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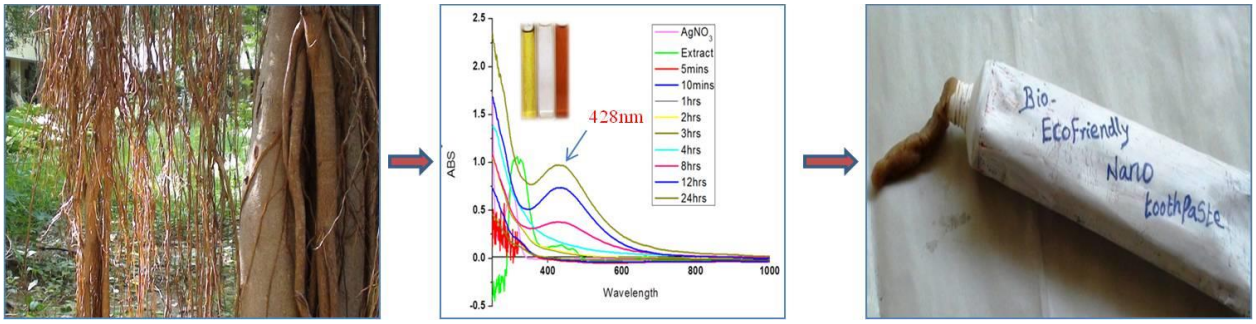
- **Novelty and Highlights:**

1. Preparation of Ag nanoparticles using the methanol extract of the banyan tree root tip.
2. The green synthesis of nanoparticles that exhibits good antibacterial activity against selected bacterial human pathogens and preparation of toothpaste.

- **Graphical Abstract:**



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Abstract

In the present study silver nanoparticles were synthesized biologically using the methanol extracts of the young banyan tree root tip. Phytochemical components in the extracts of the root tip were recorded. The stable silver nanoparticles were characterized by means UV/Visible spectrophotometer, Fourier Transform Infra Red spectroscopy and X-ray diffraction (XRD) spectra. The synthesized nanoparticles exhibited maximum absorbance at 428 nm in the UV spectra. The antimicrobial assay of the extracts of banyan tree aerial root tip increased in the presence of lower concentration of green synthesized silver nanoparticles of the five microbial pathogens used for antimicrobial assay viz., *Lactoccocus lactis*, *Bacillus subtilis*, *Pseudomonas spp*, *E.coli* and *Candida spp*. Then these nanoparticles were used as toothpaste preparation.

Keywords: Banyan tree, aerial root, green synthesis, pathogens.

Introduction

Nanotechnology is a recent developing technology, but it is practiced in before 5000 years ago in aurveda. Materials in the nanometer size range may possess beneficial properties, which can influence the development of remarkable industrial and engineering applications including biotechnological systems (Selvaraj *et al.*, 2006). Nanomaterials have been introduced very tiny particles with good biological and medical applications (Ibrahim., 2012) viz., antibacterial (Pinto *et al*., 2009; Jacob *et al*., 2011), antifungal (Jayaseelan *et al*., 2012), drug targeting and delivering (Nirmala Grace and Pandian., 2007), water purification (Mohan *et al*., 2013), preventing bio film formation (Kalimuthu *et al*., 2010), antimicrobial cotton fibers (Ravindra *et al*., 2010; Gupta *et al*., 2008), anti cancer drugs (Fidel *et al*., 2010; Vaithilingam Selvaraj and Muthukaruppan Alagar., 2007; Selvaraj *et al*., 2006), textile industries and food packaging (Mohammad *et al*., 2012; Costa *et al*., 2011) etc.

A number of approaches are available for the synthesis of nanoparticles for example, physical, chemical and biological methods and recently via green chemistry route (Mariselvam *et al*., 2013). The use of environmentally begin materials like plant leaf extract (Gopalakrishnan *et al*., 2012), bacteria (Anima Nanda and Saravanan., 2009), fungi (Jaidev and Narasimha., 2010), and enzymes (Javed Musarrat *et al*., 2010), for the synthesis of nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications of these nanoparticles. Green synthesis provides advancement over chemical and physiological method as it is cost effective, environment friendly, easily scaled up for the large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. In our study focused on the silver nanoparticles prepared from plant materials. The banyan tree aerial root tip take as a sample for using synthesis of silver nanoparticles.

We here reported the silver nanoparticles synthesized from banyan tree young aerial root tip methanol extract and characterization of synthesized nanoparticles and their antimicrobial activity against five different bacterial pathogens like *Bacillus subtilis*,

Pseudomonas spp, Lactococcus lactis, E.coli and *Candida spp*.

Materials and Methods

Plant material and preparation of infusion The banyan tree young areal root tip was collected from our college campus, Sri Paramakalyani College, Alwarkurichi, Tamilnadu, India at the month of December 2013 (Fig 1). Fresh part of aerial root tip were washed thoroughly and chopped into fine pieces. About 20 grams of chopped aerial roots were grained using mortar and pestle, 100% methanol used as a solvent to form a extract. Then this extract was filtered using Whatman No 1 filter paper without any impurities.

A part of the plant extract was air dried and then grinded into fine powder for phytochemical analysis.

Figure 1 plant source

Synthesis of silver nanoparticles and characterization Ninety millilitres of $AgNO_3$ solution was added to 10ml of plant extract and stored in a room temperature with stirring using magnetic stir. The colour of the solution was changed in to dark reddish brown indicating the silver nanoparticles formation. The synthesized NPs were characterized using UV-Visible Spectroscopy, FTIR and XRD. The prepared samples were analyzed for the rate of absorption in UV/Visible region using of UV-Visible Spectrophotometer in the range of 200 to 1000nm. IR spectra were recorded using KBr pellets (1% w/w) on a Perkin-Elmer GX FT-IR spectrometer. The Energy Dispersive X-ray analysis, the silver nanoparticle was dried on a carbon coated copper grid and performed on a HITACHI SU6600 model.

Antimicrobial assay The antibacterial activity of the green synthesized nanoparticles was tested against five microbial isolates using agar well diffusion method. The zone of inhibition was measured and expressed in millimetres. Antibacterial activity was recorded if the zone of inhibition was greater than 8mm (Mariselvam[a] *et al.*, 2012). The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active (Mariselvam[b] *et al*., 2013).

Result and Discussion

The phytochemical contents in the methanol extract of banyan tree root tip are summarized in the Table 1. The selected plant methanol extract contains carbohydrates, proteins, alkaloids, reducing sugar and tannin. The presence of reducing agents in the extract was confirmed in the phytochemical screening of the extract of the banyan tree aerial root tip. Shankar *et al.*, (2005) have reported that the reducing sugars are responsible for the reduction of silver nitrate to silver nanoparticles.

Table 1 Preliminary phytochemical study of banyan tree root tip extract.

S.No	Test for	Methanol extract of banyan tree aerial root tip
1	Carbohydrate	+
2	Protein	+
3	Amino acid	-
4	Alkaloids	+
5	Flavanoid	-
6	Terpenoids	-
7	Tannins	+
8	Saponins	-
9	Aromatic acids	-
10	Phenolic compounds	-
11	Xanthoprotein	-
12	Reducing sugar	+
13	Triterpenoids	-

Formation of silver nanoparticles reduction of silver nitrate ($AgNO_3$) during treatment with the extract of aerial root tip is evident from the colour change of the reaction mixture. The colour change in colour of the reaction mixture after 8 hours is presented in the figure 2, which indicates the formation of silver nanoparticles.

The UV/Visible spectrum (Fig 2) of the silver nanoparticles indicates that the absorption peak was in the visible region 428nm range. A single peak at the wavelength 428nm suggests a spherical size of the nanoparticles as per Mie theory.

The FTIR analysis of banyan tree root methanol extract, showed in the Figure 2b. The FTIR spectra showed strong band at 879.17cm^{-1}, 1044.76cm^{-1}, 2915.13cm^{-1} and 3340.92cm^{-1}. The band at 879.17cm^{-1}, corresponds to C-H "oop" aromatics. The band at 1044.76cm^{-1} represents C-N stretching of aliphatic amines in the extraction. The band at 2915.13cm^{-1} corresponds to C-H

stretching frequency. The band 3340.92 represents O-H stretcting in the extraction.

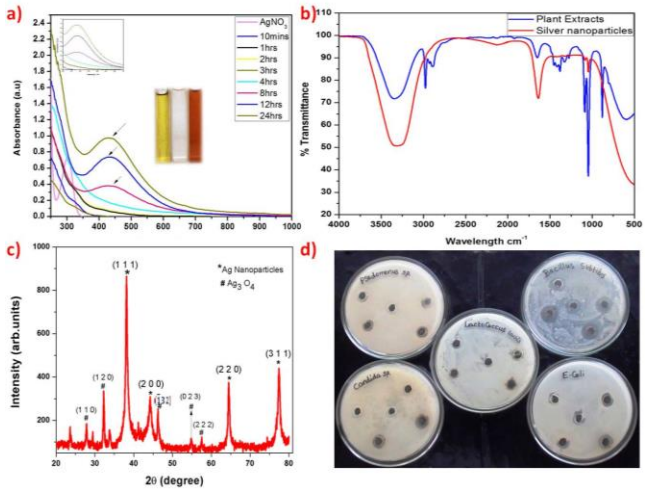

Figure 2 a - UV/Vis Spectrophotometer analysis of synthesized silver nanoparticles; b – FT-IR characterization of synthesized nanoparticles and plant source (extract); the XRD pattern of Ag nanoparticles prepared by plant extract as presented in Fig – c; d - Antimicrobial assay of silver nanoparticles using well diffusion assay method.

The FTIR spectrum of synthesized nanoparticles indicates that the nanoparticles manifest observation peaks at about 1045.21cm^{-1} and 1635.09cm^{-1}. Furthermore, the peak at 3308.06cm^{-1} were assigned O-H stretching frequency. The band at 1045.21cm^{-1} corresponds to C-O stretching frequency and this figure 2b (synthesized nanoparticles) shows stong band at 1635.09cm^{-1} to indicates the conformation of silver nanoparticles formation.

The XRD pattern of Ag nanoparticles prepared by plant extract as presented in Fig.2c. The sharp intense peaks was observed at 32.2, 38.1, 64.4 and 77.3º in the whole spectrum ranging from 10 to 80 and the corresponding planes values (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2). It is coincide with the previous literature (Spread and Christian., 1959). The secondary phase of silver nanoparticles like Ag3 O4 (Standke and Jansan., 1987) was from our spectral data and its respective crystalline planes are measured. The mean particle diameter of Ag nanoparticle was calculated from the XRD pattern using the Scherrer equation.

$$D = 0.94\,\lambda / \beta\,(\cos\theta)$$

Where β is the full width at half maximum (FWHM), λ is the X-ray wavelength (1.5406 Å) and θ is the reference peak width at angle, D is the average crystallite area size perpendicular to the sparkly planes. It reveals that the capping agents play an important character in affecting the crystal field bend, stability breaking and a special role on the modification of crystal segment of Ag nanoparticles during synthesis.

The antibacterial ability of silver is well known. The conservation of silver nitrate into silver nanoparticles using plant bio active compounds present in the aerial root tip of banyan tree resulting in highly active antibacterial nanoparticles. The green synthesized nanoparticles were treated for its antimicrobial action using five different types of microbial pathogens (Table 2 & Figure 2d). The maximum inhibition was observed for *Bacillus subtilis* (18mm), *Psedomonas spp* (14mm), *E.coli* (14mm) and *Candida spp* (15mm).

Table 2: Antimicrobial assay of green synthesized silver nanoparticles.

S.No	Microorganism	Zone of inhibition (mm)				
		25µl	50 µl	75 µl	100 µl	Standard
1	*Bacillus subtilis*	14	16	17	18	14
2	*Psedomonas spp*	11	12	13	14	10
3	*Lactococcus lactis*	10	11	12	13	11
4	*E.coli*	12	13	13	14	12
5	*Candida spp*	0	0	13	15	0

The green synthesized silver nanoparticles had inhibited the microbial growth (Table 2 & Figure 2d). These nanoparticles were highly inhibiting growth of *Bacillus subtilis* compare than the other microorganisms. The silver nanoparticles had ruptured the cell wall of the microorganisms.

Preparation of toothpaste by using green synthesized silver nanoparticles The synthesized silver nanoparticles have good inhibitory activity against the *Lactoccocus lactis*. It causes dental problems in humans. So we are prepared nano- toothpaste by using green synthesized nanoparticles (Figure 3). This silver nanoparticles as an alternative for H_2O_2 for the preparation of nano-toothpaste. This toothpaste was eco friendly, cost effective and very active against dental pathogens.

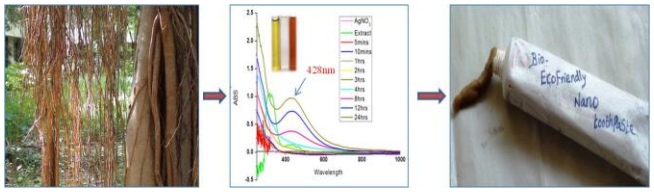

Figure 3: Prepared Eco friendly green synthesized nano toothpaste.

Conclusion

The biosynthesized silver nanoparticles have good inhibitory activity againsted tested all pathogenic microorganisms, especially it is effectively inhibit the *Lactobacillus lactis*. It causes dental problems in human. So we are prepared nano toothpaste using banyan tree

arieal root tip mediated green synthesized nanoparticles. This ecofriendly nano toothpaste have good result compare than comarcially available toothpaste and it is ecofriendly, low cost one.

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