but seem to disappear in the spectrum of Au-NPs due to its participation in the synthesis process. The Au-NPs spectrum also indicates another peak at 585 cm^{-1} caused by the stretching vibrations of the nanoparticles thus validating the formation of the nanoparticles [12,13]. Similar findings are reported by Divikaran *et al.*, who synthesised Au-NPs using Dragon fruit extract [14]. Recently, a study by *Ahmad at el.*,2019 using oil palm leaf (*Eaeis guineensis*) extract for the synthesis of gold nanoparticles revealed similar FTIR functional groups for the gold nanoparticles attributed to the presence of polyphenolic and phytochemical compounds, corresponding to O-H, C= O and N-H from amines in the extract [15].

High-Resolution Transmission Electron Microscopic (HRTEM) analysis of the nanoparticles were performed with a Tecnai G2 F2O X-Twin HRTEM, purchased from FEI Company, Hillsboro, OR, United States of America. The Au-NPs sample was dissolved in ethanol and then moved onto a HRTEM grid followed by analysis. HRTEM was used to determine the size and the shape of the bio-synthesized Au-NPs. Fig. 3(a) shows the HRTEM image of the GBPE capped Au-NPs to be predominantly poly-dispersed and spherical in shape with sizes ranging from 10 to 17 nm [16]. Fig. 3(b) shows lattice fringes of the nanoparticles which were found to be 0.114 nm, while Fig. 3(c) shows the selected area electron diffraction (SAED) illustrating the following four Braggs reflection rings, (111), (200), (220),

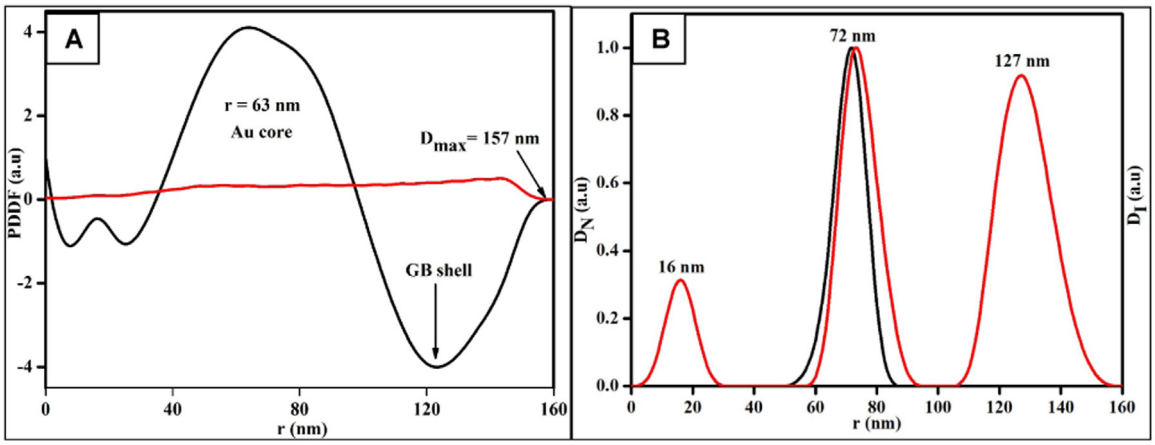

Fig. 4. (a) SAXS pair-distance distribution function (PDDF) of GBPE|AuNPs (b) Size distribution functions weighted by number (black line) and intensity (red line).

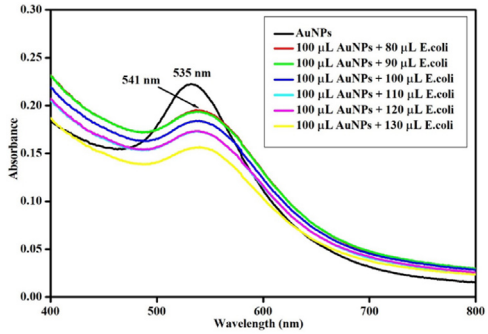

Fig. 5. Detection of *E.coli* 0157:H7 in water, GBPE|AuNPs before the interaction (Black line) with the interaction *of E.coli* at different concentrations $10^1 - 10^6$.

and (311). The SAED pattern is in good agreement with XRD (Fig. 3(d)) findings where intense Bragg reflection peaks at 38.2, 44.4, 64.6 and 77.5 where observed; with an additional peak at 81.7 corresponding to the additional (222) crystal planes [17,18]. Bindhu and Umadevi, 2014 illustrated similar HRTEM and XRD data in a study involving the synthesis of Au-NPs using *Ananas comosus* [19].

Additionally, Small Angle X-Ray Spectroscopy (SAXS) from Anton Paar Southern Africa (PTY) Ltd, Midrand, South Africa. SAX was used to determine the precise size distribution, shape, and the internal arrangement of the synthesized GBPE capped Au-NPs. SAXS analyses were outsourced and conducted by experts in this field of sample analysis in which x-ray beams were focused on the Au-NPs sample in order to allow dispersion. As seen in Fig. 4(a), the SAXS free model pair-distance distribution function (PDDF) of the GBPE capped Au-NPs revealed a characteristic profile typically observed for core-shell particles with different sizes. Fig. 4(b) reveals the size distribution weighted by number (black line) and intensity (red line) where the Au-NPs revealed highly poly-dispersed particles with average radii of (16 nm, 72 nm and 127 nm), respectively [20,21]. About 80 % of the particles appeared at 72 nm for both size distribution functions weighted by number and intensity, while the size of 16 nm is consistent with the determined values as reported by HRTEM. However, the appearance of an enhanced peak at 127 nm was ascribed to distribution by intensity. Often, colloidal suspensions consist of poly-dispersed particles and the measured scattering intensity represents the sum of the scattering intensities from particles of various sizes [21]. As such, SAXS was seen to complement the microscopic methodologies previously used since it provided structural information about a large sample of the Au-NPs [22].

Optical detection of E. coli *0157:H7*

Fig. 5 shows the UV–Vis spectra of GBPE|Au-NPs before and after their interaction with *E. coli* 0157:H7 at different concentrations. As seen from the image, the GBPE cappedcAu-NPs in the absence of *E. coli* 0157:H7, displayed an SPR band at 535 nm in water (black spectrum). However, in the presence of *E. coli* 0157:H7 in water, a reduction in the peak intensity can be observed with a redshift taking place with a new band indicated ay 541 nm. The decrease in peak intensity is the evidence of the interaction between GBPE|AuNPs and bacteria. The redshift after the addition of the bacteria, is an indication that the nanoparticles aggregate when they bind to *E. coli* 0157:H7. When the GBPE capped Au-NPs interacted with *E. coli* 0157:H7, the antioxidants in them which include, flavonoids, polyphenols and amines that surrounds the nanoparticles during synthesis, then bind to the cell wall of the bacteria

through amine, sulfhydryl (-SH) and thiol groups [22–24], which are part of the peptidoglycan,the main component of the bacterial cell wall [25,26]. After binding the bacteria consumes the GBPE capped Au-NPs then the capping molecules unbinds from the surface of gold nanoparticles, and this result in the gold nanoparticles become unstable and aggregate because the surface is no longer coated. The the Au^{+} ions are therefore released from the nanoparticles inside the bacteria, and damage the microorganism's DNA and cause oxidative stress, which leads to cell death [27–29].

It was found that as the concentration of the *E.coli* 0157:H7 increased, the SPR intensity decreased. This is caused by nanoparticle destabilisation, which is the depletion of stable nanoparticles. In this research study, the limit of detection was determined using Eq. (2). The limit of detection (LOD) is the lowest concentration that can be measured or detected, and it was found to be 10^2 CFU/mL [27–30].

$$\mathrm{LOD} = 3.3\frac{\mathrm{S}}{\mathrm{M}} \tag{2}$$

From Eq. (2), the coefficient, S represents the value of the standard deviation of the blank samples, and M is the slope of the standard curve within the concentration range.

Conclusion

A green and novel synthesis method for gold nanoparticles using a mixture of banana peel and grape extracts was achieved. The proposed procedure is simple and easy to scale up, with a green aspect, eliminating the need for any additional chemicals. The polydispersed and spherical nature of the nanoparticles were confirmed by FTIR, XRD, HRTEM and SAXS. Additionally, this method has proven to produce nanoparticles with excellent stability and prolonged shelf-life, which is a great requirement in various other applications of GBPE|AuNPs. This work presents a novel optical based sensor using green synthesized GBPE|AuNPs as a sensing medium as seen from their ability to optically detect *E.coli* 0157:H7 in water. As far as we know, the described procedure for the synthesis of Au-NPs using a mixture of grape-banana peel extracts has been demonstrated for the first time by this study.

Declaration of Competing Interest

All the authors declare that they have no known competing financial interests or personal relationships that could appear to influence the research study reported in this paper.

CRediT authorship contribution statement

R.F. Ajayi: Conceptualization, Writing – original draft, Supervision. **S. Nqunqa:** Conceptualization, Methodology. **N.P.P. Ngema:** Validation. **S.C.L. Barry:** Writing – review & editing. **U. Feleni:** Supervision, Writing – review & editing. **T. Mulaudzi:** Supervision, Writing – review & editing.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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References

[1] B. Mull, V. R. Hill, Recovery and Detection of Escherichia coli O157:H7 in Surface Water, Using Ultrafiltration and Real-Time PCR, Appl. Environ. Microbiol. 75 (2009) 3593–3597, doi:10.1128/AEM.02750-08.
[2] U. Dharmasiri, M.A. Witek, A.A. Adams, J.K. Osiri, M.L. Hupert, T.S. Bianchi, D.L. Roelke, S.A. Soper, Enrichment and detection of escherichia coli O157:H7 from water samples using an antibody modified microfluidic chip, Anal. Chem. 82 (2010) 2844–2849, doi:10.1021/ac100323k.
[3] M. Hassanisaadi, G.H.S. Bonjar, A. Rahdar, S. Pandey, A. Hosseinipour, R. Abdolshahi, Environmentally safe biosynthesis of gold nanoparticles using plant water extracts, Nanomaterials 11 (2021) 2033 (Basel), doi:10.3390/nano11082033E.
[4] H. Ismail, M.M.H. Khalil, F.A. Al Seif, F. El-Maghdoub, Biosynthesis of gold nanoparticles using extract of grape (Vitis vinifera) leaves and seeds, Prog. Nanotechnol. Nanomater. 3 (2014) 1–12.
[5] S.D. Mukundan, R. Mohankumar, R. Vasanthakumar, Comparative study of synthesized silver and gold nanoparticles using leaves extract of Bauhinia tomentosa Linn and their anticancer efficacy, Bull. Mater. Sci. 40 (2017) 335–344, doi:10.1007/s12034-017-1376-2.
[6] A. Waris, M. Din, A. Ali, S. Afridi, A. Baset, A.U. Khan, M. Ali, Green fabrication of Co and Co_3O_4 nanoparticles and their biomedical applications: a review, Open Life Sci. 16 (2021) 14–30, doi:10.1515/biol-2021-0003.
[7] A.A. Aljabali, Y. Akkam, M.S. Zoubi, K.M. Al-Batayneh, B. Al-Trad, O. Abo Alrob, A.M. Alkilany, M. Benamara, D.J. Evans, Synthesis of gold nanoparticles using leaf extract of ziziphus zizyphus and their antimicrobial activity, Nanomaterials 8 (2018) 174, doi:10.3390/nano8030174.
[8] S.S. Dash, B.G. Bag, Synthesis of gold nanoparticles using renewable Punica granatum juice and study of its catalytic activity, Appl. Nanosci. 4 (2014) 55–59, doi:10.1007/s13204-012-0179-4.

[9] Y.T. Gebreslassie, H.G. Gebretnsae, Green and cost-efective synthesis of tin oxide nanoparticles: a review on the synthesis methodologies, mechanism of formation, and their potential applications, Nanoscale Res. Lett. 16 (2021) 97, doi:10.1186/s11671-021-03555-6.
[10] S.P. Akhlaghi, B. Peng, Z. Yao, K.C. Tam, Sustainable nanomaterials derived from polysaccharides and amphiphilic compounds, Soft Matter 9 (2013) 7905–7918, doi:10.1039/c3sm50358e.
[11] H. Duan, D. Wang, Y. Li, Green chemistry for nanoparticle synthesis, Chem. Soc. Rev. 44 (2015) 5778–5792, doi:10.1039/c4cs00363b.
[12] A. Bankar, B. Joshi, A.R. Kumar, S. Zinjarde, Banana peel extract mediated novel route for the synthesis of silver nanoparticles, Colloids Surf. A Physicochem. Eng. Asp. 368 (2010) 58–63, doi:10.1016/j.colsurfa.2010.07.024.
[13] U. El-Nafaty, I. Muhammad, S. Abdulsalam, Biosorption and kinetic studies on oil removal from produced water using banana peel, Civ. Environ. 3 (2013) 125–136 http://iiste.org/Journals/index.php/CER/article/view/6125.
[14] M. Tammer, G. Sokrates, Infrared and Raman characteristic group frequencies: tables and charts, Colloid Polym. Sci. 283 (2004) 235–235, doi:10.1007/s00396-004-1164-6.
[15] D. Divakaran, J.R. Lakkakula, M. Thakur, M. Kumar Kumawat, R. Srivastava, Dragon fruit extract capped gold nanoparticles: synthesis and their differential cytotoxicity effect on breast cancer cells, Mater. Lett. 236 (2019) 498–502, doi:10.1016/j.matlet.2018.10.156.
[16] T. Ahmad, M.A. Bustam, M. Irfan, M. Moniruzzaman, H.M. Asghar, S. Bhattacharjee, Green synthesis of stabilized spherical shaped gold nanoparticles using novel aqueous elaeis guineensis (oil palm) leaves extract, J. Mol. Struct. 1159 (5) (2018) 167–173, doi:10.1016/j.molstruc.2017.11.095.
[17] a S. Khan, J. Bakht, F. Syed, Green synthesis of gold nanoparticles using acer pentapomicum leaves extract its characterization, antibacterial, antifungal and antioxidant bioassay, Dig. J. Nanomater. Biostruct. 13 (2018) 579–589.
[18] T. Ahmad, M.A. Bustam, M. Irfan, M. Moniruzzaman, H.M. Asghar, S. Bhattacharjee, Green synthesis of stabilized spherical shaped gold nanoparticles using novel aqueous elaeis guineensis (oil palm) leaves extract, J. Mol. Struct. 1159 (2018) 167–173, doi:10.1016/j.molstruc.2017.11.095.
[19] M.R. Bindhu, M. Umadevi, Antibacterial activities of green synthesized gold nanoparticles, Mater. Lett. 120 (2014) 122–125, doi:10.1016/j.matlet.2014.01.108.
[20] M. Irfan, T. Ahmad, M. Moniruzzaman, S. Bhattacharjee, B. Abdullah, Size and stability modulation of ionic liquid functionalized gold nanoparticles synthesized using elaeis guineensis (oil palm) kernel extract, Arab. J. Chem. (2016), doi:10.1016/j.arabjc.2017.02.001.
[21] N.K.R. Bogireddy, L.M. Gomez, I. Osorio-Roman, V. Agarwal, Synthesis of gold nanoparticles using coffea arabica fruit extract, Adv. Nano Res. 5 (2017), doi:10.1007/s00217-008-0973-0.
[22] T. attanawongwiboon, S. Soontaranon, K. Hemvichian P. Lertsarawut, S. Laksee, R. Picha, Study on particle size and size distribution of gold nanoparticles by TEM and SAXS, Radiat. Phys. Chem. 191 (2022) 109842, doi:10.1016/j.radphyschem.2021.109842.
[23] F. Fatima, S.R. Verma, N. Pathak, P. Bajpai, Extracellular mycosynthesis of silver nanoparticles and their microbicidal activity, J. Glob. Antimicrob. Resist. 7 (2016) 88–92, doi:10.1016/j.jgar.2016.07.013.
[24] A. Typas, M. Banzhaf, C.A. Gross, W. Vollmer, From the regulation of peptidoglycan synthesis to bacterial growth and morphology, Nat. Rev. Microbiol. 10 (2012) 123–136, doi:10.1038/nrmicro2677.
[25] G.W. Liechti, E. Kuru, E. Hall, A. Kalinda, Y.V. Brun, M. Vannieuwenhze, A.T. Maurelli, A new metabolic cell-wall labelling method reveals peptidoglycan in chlamydia trachomatis, Nature 506 (2014) 507–510, doi:10.1038/nature12892.
[26] B. Reidy, A. Haase, A. Luch, K.A. Dawson, I. Lynch, Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications, Materials 6 (2013) 2295–2350 (Basel), doi:10.3390/ma6062295.
[27] X. Yang, Y. Dang, J. Lou, H. Shao, X. Jiang, D-alanyl-D-alanine-modified gold nanoparticles form a broad-spectrum sensor for bacteria, Theranostics 8 (2018) 1449–1457, doi:10.7150/thno.22540.
[28] M.I. Din, F. Arshad, Z. Hussain, M. Mukhtar, Green adeptness in the synthesis and stabilization of copper nanoparticles: catalytic, antibacterial, cytotoxicity, and antioxidant activities, Nanoscale Res. Lett. 12 (2017), doi:10.1186/s11671-017-2399-8.
[29] X. Xu, Y. Yuan, G. Hu, X. Wang, P. Qi, Z. Wang, Q. Wang, X. Wang, Y. Fu, Y. Li, H. Yang, Exploiting pH-regulated dimer-tetramer transformation of concanavalin A to develop colorimetric biosensing of bacteria, Sci. Rep. 7 (2017) 1–8, doi:10.1038/s41598-017-01371-6.
[30] S. Nqunqa, Optical and Microarray Silver-Gold Based Sensors for the Detection of *E.coli* 0157:H7 in Seawater, Master's Thesis, University of the Western Cape (2021).http://etd.uwc.ac.za/xmlui/handle/11394/8256.