Antibacterial Activity of Biosynthesized Nanoparticles using Tamarind Shell Extract.

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ABSTRACT:

Microbial antibiotic resistance is increasing due to release of hazardous chemicals into the environment. Anthropogenic activities have made the microbial world more resistance towards the conventional antibiotics. The biosynthesized nanoparticles are challenging particles and bactericidal in nature in treating waste water. Nano size of the particles has high caliber in removing the pollutants from the water by the adsorption phenomenon. . The present study emphasizes on the green synthesis of nanoparticles from the tamarind shell extract, an agricultural waste, commonly used as a fuel by direct burning. Tamarind shell, being rich in calcium and other organic compound, is used as reducing and capping agent in converting the silver to silver nanoparticles which is then characterized using XRD showing cubic crystalline structure, FTIR depicting the functional group, UV-Vis with a peak at 430nm spectrophotometer and TEM showing an average particle size of 25nm.the characterized silver nanoparticles were subjected to the antibacterial activity. the antibacterial sensitivity were recorded on gram negative and gram positive bacteria like *E-coli*, *Streptococcus spp*, *Staphylococcus aureus*, *Klebseilla spp* and *Pseudomonas aeruginosa* . Thus the result obtained proves the ability of silver nanoparticles as the future prospects of research in medicine and pollution remediation.

Key words- Biosynthesis of nanoparticles; Silver nanoparticles, Tamarind shell extract, Antibacterial activity.

1. Introduction

Antibiotics have been widely used and accepted due to its effective treatment of the chronic infections caused by bacteria. Silver nanoparticles constitute a very promising approach for the development of new antimicrobial systems. Nontoxic Nano-materials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials [1]. Biosynthesized nanoparticles have been promisingly shown its antimicrobial activity against bacterial species like *E-coli, Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtili*s [2-3]

Nanoparticles are the ultrafine structure ranging from 10 nm to 100 nm widely seen its application in various field of medicine, electronics, environment etc. Nano scale devices are being used for enhanced sensing, treating and remediating environmental contaminants. There are physical, chemical and biological procedures to synthesize the nanoparticle out of these three, chemical processes is being widely used due to its quick reaction and short time reaction for synthesis of large quantity of nanoparticles. However capping agents are required for stabilization and also the chemical reagents used generally for nanoparticles synthesis are toxic and the remnants after reaction are not very eco- friendly [4]. Various plant materials are used as a source for synthesis of nanoparticles which includes plants, fungi, bacteria, algae etc [5].

Plants and plant products have been exploited for their unique phyto-chemicals present in them like proteins, vitamins, enzymes, amino acids and other organic compounds which are environmentally safe and serve as ideal tool for enhanced medicinal and pollution remedial applications. It is studied and reported that polysols such as terpenoids, polysaccharides and flavones help in bioreduction, stabilization and capping mechanisms of stable silver nanoparticles [6]

Tamarind pulp is a commonly used in Indian culinary and is high in tartaric acid, sugar, B and C vitamins, carotenoids [7-8]*T. indica* fruit extract is not only used in culinary but also studies reveal that it is used in synthesis of nanoparticles without the addition of any external surfactant, capping agent or template [9]. Tamarind shell powder, a cheap agro based waste product, is a known adsorbent evaluated to remove COD, TS, turbidity and sulphate, malachite dye, metals like Cu and Cr (VI) [10-13]. The adsorbent prepared from tamarind fruit shell has a significant capacity for adsorption of Chromium (VI) & Nickel (II) ions from aqueous solution & can be employed effectively as a low cost adsorbent [14]. Tamarind shell is composed majorly of fiber, tannin, tartaric acid and calcium. Not only the tamarind shell but the tamarind seed is also put to use in removal of pollutant. It is reported in the study that tamarind seed powder is used as a coagulant and flocculent for reducing COD from waste water obtained from the detergent industry [15]. The studies have shown that tamarind shell is used as a low cost adsorbent for removal of methylene blue from aqueous solution[16] . Tamarind shell can be studied further for its potential uses in water treatment processes.

2. **Experimentation:**

2.1 Plant Extract Preparation

Tamarind shell is collected post-harvest season. Pulp was completely separated from the tamarind shells (TS). The TS is washed with tap water to remove the dust particles and remnants of the pulp, a final wash is given by the distilled water. The TS is dried in sun for 2 days. To remove the complete moisture it is dried in hot air oven for 4 hours at 60^{o}C. The TS is pounded firstly in mortar and pestle and then grinded to fine particles using a domestic grinder. The tamarind shell powder was sieved to get the uniform particle size. 10 gram of powder was suspended into 100 ml distilled water to make the tamarind shell extract. The mixture was heated on a hot plate for 10 minutes and the filtered using Whatmann paper 1. The filtrate was collected and labeled as Tamarind Shell Extract (TSE). The TSE is further used for the synthesis of silver nanoparticles.

2.2 Synthesis of Silver Nanoparticles using plant extract

The TSE was added to 1 mM silver nitrate solution and heated at 80^{o}C for 30 minutes on hot plate using magnetic stirrer. The color change is observed from pale yellow to brown color. The color change indicates the synthesis of nanoparticles.[17] in their studies. The synthesized nanoparticles were further studied and analyzed using UV-Vis Spectrophotometer, XRD, FTIR and TEM.

2.3 Antibacterial Assay for TSE-Silver Nanoparticles

The silver nanoparticles thus synthesized were subjected to antibacterial activity by agar well-plate method to check the efficiency of minimal inhibition zone [18]. The isolates *Esherichia coli, Streptococcus spp, Staphylococcus aureus, Klebsiella spp and Pseudomonas aeruginosa* were used for antibacterial assay. The antibacterial activity was done in triplicates and mapped to check the efficiency of biologically synthesized silver nanoparticles with that of silver nitrate.

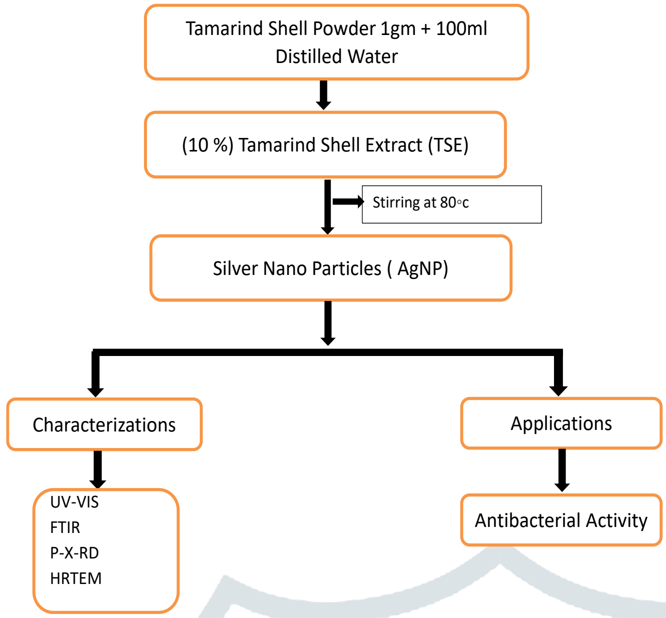

Fig 1. Flowchart -synthesis of silver nanoparticles using Tamarind Shell Extract

3. Results and Discussion

3.1 X-Ray Diffraction

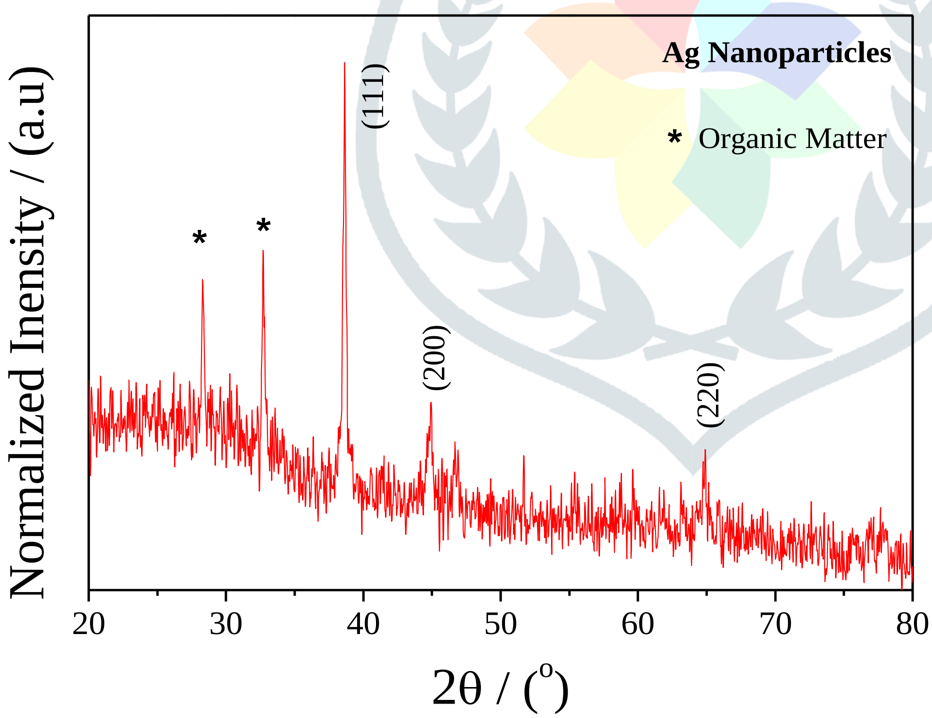

Fig. 2. P-XRD patterns of Ag nanoparticles

The TSE sample was centrifuged at 3000 rpm for 10 minutes to concentrate the AgNPs. The supernatant was discarded and only thick brown liquid was used further for making the thin films of AgNPs on the slide with multiple layers and allowed to air dry. Annealing of the slides was done at 280°C for 6 hours in the muffle furnace. The slides were used for studying the crystallinity of the AgNPs using X-ray Diffractometer (P-

XRD). P-XRD patterns of AgNPs (Fig. 2) is observed that diffractions at peaks around 38.6°, 44.8° and 64.7° in 2θ are depicting (111), (200) and (220) planes of face centered cubic silver nanoparticles using JCPDS card no. 04-0783. In addition to these AgNP crystal reflections, other peaks are also seen and are attributed to be the organic residues of TSE. The average crystallite size of AgNPs is calculated using Scherer's formula.

$$D = \frac{k\lambda}{\beta Cos\theta}$$

Where, k is the Scherer's constant, λ is the wavelength of X-rays used (λ = 1.5418 Å) for Cu K_α target, β is the observed FWHM for (111) peak, and θ is the Bragg's angle.

The estimated average crystallite size using the Scherer's formula found to be 25 nm. TSE is the potential reducing agent revealing the formation of crystalline silver nanoparticles.

3.2 FTIR

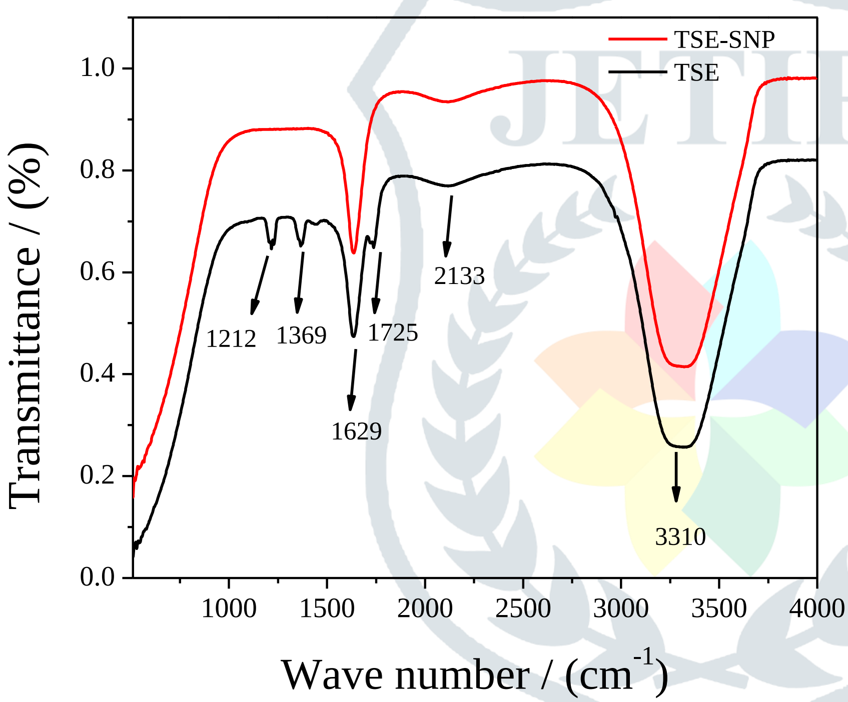

Fig. 3. FTIR spectra of Tamarind shell extract (TSE) and synthesized silver nanoparticles(SNP)

FTIR spectrum was used to identify the possible bio-molecules responsible for the reduction of the silver ions to silver nanoparticles and capping of the bio-reduced AgNPs.

The TSE and synthesized AgNPs were subjected to FTIR in the range of 400 – 4000 cm^{-1} and the corresponding spectra (Fig 3). The spectra produced by FTIR in TSE show six characteristic bands at and around 1212, 1369, 1629, 1725, 2133 and 3130 cm^{-1}. Out of six three of TSE-AgNPs specific bands at 1629, 2133 and 3130 cm^{-1}. In TSE, the bands are in region of 1200 – 1370 cm^{-1} and are assigned to C–C and C–N stretching and bending vibrations of the carbonyl group or correspond to N-H (bond) of secondary [19-21]whereas, the band at 1725 cm^{-1} is assigned to the stretching vibration of N-H group (primary amides)[22]. The bands at 1630, 2120 and 3300 cm^{-1} are attributed to the stretching vibrations of C = C bond, stretching alkyne bonds and O-H stretching vibrations of absorbed water at the surface of Ag nanoparticles respectively.[22]

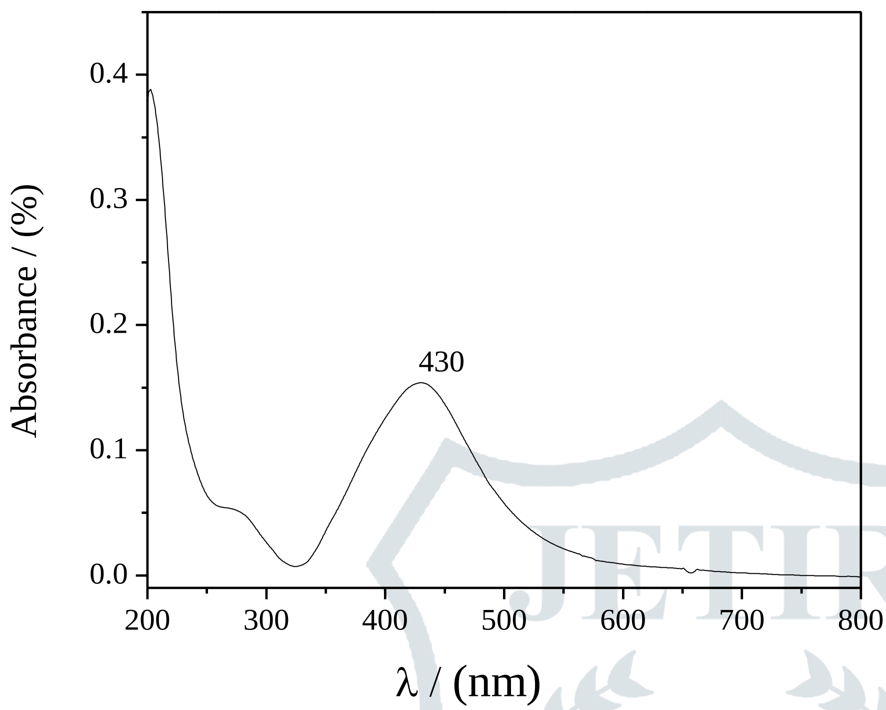

Fig. 4. UV-Visible Absorbance spectra of Silver Nanoparticles (AgNPs)biosynthesized from Tamarind Shell Extract

3.3 UV-Visible

The UV-Vis spectrum of nanoparticles was recorded from the reaction medium as a function of reaction time of 30 minutes at 80°C using 3% TSE with 1mM $AgNO_3$. The sample showed maximum absorption peak ranging between 200-450nm. The maximum peak of synthesized nanoparticles was at 430nm (Fig 4). The absorption spectra show the band edge at 260 nm corresponds to the band gap energy of AgNPs. Using the absorption edge wavelength, the band gap energy (E_g) is calculated using the formula;

$$E_g = \frac{hc}{\lambda}\,\text{eV}$$

Where, h is the Planck's constant, c is the wavelength of light and λ is the absorption edge wavelength from absorption spectra. The band gap energy of prepared silver nanoparticles is found to be 4.76 electron Volt(eV) .Due to bio-reduction in tamarind shell extract, the band gap energy gets reduced which confirms that, bio-reduction yields smaller sized AgNPs, Thus the corresponding bands(260-450) gap can be tuned, which could be potential for optical applications as well. In addition, the color change observed during the synthesis (bioreduction), confirms the formation of silver nanoparticles. The observed color change could be due to the excitation of surface plasmon vibrations / resonance (SPR), which is a characteristic feature for the silver nanoparticle. It is seen that, the absorption spectra for prepared AgNPs shows a clear characteristic absorption peak at/around 430 nm called SPR peak of silver nanoparticles, which further confirms the formation of silver nanoparticles due to bioreduction by means of Tamarind Shell Extract.

3.4 HR-TEM

TEM images(Fig-4) of synthesized nanoparticles from TSE show the particle size in the range of 2 nm -100 nm depictingthat, the particles are spherical in shape with an average size of 25 nm. The particle size is estimated using Image J software. The fringe width, d-spacing that is interplanar spacing is found to be 2.35 nm in dimension. The obtained d-spacing values was compared with JCPDS pattern no. (04-0783) to assign the (h,k,l) values. Comparing the h,k,l values it was found to be (111) indicating the orientation of crystallites along that direction and confirming crystalline cubic structure. Selected area electron diffraction pattern (SAED) clearly exhibits ring patterns representing polycrystalline nature of AgNP. Polycrystalline nature of AgNP confirms the cubic nano structure.

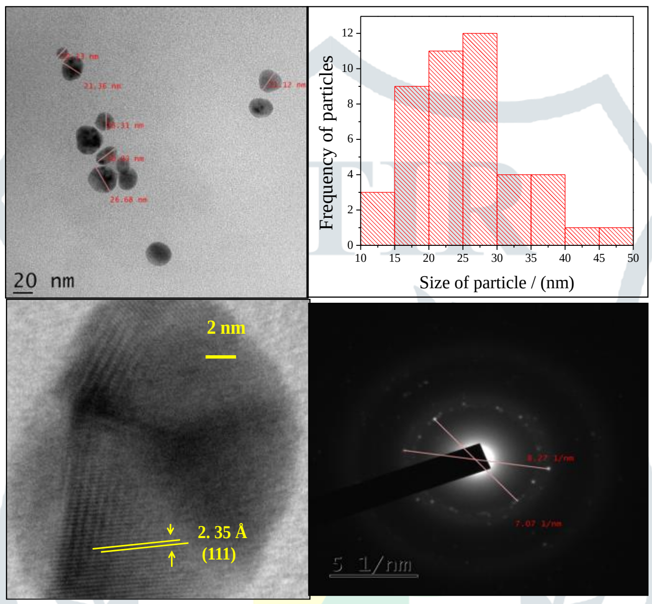

Fig 4. HR-TEM micrographs showing (a) size distribution of Ag nanoparticles; (b) corresponding histogram; (c) HR-TEM image showing *d*-spacing and (d) SAED pattern of Ag nanoparticles

3.5 Antibacterial Activity

As per the results obtained from the antibacterial activity, it is found that all the marker organisms commonly found as water-borne pathogens, were positively been affected by the test samples. Test samples namely tamarind shell extract (TSE), biosynthesized silver nanoparticles (AgNPs) and silver nitrate (1 mM $AgNO_3$) subjected for antibacterial test showed a noticeable result in terms of the zone of inhibition .

Silver nitrate, the potential bactericidal property possessing compound proved to show 9 mmof zone of inhibition for *E.coli*, *Streptococcus spp* and *Staphylococcus aureus*. 0.4 mm for *Klebsiella spp*. 10mm for *Pseudomonas aeruginosa* (plate 1) This indicates that the ($AgNO_3$) can be used as a potential antibacterial compound. Similar results were reported [23-26]

Organic extract of Tamarind Shell an agro based waste was found to show no antibacterial activity, whereas when it was subjected to silver nitrate for the synthesis of silver nanoparticles found to show a remarkable antibacterial activity. It is found that the TSE acted as a reducing agent in the synthesis of silver nanoparticles. It clearly enumerates that zone of inhibition with silver nanoparticles is more than the silver nitrate (Tab-1). The AgNP may destruct the structure of the bacterial cell interfering its enzymatic activity eventually killing the organism [27] in their report. Also similar results were found and reported by Bindhu and Umadevi, [28]

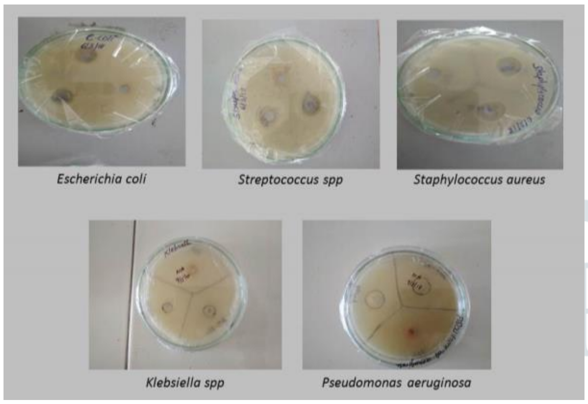

Plate-1 zone of inhibition shown by various microorganisms

Organism	$AgNO_3$ Zone of inhibition in (mm)	Inhibition zone Zone of of AgNP(mm) synthesized by TSE
Escherichia coli	9	11
Streptococcus spp	9	11
Staphylococcus aureus	9	12
Klebsiella spp	4	8
Pseudomonas aeruginosa	10 mm	11mm

Table.1 Zone of inhibition by $AgNO_3$ and AgNP on different bacterial organism

4. Conclusion:

The present study emphasized on antibacterial activity of the synthesized silver nanoparticles using Tamarind shell agricultural waste, having high potential of reducing the silver into silver nanoparticles. The Tamarind shell is used as reducing agent which is ecofriendly, inexpensive and easily available. The synthesized silver nanoparticles are characterized by UV-Vis Spectrophotometer, FTIR, XRD, TEM which clearly indicates the presence of silver nanoparticles with an average size of 25nm, with crystalline structure. The synthesized nanoparticles were tested for its antibacterial activity for 5 different species and which showed the maximum zone of inhibition for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (12mm) and least for *Klebsiella spp (8mm)*. Thus the results obtained can be explored in the field of environmental remediation and medicine as silver nanoparticles prove to be potential candidates.

5. References

1. Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science*, *275*(1), 177-182

2.Ojo, O. A., Oyinloye, B. E., Ojo, A. B., Afolabi, O. B., Peters, O. A., Olaiya, O., ... & Osunlana, O. (2017). Green synthesis of silver nanoparticles (AgNPs) using Talinum triangulare (Jacq.) Willd. leaf extract and monitoring their antimicrobial activity. *Journal* of Bionanoscience, 11(4), 292-296

3. Jackson, T. C., Uwah, T. O., Ifekpolugo, N. L., & Emmanuel, N. A. (2018). Comparison of Antimicrobial Activities of Silver Nanoparticles Biosynthesized from Some Citrus Species. American Journal of Nano Research and Applications, 6(2), 54.

4. Vithiya, K., & Sen, S. (2011). Biosynthesis of nanoparticles. International Journal of Pharmaceutical Sciences and Research, 2(11), 2781

5.Li, X., Xu, H., Chen, Z. S., & Chen, G. (2011). Biosynthesis of nanoparticles by microorganisms and their applications. Journal of Nanomaterials, 2011

6. J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, H. Ning,J. Hong, C. Chen, Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf, Nanotechnology 18 (105) (2007) 10413

7. De Caluwé E., Halamová K., Van Damme P., Tamarindus indica L. – A review of traditional uses, phytochemistry and pharmacology, in: Juliani H.R., Simon J.E., Ho C.T. (Eds.), African natural plant products: discoveries and challenges in quality control, Am. Chem. Soc. Symp. Ser. 1021, Am. Chem. Soc., Wash., D.C., U.S.A., 2009.

8. Grollier C., Debien C., Dornier M., Reynes M., Principales caractéristiques et voies de valorisation du tamarin, Fruits 53 (1998) 271– 280

9. Jayaprakash, N., Vijaya, J. J., Kaviyarasu, K., Kombaiah, K., Kennedy, L. J., Ramalingam, R. J., & Al-Lohedan, H. A. (2017). Green synthesis of Ag nanoparticles using Tamarind fruit extract for the antibacterial studies. Journal of Photochemistry and Photobiology B: Biology, 169, 178-185

10. Rajeshkannan, R., Rajasimman, M., & Rajamohan, N. (2011). Decolourization of malachite green using tamarind seed: optimization, isotherm and kinetic studies. Chemical Industry and Chemical Engineering Quarterly/CICEQ, 17(1), 67-79

11 Gupta, S., & Babu, B. V. (2010). Experimental investigations and theoretical modeling aspects in column studies for removal of Cr (VI) from aqueous solutions using activated tamarind seeds. Journal of Water Resource and Protection, 2(08), 706.

12. Andal, N. M., & Charulatha, S. (2014). Experimental Optimization of an Agricultural Waste in the Uptake of Cu (II) Ions. Chemical Science Review and Letters, 2(8), 615-619.

13.Sivakumar, D., Balasundaram, V., Venkatesan, G., & Saravanan, S. P. (2014). Effect of tamarind kernel powder for treating dairy industry wastewater. Pollution Research, 33, 519-523

14. Pandharipande, S. L., & Kalnake, R. P. (2013). Tamarind fruit shell adsorbent synthesis, characterization and adsorption studies for removal of Cr (VI) & Ni (II) ions from aqueous solution. International Journal of Engineering Science & Emerging Technologies, 4(2), 83-89.

15.Ronke, R. A., Saidat, O. G., & Abdulwahab, G. (2016). Coagulation-Flocculation Treatment of Industrial Wastewater Using Tamarind Seed Powder. International Journal of ChemTech Research, 9(5), 771-780.

16.Saha, P. Water Air Soil Pollution (2010) 213: 287. https://doi.org/10.1007/s11270-010-0384-2

17.Krishnaraj, C., Jagan, E. G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P. T., & Mohan, N. (2010). Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces B:* Biointerfaces, 76(1), 50-56.

18. Heatley, N. G. (1944). A method for the assay of penicillin. Biochemical Journal, 38(1), 61

19. Schreurs, W. J., & Rosenberg, H. (1982). Effect of silver ions on transport and retention of phosphate by Escherichia coli. Journal of Bacteriology, 152(1), 7-13

20. T. Santhosh kumar, A. A. Rahuman, G. Rajkumar, S. Marimuthu, A. Bagavan, C. Jayaseelan, A. A. Zahir, G. Elango and C. Kamaraj, Parasitol. Res. 108 (2011) 693–702

21.R. Koyyati, V. Nagati, R. Nalvothula, R. Merugu, K. R. Kudle, P. Marx and P. R. M. Padigya, Int. J. Res. Pharm. Sci., 5(1) (2014) 32-39

22.Vigneshwaran, N., Ashtaputre, N. M., Varadarajan, P. V., Nachane, R. P., Paralikar, K. M., & Balasubramanya, R. H. (2007). Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus. Materials letters, 61(6), 1413-1418

23 .Krithiga J, Briget Mary M (2015) Synthesis of Agnps of Momordica charantia Leaf Extract, Characterization and Antimicrobial Activity. Pharm Anal Acta 6: 427

24. Ikram, S. (2015). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract

25. Mariselvam, R., Ranjitsingh, A. J. A., Nanthini, A. U. R., Kalirajan, K., Padmalatha, C., & Selvakumar, P. M. (2014). Green synthesis of silver nanoparticles from the extract of the inflorescence of Cocos nucifera (Family: Arecaceae) for enhanced antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 129, 537-541.

26. Prasad, T. N. V. K. V., & Elumalai, E. K. (2011). Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine, 1(6), 439-44

27. Arokiyaraj, S., Vincent, S., Saravanan, M., Lee, Y., Oh, Y. K., & Kim, K. H. (2017). Green synthesis of silver nanoparticles using Rheum palmatum root extract and their antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. Artificial cells, nanomedicine, and biotechnology, 45(2), 372-379.

28. Bindhu, M. R., & Umadevi, M. (2015). Antibacterial and catalytic activities of green synthesized silver nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *135*, 373-378.