Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.16.048

Assessment Of Antioxidant and Anti-inflammatory Properties Of Gold Nanoparticles Synthesized Using Pterocarpus Santa- An In Vitro Study

Paladugu Devi Navya[1], Gurumoorthy Kaarthikeyan[2]*, S Rajeshkumar[3], Bhavana Garapati[4]

[1]Postgraduate student, Department of Periodontics. Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University,Chennai, India.

[2]Professor and Head of the department, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences,(SIMATS), Saveetha University, Chennai, India

[3]Associate Professor, Nano biomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences,(SIMATS), Saveetha University, Chennai, India

[4]Post graduate student, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University,Chennai, India.

***Corresponding author:** Gurumoorthy Kaarthikeyan, Professor and Head of the department, Department of Periodontics, 162, Poonamallee High Road, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University,Chennai, 600077,Tamil Nadu, India, Email: kaarthikeyan@saveetha.com

Submitted: 24 April 2023; Accepted: 17 May 2023; Published: 13 June 2023

ABSTRACT

Background: Pterocarpus santa which is popularly known as red sandal or yerra chandanam or lal chandan, is one of most expensive plant in the world, which is used for its anti inflammatory, antipyretic and anti hyperglycemic properties from the ancient times. Aim of the present study is to synthesize, characterize and assess antioxidant and anti-inflammatory properties of red sandal gold nanoparticles.

Materials and methods: Preparation and characterisation of red sandal gold nanoparticles (AuNP) and its characterisation by UV spectroscopy, transmission electron microscopy (TEM) and fourier-transform infrared spectroscopy (FTIR). Antioxidant and anti-inflammatory activity of gold nanoparticles were assessed using DPPH assay and BSA method respectively.

Results: The production of AuNPs was monitored using visual color change and UV–visible spectroscopy and Pterocarpus santa mediated gold nanoparticles are spherical in shape and size of 2-35 nanometers shown in TEM analysis. Biosynthesized red sandal AuNP showed 83% highest inhibitory activity of DPPH radical at highest concentration of 50µg/ml. Highest inhibition and maximum protective activity of red sandal AuNP was 80.5% at concentration of 50µg/mL.

Conclusion: Red sandal mediated green synthesis of gold nanoparticles exhibited good antioxidant and anti-inflammatory properties and can be applied in regenerative periodontal therapy in the future studies.

Keywords: *Gold Nanoparticles, Pterocarpus santa, A-PRF+, Periodontal Regeneration, antioxidant and anti-inflammatory properties*

INTRODUCTION

Nano means small, recently the word nanotechnology came into the light of dentistry especially in periodontics for various purposes like drug delivery, tissue engineering, biofilms, bone replacement materials, tooth repair and in implants. Nanotechnology is the combination of science and engineering, which is used to prepare, characterize and apply these materials for the human benefits. The materials synthesized using these nanotechnology should contain the smallest functional group at least in one dimension. Richard.P.Feynman, father of nanotechnology, suggested that these nanotechnology can be used to produce tools and instruments in precise microscopic structures. [1]

A nanoparticle is a small particle, ranging from 1-100 nanometers in size. Nanoparticles, which can't be seen through the naked eye, can express different physical and chemical properties than larger materials. The top down, bottom up, and biomimetic approaches to nanomaterial synthesis are all used. Nanoparticles are widely used in various biomedical applications, particularly in dentistry.[2] The use of nanoscale particles in periodontics can be divided into three categories: prevention of periodontal disease, diagnosis/detection and in treatment of periodontal disease. The various applications of nanomaterial in periodontics includes bone grafts,[3,4,5] membranes of nanoscale,[6] local drug delivery, wound healing agents, management of biofilms , nano coating on implant surfaces and nanorobotics. Gold nanoparticle (AuNP) of herbal products is one of the recent innovations, commonly used in various medical applications today. These particles have been shown to be highly biocompatible and also to have favorable properties like antimicrobial, antioxidant and anti-inflammatory properties. As an implication of their significant existence, spherical gold nanoparticles (AuNPs) draw intense attention.[7] The specific intrinsic characteristics like optical, physicochemical properties of these AuNPs can be changed by changing the properties of particles, such as their form, scale, and aspect ratio or climate.[7,8]

Pterocarpus santalinus with the common name red sanders, red saunders, red sandalwood, rake chandan and lal chandan, is a species of Pterocarpus commonly grown in the eastern ghats of south India. This herbal plant has enormous medicinal properties and is commonly used for treating fever, hemorrhage, and dysentery by the tribes of eastern ghat ranges in India [9,10]. The phytochemical examination of Pterocarpus santa reveals that it includes glucose, steroids, tannins, phenols, flavonoids, glycerides, and glycosides, among other things. The green synthesis of red sandal gold nanoparticles is described for the first time in this paper. This method is simple, rapid, inexpensive and eco friendly. The literature review of previous studies showed the green synthesis of curcumin nanoparticles and its characterisation.[11,12] Similarly, the green synthesis of nanoparticles from zinc oxide in conjugation with red sandal powder was reported[13]. The aim of this study was to synthesize the red sandal nanoparticles and its characterisation and to check the antioxidant and anti-inflammatory effect of the gold nanoparticles synthesized by pterocarpus santa.

MATERIALS AND METHODS

Synthesis and characterisation of gold nanoparticles: A gramme of finely grinded, meshed powder of dried wood (red sandal), 100 mL of deionised water was mixed and boiled at 90 °C for 10-15 minutes. Solution was allowed to cool and passed on filter paper through 0.2 μm of cellulose nitrate membrane. 200 microliter of red sandal aqueous extract was applied to 2 mL of 0.01 M $HAuCl_4$ and was thoroughly mixed by manual shaking. The solution was transferred to a fresh and neat beaker and kept on an orbital shaker for 2 days. The color change was measured for every 3 hours and UV-spectroscopy was performed at 12, 24 and 46 hours. The synthesis of AuNPs was spontaneous, and color shifts from pinkish red to dark maroon color were observed in the figure 1. Characterisation of the gold nanoparticles was done by UV-Visible spectrophotometer and TEM analysis. Further characterization was accomplished by FTIR

Antioxidant property

This property of the Red Sandal AuNPs was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

Five test tubes each contained 10 mL, 20 mL, 30 mL, 40 mL, and 50 mL of the nanoparticle for the test group. One milliliter of DPPH was added to each test tube. The test tube containing 10 mL, 20 mL, 30 mL, 40 mL, and 50 mL of nanoparticles, respectively, received additions of 1990 mL, 1980 mL, 1970 mL, 1960 mL, 1950 mL, and 50% methanol solution. In the control group, 2 mL of methanol solution received 1 mL of DPPH added to it. The standard medium utilized was ascorbic acid. For around 20 minutes, the test tubes were incubated in a dark cabinet. The UV Spectrophotometer was used to detect absorbance at 517 nm. Control Absorbance − Sample Absorbance × 100

Absorbance of sample solution was used to calculate Inhibition Percentage:

(Absorbance of control-Absorbance of sample/ Absorbance of control) x100

Absorbance of control is absorbance of DPPH and methanol and Absorbance of sample is the absorbance of DPPH and sample extract.

Estimating Anti-inflammatory property

In the test group, 5 test tubes each contained 10 mL, 20 mL, 30 mL, 40 mL, and 50 mL of the nanoparticles. 2 ml of 1% Bovine Serum Albumin (BSA) were added to each test tube. The test tube holding 10 litres, 20 litres, 30 litres, 40 litres, and 50 litres of nanoparticles, respectively, received 390 litres, 380 litres, 370 litres, 360 litres, and 350 litres of distilled water. In the control group, 2 mL of BSA solution was mixed with 2 mL of dimethyl sulphoxide (DMSO). In the control group, diclofenac sodium was divided into five test tubes at concentrations of 10, 20, 30, 40, and 50 mL, respectively.

% Inhibition was calculated using the following formula:

% of inhibition = Control OD − Sample OD × 100

Statistical Analysis

All results were presented as the mean and standard deviations. Independent t test was performed to see the significant difference between control and test group. P values of < 0.05 were considered statistically significant.

RESULTS

Characterization Of Aunps

Uv-Spectroscopy

The production of AuNPs was monitored using visual color change and UV–visible spectroscopy. There was a quick change in the color from pinkish red to dark moron when the chloroauric acid solution was added to the extract suggesting the production of AuNPs. Surface plasmon resonance (SPR), which is created by the collective oscillation of free conductive electrons driven by an interacting electromagnetic field in metallic NPs, could explain the visible color shift. The UV–visible spectrum reveals two bands, one in the visible region about 100 nm and the other in the near-infrared region, both of which indicate anisotropic NP production seen in figure 1.

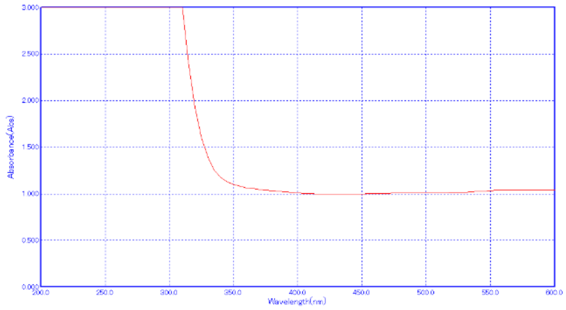

FIGURE 1: UV visible spectroscopy showing the peaks of gold nanoparticles.

Transmission Electron Microscope Analysis

The scale and morphology of the AuNPs were determined by the Transmission Electron Microscope (TEM). 1mL of AuNPs containing reaction mixture is diluted to 5mL sonicated using an ultrasonic bath and falling Ultra-thin Cu on the Cu grid Holey C film and permitted to vacuum dry. TEM pictures showed that Pterocarpus santa mediated gold nanoparticles are spherical in shape and size of 2-35 nanometers was mentioned in figure 2.

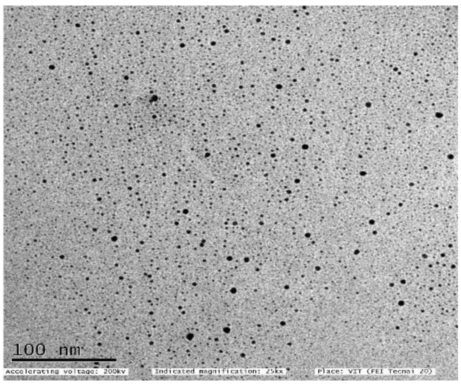

FIGURE 2: Transmission electron microscope image of gold nanoparticles

Fourier-Transform Infrared Spectroscopy (Ftir)

The characterization of green synthesized gold nanoparticles (AuNPs) was analyzed by FTIR. The different functional groups involved in the conversion of gold salt into biocompatible AuNPs of Pterocarpus santalinus is shown in figure 3. The broad absorbance band at 3283 cm^{-1} is the characteristic of the OH stretch of the alcohol. The bands at 1636 cm^{-1} correspond to C=C and those at 1366 cm^{-1} correspond to the C-H bending modes of methyl . This result clearly indicates that AuNPs are synthesized from P. santalinus extract.

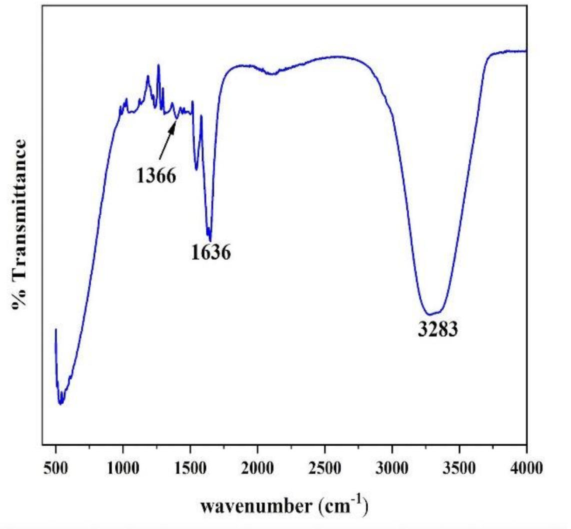

FIGURE 3: Different functional groups of gold nanoparticles.

Anti-Oxidant

Fig. 4 displays the DPPH free radical scavenging activity of various doses of greenly produced AuNP. Using free radical DPPH, red sandal AuNp was tested for antioxidant properties. By using hydrogen or an electron, DPPH that is fluorescent violet is converted to a yellow or colourless solution. In plant extracts, antioxidants are biomolecules with functional groups on their AuNp surfaces that interact with free oxygen radicals to decrease DPPH. At its greatest concentration of 50 g/ml, biosynthesized red sandal AuNP exhibited the best DPPH radical inhibition activity, 83%. Dose-dependent antioxidant activity that was comparable to ascorbic acid's DPPH-scavenging activity was reported (Standard).

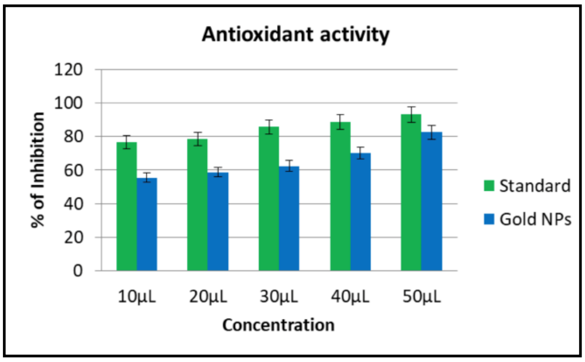

FIGURE 4: Antioxidant activity of Red sandal AuNps compared with standard: DPPH free radical scavenging activity.

Anti-Inflammatory

Inhibition of protein denaturation was seen at various doses of methanolic red sandal AuNP extract (Fig. 5). Red sandal AuNP had protein denaturation inhibitory action that was comparable to synthetic, commercially available anti-inflammatory medication Diclofenac at 75.5%, 83.4%, 83.5%, 88.2%, and 89.5%, respectively. Red sandal AuNP has a maximal inhibitory and protective activity of 80.5% at a concentration of 50 g/mL. Anti-inflammatory activity rose with increasing extract concentration and was comparable to standard.

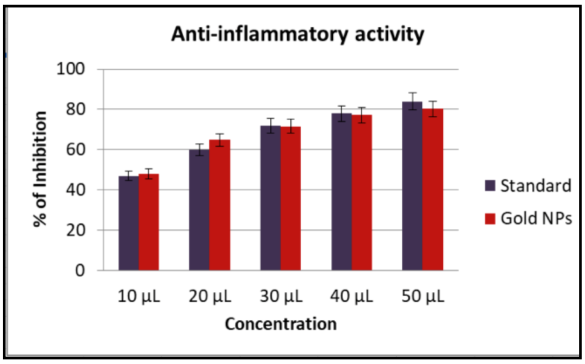

FIGURE 5: Anti-inflammatory activity of Red sandal AuNPs compared with standard: Bovine serum albumin assay.

DISCUSSION

In the present study, preparation of gold nanoparticles from Pterocarpus santa was confirmed by UV spectrophotometer and morphology of nanoparticles was confirmed by transmission electron microscope. Pterocarpus santalinus is used as an alternative medicine for treating diabetes in Ayurveda.[14–16] It is also used in the treatment of diarrhea, ulcers, headaches and in treating inflammatory conditions.[17] This herbal product is understood to prevent the development of T-cell proliferation and tumor necrosis factor alpha.[18] This nature of red sandal can be used as an anti-inflammatory property against periodontal destruction. FTIR results show peaks at different functional groups of gold nanoparticles. Similar trend was also observed by Keshavamurthy et al., (2017).[19] Similar results were found in another study using silver nanoparticles.[20] TEM pictures showed that Pterocarpus santa mediated gold nanoparticles are spherical in shape and size of 2-35 nanometers. This is in accordance with another study using curcumin nanoparticles.[11]

Study conducted by Arokyaraj et al on antifungal activity of Pterocarpus santa, concluded that red sanders can act as a potential antifungal medication against various fungi strains [21,22]. A study conducted by P.S. Warakagoda et al to check the propagation of red sanders through tissue tissue culture shows positive results after 4 weeks.[21] Another study conducted by Arokyaraj et al 2008 on red sanders shows the antimicrobial, anti-inflammatory properties of the same.

The size of AuNPs determines their chemical and physical properties.[23] These properties promote functionalization with thiols, phosphates, and amines, allowing them to be used as drug delivery devices as well as in biodetection.[24] Targeted drug delivery would provide safe therapeutic alternatives for a variety of diseases. With logical drug delivery platform design, rapid progress in targeted drug delivery can be achieved. AuNPs has gotten a lot of attention as drug delivery platforms and proper design methodologies that take physiologic compatibility into account are desperately needed at this hour.[25] The nanoparticles are extremely biocompatible and endowed with advantageous traits like antibacterial properties. Nanoparticles will bind to bacteria's cell membrane and render it porous, causing the cell membrane's permeability to shift and cell death to occur.[26]

By assessing the antioxidants' capacity to scavenge DPPH free radicals, their anti-oxidant properties were examined. The strongest antioxidant was ascorbic acid (Standard). The antioxidant potential of red sandal AuNPs was good and comparable to that of the accepted reference. While AuNPs polyhedral in shape bio-produced using the Acinetobacter SW30 isolate did not exhibit antioxidant properties [26], our study's biosynthesized red sandal AuNP, which were mostly spherical, demonstrated 83% of the highest inhibitory activity of the DPPH radical at the highest concentration of 50 g/ mL. Spherical nanoparticles demonstrated better antioxidant activity up to 45%. Antioxidant activity that was dose dependent was reported. By scavenging free radicals, plant extracts with hydroxyl groups in phenolic compounds might delay the oxidation of lipids and boost antioxidant activity. In contrast to our work, which found greater potential at lower doses, a previous study found that produced Acanthopho-raspicifera AuNPs had the maximum antioxidant activity, with 62.8% at 500 g/mL. Similar to our work, 35-96% of DPPH activity was inhibited at 256 mg/mL.[27]

The ability of bovine serum albumin to prevent protein denaturation was examined. As compared to control diclofenac, red sandal AuNP was most effectively inhibited by 80.5% at a concentration of 50 g/mL. More anti-inflammatory effects result from an increase in phytochemical content. Effect was seen at much lower dosages of gold nanoparticles (40 and 80 mg/kg), and it was comparable to the usual painkiller diclofenac sodium. By reducing endothelial leukocyte contact, leukocyte inflow to neighboring tissues, and a significant reduction in chemotaxis, gold nanoparticle.

CONCLUSION

Periodontal regeneration is one of the toughest procedures to gain the lost tissues and the most beautiful thing if achieved. To enhance the regeneration various procedures and adjuncts came into the light. Adding the benefits of nanoparticles and herbs to the PRF is one of the advanced procedures to enhance PRF properties for regeneration. Incorporation of nanoparticles from the red sandal in A-PRF+ may aid in improving properties like anti-microbial, antioxidant, mechanical properties and controlled growth factor release. All the mentioned properties may help in periodontal regeneration and tissue repair. The present study has proven the green gold nanoparticle synthesis of Pterocarpus santa. Further research has to be done to check the growth factor release and cytotoxic effects.

REFERENCES

1. Richard Feynman, the futurists and the nanotechnology initiative. Physics Today. 2010.
2. Heuer AH, Fink DJ, Laraia VJ, Arias JL, Calvert PD, Kendall K, et al. Innovative Materials Processing Strategies: a Biomimetic Approach. Vol. 255, Science. 1992. p. 1098–105.
3. Jain R. Comparison of Nano-Sized Hydroxyapatite and b -Tricalcium Phosphate in the Treatment of Human Periodontal Intrabony Defects. Journal Of Clinical And Diagnostic Research. 2014.
4. Mazor Z, Horowitz R, Chesnoiu-Matei I, Mamidwar S. Guided Bone Regeneration Using Nanocrystalline Calcium Sulfate Bone Graft in an Extraction Socket: A Case Report . Vol. 4, Clinical Advances in Periodontics. 2014. p. 49–55.
5. Mazor Z, Horowitz R, Chesnoiu-Matei I, Mamidwar S. Guided Bone Regeneration Using Nanocrystalline Calcium Sulfate Bone Graft in an Extraction Socket: A Case Report . Vol. 4, Clinical Advances in Periodontics. 2014. p. 49–55.
6. Han DH, Hong KS, Chung CH, Yim SB. A comparative study for guided bone regeneration of silk fibroin nanomembrane(NanoGide-S™). Vol. 38, The Journal of the Korean Academy of Periodontology. 2008. p. 475.
7. Rajeshkumar S, Tharani M, Jeevitha M, Santhoshkumar J. Anticariogenic Activity of Fresh Aloe Vera Gel Mediated Copper Oxide Nanoparticles . Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3664.
8. Elahi N, Kamali M, Baghersad MH. Recent biomedical applications of gold nanoparticles: A review. Vol. 184, Talanta. 2018. p. 537–56.
9. G S, Swetha G, Balaji S, Rajesh S, Thangavelu L. Preparation of Mouthwash Using Red Sandal Mediated Silver Nanoparticles and Its Antimicrobial Activity - An In Vitro Study . Vol. 12, Journal of Complementary Medicine Research. 2021. p. 74.
10. Hermanns N, Kulzer B, Kohlmann T, Jacob S, Landgraf W, Theobald K, et al. Treatment satisfaction and quality-of-life between type 2 diabetes patients initiating long- vs. intermediate-acting basal insulin therapy in combination with oral hypoglycemic agents – a randomized, prospective, crossover, open clinical trial. Vol. 13, Health and Quality of Life Outcomes. 2015.
11. Dharman S, Reader, Department of Oral Medicine & Radiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, et al. Synthesis and Characterisation Of Novel Turmeric Gold Nanoparticles and Evaluation Of Its Antioxidant, Anti-Inflammatory, Antibacterial Activity For Application In Oral Mucositis-An Invitro Study. International Journal of Dentistry and Oral Science. 2021. p. 2525–32.
12. Manju S, Sreenivasan K. Gold nanoparticles generated and stabilized by water soluble curcumin–polymer conjugate: Blood compatibility evaluation and targeted drug delivery onto cancer cells. Vol. 368, Journal of Colloid and Interface Science. 2012. p. 144–51.
13. Kitture R, Chordiya K, Gaware S, Ghosh S, More PA, Kulkarni P, et al. ZnO Nanoparticles-Red Sandalwood Conjugate: A Promising Anti-Diabetic Agent. J Nanosci Nanotechnol. 2015 Jun;15(6):4046–51.
14. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. Vol. 106, Journal of Ethnopharmacology. 2006. p. 1–28.
15. Mukherjee PK, Nema NK, Pandit S, Mukherjee K. Indian Medicinal Plants with Hypoglycemic Potential. Bioactive Food as Dietary Interventions for Diabetes. 2013. p. 235–64.
16. Vedavathy S, Rao KN, Rajaiah M, Nagaraju N. Folklore Information from Rayalaseema Region, Andhra Pradesh for Family Planning and Birth Control. Vol. 29, International Journal of Pharmacognosy. 1991. p. 113–6.
17. Krishnaveni KS, Srinivasa Rao JV. A new isoflavone glucoside from Pterocarpus santalinus. J Asian Nat Prod Res. 2000;2(3):219–23.
18. Cho JY, Park J, Kim PS, Yoo ES, Baik KU, Park MH. Savinin, a Lignan from Pterocarpus santalinus Inhibits Tunror Necrosis Factor-.ALPHA. Production and T Cell Proliferation . Vol. 24, Biological and Pharmaceutical Bulletin. 2001. p. 167–71.
19. Keshavamurthy M, Srinath BS, Ravishankar Rai V. Phytochemicals-mediated green synthesis of gold nanoparticles using Pterocarpus santalinus L. (Red Sanders) bark extract and their antimicrobial properties. Vol. 36, Particulate Science and Technology. 2018. p. 785–90.
20. joseph D, Baskaran S, Nagarajan M, Sivasubramnian S. Synthesis And Characterization Of Silver Nanoparticle From Couroupita Guianensis Leaf Extract And Its Effect On Clinical Pathogens . Asian Journal of Pharmaceutical and Clinical Research. 2020. p. 117–21.
21. Warakagoda PS, Subasinghe S. In vitro propagation of Pterocarpus santalinus L. (Red Sandalwood) through tissue culture. Vol. 41, Journal of the National Science Foundation of Sri Lanka. 2013. p. 53.

22. Arokiyaraj S. Free radical scavenging activity and HPTLC finger print of Pterocarpus santalinus L. – an in vitro study [Internet]. Vol. 1, Indian Journal of Science and Technology. 2008. p. 1–3.
23. Papasani MR, Wang G, Hill RA. Gold nanoparticles: the importance of physiological principles to devise strategies for targeted drug delivery. Vol. 8, Nanomedicine: Nanotechnology, Biology and Medicine. 2012. p. 804–14.
24. Daniel MC, Astruc D. Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. Vol. 104, Chemical Reviews. 2004. p. 293–346.
25. Rk S, Sanjukta RK. Green Synthesis of Silver Nanoparticles using Plants [Internet]. Vol. 2, International Journal of Nanomedicine and Nanosurgery (ISSN 2470-3206). 2016.
26. Priyanka, Priyanka P, Revanth Kumar TR. Real-time Facial Expression Recognition System using Raspberry Pi. NCTET-2K17. 2017.
27. Donga S, Bhadu GR, Chanda S. Antimicrobial, antioxidant and anticancer activities of gold nanoparticles green synthesized using Mangifera indica seed aqueous extract. Vol. 48, Artificial Cells, Nanomedicine, and Biotechnology. 2020. p. 1315–25.