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Synthesis and Characterization of Gold Nanoparticles using Plant Extract of *Terminalia arjuna* with Antibacterial Activity

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Abstract

The use of plant extracts for nanoparticles synthesis are green, economical and cost effective approach. The present study reports the bio-synthesis of gold nanoparticles (Au NPs) using leaf extract of Terminalia arjuna. After exposing the gold ions to aqueous solution of leaf extract, rapid reduction of gold ions into gold nanoparticles is observed within few minutes. The characterization of biosynthesized Au NPs were carried out by ultraviolet-visible spectroscopy (UV-Vis), transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDX) techniques. UV-visible spectrum of the aqueous medium containing gold nanoparticles showed a peak of 530 nm. TEM analysis was performed to examine the size and shape of the biosynthesized gold nanoparticles. TEM analysis indicated that gold nanoparticles were well dispersed and ranged between 15 to 30 nm in size. Antibacterial activity of the biosynthesized Au NPs was studied against common human pathogens such as Staphylococcus aureus (NCIM 5021), Pseudomonas aeruginosa (NCIM 5029), and Salmonella typhimurium (NCIM 2501) by agar well diffusion method. This method exploits the economical and greener approach for the synthesis of metallic nanoparticles.

Keywords: *Terminalia arjuna, Au NPs, TEM, Antibacterial activity, Green synthesis.*

1. INRODUCTION

Currently, nanoparticles (NPs) have drawn significant attention because of their valuable properties and their applications in various fields such as medicinal, sensor, catalytic, electronic and optical [1]. Nanotechnology has major applications in biomedical research for diagnostic as well as therapy. Au NPs are used in magnetic resonance imaging; X-ray computed tomography, drug delivery, cancer diagnosis and photo thermal therapy [2, 3, 4]. Various chemical and physical approaches are usually employed to synthesize well- defined NPs with desirable sizes and shapes. The chemical synthesis route of NPs involves pyrolysis, inert gas condensation, laser ablation, hydrothermal and solvo-thermal processes [5, 6, 7]. Due to extreme conditions of synthesis, chemical toxicity and expensive physical methods, greener routes for NP synthesis are explored over last decade.

Green-mediated synthesis and character-izeation of NPs have emerged as a significant division of nanotechnology, especially for noble metals such as gold, platinum, and palladium [8, 9]. The synthesis of NPs using biological materials could be a sophisticated alternative to chemical and physical methods. In general, biological organisms, such as bacteria, fungi, algae and plants represent a development of an eco-friendly and cost-effective approach [10, 11, 12]. A great deal of effort has been devoted towards the biosynthesis of metal nanoparticles using bacteria, fungi, actinomycetes, yeast and viruses [13, 14, 15, 16, 17]. The potential of Au NPs synthesized using *B. safensis* was investigated for catalytic and biomedical applications such as, dye degradation, antifungal, anticoagulant and thrombolytic agents [18, 19]. Moreover, the biosynthesis of metallic nanoparticles also carried out using agro-waste materials, enzymes and pigments [20]. Additionally, few reports also documented the arthropods and their metabolites can be used as excellent candidates for the green synthesis of metallic nanoparticles [21]. In addition to the above-mentioned methods, phyto-synthesis is not only advantageous but also profitable approach [22, 23, 24]. In comparison to microorganisms, the phyto-synthesis method is devoid of complex and multistep operations like microbial isolation, culturing, maintenance etc., and also is a very rapid and cost-effective method that can be easily scaled up for bulk production [25]. Moreover, it has been noticed that the rate of nanoparticles synthesis is faster using plants than microbes, and the produced NPs are considerably more stable [26, 27]. Plants are known to harbour a spacious range of metabolites and can be play a vital role in nanoparticles biosynthesis [28, 29, 30]. Gopinath et al. (2013) also reported that *Terminalia arjuna* (*T. Arjuna*) leaf extract contains arjunetin, leucoan-thocyanidins and tannins like metabolites, which are mainly responsible for the bio-reduction of Au NPs [31, 32]. However, their potential is yet to be fully utilized for synthesizing metallic NPs. It is evident from the literature that plants have been utilized successfully for quick and extracellular biosynthesis of metallic NPs [33, 34]. Shankar et al. (2003) reported that the Ag and Au NPs can be rapidly and effectively synthesized using plant extracts, comparable to those of chemical methods [35, 36, 37]. Additionally, Au NPs obtained from the leaf, seed, seed shell and pod extract of *Cola nitida* displayed potent catalytic, anti-coagulant and thrombolytic activities [38, 39]. Moreover, the important applications of Au NPs towards environmental and biological fields are reported for antibacterial, anti-diabetic, antifungal, and larvicidal activities [40]. There are numerous advantages of green synthesis of nanoparticles over conventional methods. It is very easy, rapid, cost-effective and somewhat consistent [41, 42]. Here, in this study an economical and cost effective method for the plant extract mediated synthesis of Au NPs is reported. In this method, the reduction of $HAuCl_4$ solution using the aqueous leaf extracts of *T. arjuna* is carried out. The bio-reduction process was monitored by the UV-visible spectroscopy, and the crystalline structure was investigated by the EDX and TEM technique. This article accounts the efficient synthesis of Au NPs using the leaf extract of *T. arjuna* and their applications in antimicrobial activity against common pathogens.

2. MATERIALS AND METHODS

2.1. Microorganisms, Chemicals and Glassware

The standard bacterial strains of *Staphylococcus aureus* (NCIM 5021), *Pseudomonas aeruginosa* (NCIM 5029) and *Salmonella typhimurium* (NCIM 2501) were procured from the National Collection of Industrial Microorganisms (NCIM), Pune, India; to check the antibacterial activity of Au NPs. Auric acid

were obtained from Sigma–Aldrich. All glassware's were washed with distilled water and dried in oven.

2.2. Maintenance of the Microorganisms

The bacterial strains of *S. aureus* (NCIM 5021); *P. aeruginosa* (NCIM 5029) and *S. typhimurium* (NCIM 2501) were maintained on nutrient agar slants and stored at 4°C with regular sub culturing after every two months.

2.3. Identification of Plant and Collection of Leaves

The plants of *T. arjuna* were identified by observing morphological features. The *T. arjuna* is about 20–24 meters tall; develops a wide canopy at the crown, from which branches fall downwards. The elliptical, conical leaves formed by plants are green on the top and develops a pale yellow flowers which appear in March and June (Figure 1) [43, 44]. Fresh leaves of plants were collected from trees in campus of Lokmangal Biotechnology College Wadala, North Solapur.

Figure 1. *Fresh leaves of T. Arjuna.*

2.4. Preparation of Plant Extracts

Au NPs were prepared by using *T. arjuna* plant extract. Collected plant leaves were surface washed thoroughly with distilled water. Then, the leaves were shade dried. The dried leaves were grinded into fine powder; 5 g of fine powder was dissolved in 100 mL of distilled water and kept in boiling water bath for 15 min. The content was centrifuged for 10 min. for the removal of cell debris. Supernatant was used as leaf extract. After cooling, the extracts were filtered with filter paper (Whatman No. 1) and stored in refrigerator at 4°C. The filtrate was used for the preparation of Au NPs.

2.5. Synthesis of Au NPs

The filtered solution was treated as source extract and used in subsequent procedures. To the 95 mL of 1 mM aqueous $HAuCl_4$ solution, 5 mL of leaf extract was added with the ratio of 20:1, the sample was kept at room temperature, until the colour of aqueous solution changed from light yellow to bright red colour [40].

2.6. Physico-chemical Characterization

The formation and stability of Au NPs in aqueous solution was confirmed by using UV-Vis spectrophotometer. The mixture of leaf extract and $HAuCl_4$ solution was subjected to UV–Vis spectroscopy analysis. The bio-reduction of pure Au^{3+} ions was done with the leaf extract of *T. arjuna*. The reaction was monitored by periodic sampling of the 1 mL aliquots and the optical absorbance of Au NPs suspended in distilled water was recorded on UV-Vis Spectrophotometer (UV-Vis 1601 Schimodzu spectrophotometer, Kyoto, Japan) in 200-800 nm wavelength range. To examine the size and shape of the biosynthesized Au NPs produced by using *T. arjuna*, transmission electron microscopy (TEM) analysis of the sample was done using a Philips CM 200 (Philips, Amsterdam, The Netherlands).The instrument was operated at an accelerating voltage of 200 kV with a resolution of 0.23 nm. A drop of the solution was placed on carbon-coated copper grid and later exposed to infrared light (45 min) for solvent evaporation. Further, the elemental composition of the synthesized sample was also determined by Energy Dispersive X-ray Spectroscopy (EDX).

2.7. Antibacterial Activity

The plant mediated Au NPs were tested for antibacterial effect against common human pathogens viz. *S. aureus* (NCIM 5021), *P. aeruginosa* (NCIM 5029), and *S. typhimurium* (NCIM 2501). To test antibacterial effect, pure bacterial cultures were uniformly spreads on sterile nutrient agar plates. The 10 mm diameter wells were made on the nutrient agar plates using a sterile cork borer. The 100 µL solution of green synthesized Au NPs was added in the well whereas, *T. arjuna* leaf extract was used as a control. After 24 h, inhibition zones around the wells were measured in millimetre (mm).

3. RESULTS AND DISCUSSION

T. arjuna belongs to Combretaceae family and it is a large evergreen tree with spreading crown, drooping branches. *T. arjuna* bark has been used for the treatment of heart failure and hyper-cholesterolemia. In addition, it also possesses antibacterial properties. In this present study, we have reported the green synthesis and characterization of Au NPs using *T. arjuna* leaf extract with antibacterial activity. The water soluble ingredients present in the extract are responsible for reduction of metal ions and efficient stabilization of NPs.

3.1. Synthesis of Au NPs

The present study focused on the simple, one pot, convenient and green synthesis of Au NPs using *T. arjuna* leaf extract. The solution containing Au NPs was confirmed by the formation of red colour (Figure 2). The colour change from yellow to ruby red indicates the formation of Au NPs. The appearance of a ruby red colour after 15 min. of incubation time confirms the reduction of gold chloride into Au NPs. This data is in good agreement with the report documented by Gopinath et al. (2013) on Au NPs synthesis from *T. arjuna* leaf extract. Authors reported the color of the solution was changed within 15 min. from yellow to dark red which indicates the rapid formation of the Au NPs [31]. Other literature on Au NPs synthesis from *T. arjuna* plant also supports with our proposed data [32, 33, 44]. Further, UV-Vis spectroscopic analysis was carried out to confirm the formation of Au NPs.

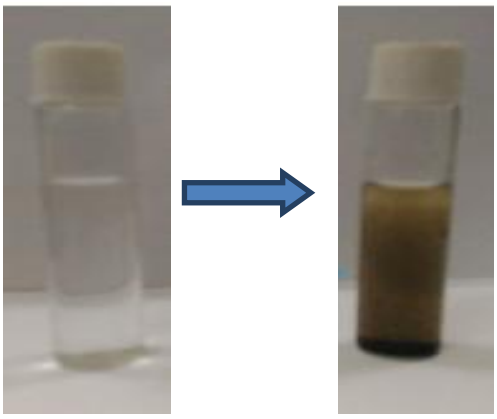

Figure 2. *Colour change observed (a) before and (b) after formation of Au NPs.*

3.2. UV-Vis Spectra of Au NPs

UV-Vis spectroscopy is one of the most important techniques used to identify the formation and stability of the Au NPs in aqueous solution. The reaction was carried out at room temperature on spectro-photometer at a resolution of 1 nm. The production and stabilization of the reduced Au NPs in the colloidal solution was monitored by UV-Vis spectrophotometer analysis. The Au NPs are known to exhibit maximum absorbance in the range of 400-700 nm. The synthesis of Au NPs was monitored at different time intervals such as 5, 10, 15, 20, 25 min. The Au NPs synthesized by *T. arjuna* are positioned at 530 nm (Figure 3). Initially, at 5 minutes, the Au NPs were absorbed slowly, and the absorbance was gradually increased up to 25 min. After 25 min., there was no absorbance which indicated that the Au NPs synthesis process was completed. The UV–vis spectra recorded by Gopinath et al. (2013) during the rapid reduction process at various time intervals showed absorption peak at 530 nm which corresponds to the wavelength of the surface plasmon resonance of Au NPs [31]. The UV–vis spectra of Au NPs obtained from *T. arjuna* fruit extract

showed an absorption peak at 523 nm [32]. The recent report documented by Suganthy et al. (2018) recorded the UV-visible absorption at 536 nm for Au NPs obtained from *T. arjuna* bark extract [44].

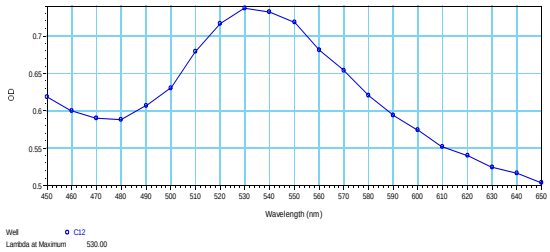

Figure 3. *UV-Visible spectrum of Au NPs.*

3.3. Transmission Electron Microscopy (TEM)

The shape, size and morphology of the synthesized Au NPs were elucidated with the help of transmission electron microscopy. The TEM image further ascertains that the Au NPs are predominantly spherical in morphology with their sizes ranging from 15–30 nm (Figure 4). The recent report of Gopinath et al. (2013) documented the TEM image of Au NPs obtained from *T. arjuna* leaf extract are mostly spherical in shape and the sizes ranging from 20–50 nm with an average size of 20 nm [31]. Other report for Au NPs obtained from *T. arjuna* fruit extract described spherical morphology with an average size of about 25 nm [32]. The Au NPs synthesized from *T. arjuna* bark produced a particles with different shapes predominantly triangular, pentagonal and spherical shaped with an average of 14 nm [34, 44]. From above literature, it has been suggested that *T. arjuna* is an versatile plant for synthesis of Au NPs of various sizes and shaped.

3.4. Energy Dispersive X-ray Spectroscopy (EDX) Studies

The presence of the elemental gold can be seen in the plot of EDX analysis, which indicates the reduction of gold ions to elemental gold. The peak for Au NPs was recorded with emission energy at 2.12 keV and some other signals also observed from Cl and Cu is shown in Figure 5. Similarly, *T. arjuna* fruit extract derived Au NPs illustrated an EDX spectrum at the energy of 2.12 keV [32].

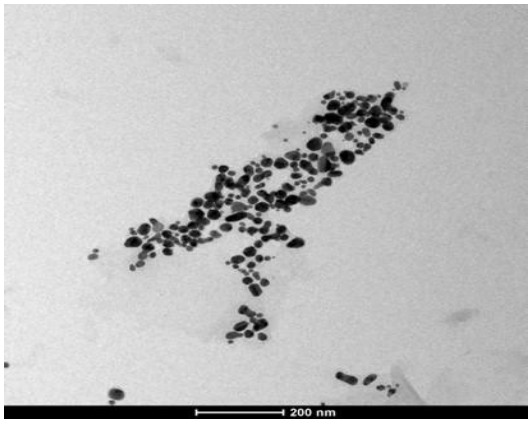

Figure 4. *The Transmission electron microscopy of Au NPs of T. arjuna*

Although, EDX spectrum of Au NPs obtained from *T. arjuna* bark extract showed strong signal for gold ions [34, 44, 45]. Hence, the present data of Au NPs obtained from *T. arjuna* leaf extract is in well accordance with the previous reported literature.

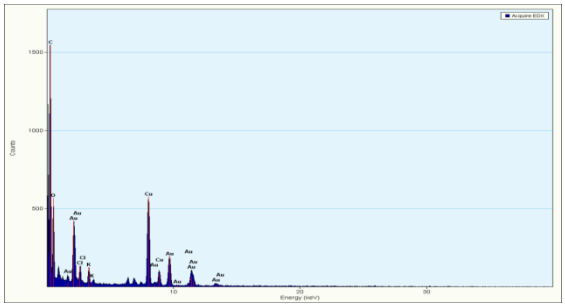

Figure 5. *EDX image of Au NP produced by T. arjuna*

3.5. Antibacterial Activity

The biosynthesized Au NPs from *T. arjuna* leaf extract were tested for their antibacterial activities against common human pathogens such as *S. aureus* (NCIM 5021), *P. aeruginosa* (NCIM 5029) and *S. typhimurium* (NCIM 2501) using agar well diffusion method. The Au NPs showed good antibacterial activity against common

bacterial pathogens (Table 1). The potency of nanoparticles demonstrated as an antibacterial against bacteria. These results may serve as a guide for studying the antibacterial effect of Au NPs against other human and agricultural pathogens [46, 47]. The suggested mechanism of antibacterial activity of the Au NPs is that NPs penetrated inside the cell wall of bacteria. Further, it will form aggregates inside the cell cytoplasm.

Table 1. *Antibacterial activity of Au NPs in terms of zone of inhibition*

Sr. No.	Name of organism	Diameter of zone of inhibition (mm) *Terminalia arjuna*
1	*Staphylococcus aureus* (NCIM 5021)	20
2	*Pseudomonas aeruginosa* (NCIM 5029)	18
3	*Salmonella typhimurium* (NCIM 2501)	16

The formation of Reactive Oxygen Species (ROS) causes the oxidative stress on microbial cells resulting into the loss of cell membrane integrity [48].

4. CONCLUSION

The aim of this work was to develop a simple and efficient synthesis process for Au NPs using the dried leaves of *T. arjuna* plant. The characterizations from UV-Vis, TEM and EDX, support the identity of the NPs. The EDX analysis strongly suggests the formation of Au NPs. Considering its antibacterial activity against human pathogens, these Au NPs may be used in food and pharmaceuticals industries. The plant mediated biosynthesis of Au NPs is non-toxic compared to other reports. Also, this method has advantages: the process is easy since it can be scaled, and it is also economically possible may be used in controlling human and agricultural pathogens.

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REFERENCES

1. Mohanpuria, P., Ran, K. N., Yadav, S. K. (2008). “Biosynthesis of nanoparticles technological concept and future applications”, *J. Nanopart. Res.,* 10: 507–517.
2. Alric, C., Taleb, J., Le Duc, G., Mandon, C., Billotey, C., Meur-Herland, A. L., Brochard, T., Vocanson, F., Janier, M., Perriat, P., Roux, S., Tillement, O. (2008). “Gadolinium chelate coated gold nanoparticles as contrast agents for both X-ray computed tomography and magnetic resonance imaging”, *J. Am. Che. Soc.,* 138: 5908–5915.
3. Cail, W., Gao, T., Hong, H., Sun, J. (2008). “Applications of gold nanoparticles in cancer nanotechnology”, *Nanotechnol. Sci. Appl*., 1: 17–32.
4. Ghosh, P., Han, G., De, M., Kim, C. K., Rotello, V. M. (2008). “Gold nanoparticles in delivery applications”, *Adv. Drug Deliv. Rev*., 60: 1307–1315.
5. Rao, C. N. R., Muller, A., Cheetham, A. K. (2004). “The Chemistry of Nanomaterials: Synthesis. Properties and Applications”, *Wiley-VCH, Weinheim*, 1–11.
6. Moreno-Álvarez, S. A., Martínez-Castañón, G. A., Niño-Martínez, N., Reyes-Macías, J. F., Patiño-Marín, N., Loyola-Rodríguez, J. P., Ruiz, F. (2010). “Preparation and bactericide activity of gallic acid stabilized gold nanoparticles”, *J. Nanopart. Res*., 12: 2741–2746.
7. LaVan D. A., McGuire T., Langer R. (2003). “Small scale systems for in vivo drug delivery”, *Nat. Biotechnol*., 21: 1184-1191.
8. Yuqing, M., Sun, K., Qiu, J., Liu, J. (2009). “Preparation and characterization of gold nanoparticles using ascorbic acid as reducing agent in reverse micelles”, *J. Mater. Sci.,* 44: 754–758.
9. Mittal, J., Batra, A., Singh, A., Sharma, M. M. (2014). “Phytofabrication of nanoparticles through plant as nanofactories”, *Adv. Nat. Sci.: Nanosci. Nanotechnol.,* 5: 043002.
10. Malarkodi, C., Annadurai, G. (2012). “A novel biological approach on extracellular synthesis and characterization of semiconductor zinc sulfide nanoparticles”, *Appl. Nanosci*., 3: 389-395.

11. Gnanajobitha, G., Annadurai, G., Kannan, C. (2012). "Green synthesis of gold nanoparticle using *Elettaria cardamomom* and assesment of its antimicrobial activity", *Int. J. Pharma Sci. Res.,* 3: 323–330.
12. Rajeshkumar, S., Kannan, C., Annadurai, G. (2012). "Synthesis and characterization of antimicrobial gold nanoparticles using marine brown seaweed *Padina tetrastromatica*", *Drug Invention Today,* 4: 511-513.
13. Husseiny, M. I., El-Aziz, M. A., Badr, Y., Mahmoud, M. A. (2007). "Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*", *Spectrochim. Acta,* 67: 1003−1006.
14. Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I., Parishcha, R., Ajaykumar, P. V., Alam, M., Kumar, R., Sastry, M. (2001). "Fungus-mediated synthesis of gold nanoparticles and their immobilization in the mycelia matrix: A novel biological approach to nanoparticles synthesis", *Nano Lett.*, 1: 515−519.
15. Ahmad, A., Senapati, S., Khan, M. I., Kumar, R., Sastry, M. (2003). "Extracellular biosynthesis of monodisperse gold nanoparticles by novel extremophilic actinomycete, *Thermomonospora sp.*", *Langmuir*, 19: 3550−3553.
16. Kowshik, M., Arhtaputre, S., Kharrazi, S., Vogel, W., Urban, J., Kulkarni, S. K., Paknikar, K. M. (2003). "Extracellular synthesis of gold nanoparticles by a gold-tolerant yeast strain MKY3", *Nanotechnol*., 14: 95−100.
17. Shenton, W., Douglas, T., Young, M., Stubbs, G. (1999). "Mann, Inorganic−organic nanotube composites from template mineralization of tobacco mosaic virus", *Adv. Mater*., 11: 253−256.
18. Ojo, S. A., Lateef, A., Azeez, M. A., Oladejo, S. M., Akinwale, A. S., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Gueguim-Kana, E. B., Beukes, L. S. (2016). "Biomedical and catalytic applications of gold and silver-gold alloy nanoparticles biosynthesized using cell-free extract of *Bacillus safensis* LAU 13: antifungal, dye degradation, anti-coagulant and thrombolytic activities", *IEEE Trans. Nanobiosci*., 15: 433-442.
19. Oladipo, I. C., Lateef, A., Elegbede, J. A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Akinboro, A., Gueguim-Kana, E. B., Beukes, L. S., Oluyide, T. O. Atanda, O. R. (2017). "Enterococcus species for the one-pot biofabrication of gold nanoparticles: characterization and nanobiotechnological applications". *J. Photochem. Photobiol., B*., 173: 250-257.
20. Adelere, I. A., Lateef, A. (2016). "A novel approach to the green synthesis of metallic nanoparticles: the use of agro-wastes, enzymes, and pigments". *Nanotechnol. Rev*., 5: 567-587.
21. Lateef, A., Ojo, S. A., Elegbede, J. A. (2016). "The emerging roles of arthropods and their metabolites in the green synthesis of metallic nanoparticles". *Nanotechnol. Rev*., 5: 601-622.
22. Harris, A. T., Bali, R. (2008). "On the formation and extent of uptake of gold nanoparticles by live plants", *J. Nanopart. Res*., 10: 691−695.
23. Thakkar, K. N., Mhatre, S. S., Parikh, R. Y. (2010). "Biological synthesis of metallic nanoparticles", *Nanomed.,* 6: 257−262.
24. Iravani, S. (2011). "Green synthesis of metal nanoparticles using plants", *Green Chem*., 13: 2638−2650.
25. Shankar, S. S., Rai, A., Ahmad, A., Sastry, M. (2004). "Rapid synthesis of Au, Ag, and bimetallic Au core−Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth", *J. Colloid Interface Sci.,* 275: 496−502.
26. Jha, A. K., Prasad, K., Kulkarni, A. R. (2009). "Plant system: nature's nano factory", *Colloids Surf. B,* 73: 219−223.
27. Dudhane, A. A., Waghmode, S. R., Bhosale, M. A., Mhaindarkar, V. P. (2017). "*Caesalpinia pulcherrima* mediated green synthesis of silver nanoparticles: evaluation of their antimicrobial and catalytic activity", *Int. J. Nanopart*., 9: 153-165.
28. Bhattacharya, D., Gupta, R. K. (2005). "Nanotechnology and potential of microorganisms", *Crit. Rev. Biotechnol*., 25: 199−204.
29. Mandal, D., Bolander, M. E., Mukhopadhyaya, D., Sarkar, G., Mukherjee, P. (2006). "The use of microorganism for the formation of metal nanoparticles and their applications", *Appl. Microbiol. Biotechnol*., 69: 485−492.
30. Shankar, S. S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A., Sastry, M. (2004). "Biological synthesis of triangular gold nanoprisms", *Nat. Mater*., 3: 482−488.
31. Gopinath, K., Venkatesh, K. S., Ilangovan, R., Sankaranarayanan, K., Arumugam, A. (2013). "Green synthesis of gold nanoparticles from leaf extract of *Terminalia arjuna*, for the enhanced mitotic cell division and pollen germination activity", *Ind. Crops Prod.,* 50: 737-742.
32. Gopinath, K., Gowri, S., Karthika, V., Arumugam, A. (2014). "Green synthesis of gold nanoparticles from fruit extract of *Terminalia arjuna*, for the enhanced seed germination activity of *Gloriosa superb"*. *J. Nanostruct. Chem*., 4: 115.
33. Singh, J., Kumar, S., Rathi, B., Bhrara, K., Chhikara, B. S. (2015). "Therapeutic analysis of *Terminalia arjuna* plant extracts in combinations with different metal nanoparticles". *Mater. NanoSci*., 2: 1-7.

34. Ahmed, Q., Gupta, N., Kumar, A., Nimesh, S. (2017). "Antibacterial efficacy of silver nanoparticles synthesized employing *Terminalia arjuna* bark extract". *Artif. Cells Nanomed. Biotechnol*., 45: 1192-1200.
35. Kasthuri, J., Kathiravan, K., Rajendiran, N. (2009). "Phyllanthin-assisted biosynthesis of gold and gold nanoparticles: a noval biological approach", *J. Nanopart. Res*., 11: 1075−1085.
36. Shankar, S. S., Ahmad, A., Pasricha, R., Sastry, M. (2003). "Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes", *J. Mater. Chem.*, 13: 1822−1826.
37. Shankar, S. S., Ahmad, A., Sastry, M. (2003). "Geranium leaf assisted biosynthesis of gold nanoparticles", *Biotechnol. Prog*., 19: 1627−1631.
38. Lateef, A., Ojo, S. A., Folarin, B. I., Gueguim-Kana, E. B., Beukes, L. S. (2016). "Kolanut (*Cola nitida*) mediated synthesis of silver–gold alloy nanoparticles: Antifungal, catalytic, larvicidal and thrombolytic applications". *J. Cluster Sci*., 27: 1561-1577.
39. Lateef, A., Ojo, S. A., Elegbede, J. A., Akinola, P. O., Akanni, E. O. (2018). "Nanomedical Applications of Nanoparticles for Blood Coagulation Disorders". *In Environ. Nanotechnol*. (Pp. 243-277). Springer, Cham.
40. Ghosh, S., Patil, S., Ahire, M., Kitture, R., Gurav, D. D., Jabgunde, A. M., Kale, S., Pardesi, K., Shinde, V., Bellare, J. Dhavale, D. D. (2012). "*Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential". *J. Nanobiotechnol*., 10: 17.
41. Pal, S., Mondal, S., Maity, J., Mukherjee, R. (2018). "Synthesis and Characterization of ZnO Nanoparticles using *Moringa Oleifera* Leaf Extract: Investigation of Photocatalytic and Antibacterial Activity", *Int. J. Nanosci. Nanotechnol*., 14: 111-119.
42. Ahmed, S., Ahmad, M., Swami, B. L., Ikram, S. (2016). "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise", *J. Adv. Res.*, 7: 17–28.
43. Ahmed, S., Ikram, S. (2015). "Silver nanoparticles: one pot green synthesis using *Terminalia arjuna* extract for biological application", *J. Nanomed. Nanotechnol.*, 6: 309.
44. Suganthy, N., Ramkumar, V. S., Pugazhendhi, A., Benelli, G., Archunan, G. (2017). "Biogenic synthesis of gold nanoparticles from *Terminalia arjuna* bark extract: assessment of safety aspects and neuroprotective potential via antioxidant, anticholinesterase, and antiamyloidogenic effects", *Environ. Sci. Pollut. Res*., 25: 1-16.
45. Biswas, M., Biswas, K., Karan, T. K., Bhattacharya, S., Ghosh, A. K., Haldar, P. K. (2011). "Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf", *J. Phytology*, 3: 33–8.
46. Hadi, S. M., Bagherieh-Najjar, M. B., Kohan B. E., Mianabadi, M. (2018). "Ag-Conjugated Nanoparticle Biosynthesis Mediated by Rosemary Leaf Extracts Cor-rlates with Plant Antioxidant Activity and Pro-tein Content". *Int. J. Nanosci. Nanotechnol*., 14: 319-325.
47. Pal, S., Mondal, S., Maity, J., Mukherjee, R. (2018). "Synthesis and characterization of ZnO nanoparticles using *Moringa oleifera* leaf extract: Investigation of photocatalytic and antibacterial activity". *Int. J. Nanosci. Nanotechnol*., 14: 111-119.
48. Mohamed, M. M., Fouad, S. A., Elshoky, H. A., Mohammed, G. M., Salaheldin, T. A. (2017). "Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis"*. *Int. J. Vet. Sci. Med*., 5: 23-29.