

Hydroxyapatite Herbal Nanorods for Biomedical Application

Suganya .K¹, Atchaya .P², Suba Sri .M³, Mekala .M⁴

¹Associate Professor, Department of Microbiology, Sri Ramakrishna College of Arts and Science for women, Coimbatore, Tamilnadu, India

^{2,3}Research Scholar, Department of Microbiology, Sri Ramakrishna College of Arts and Science for women, Coimbatore, Tamilnadu, India

⁴Associate Professor, Department of Microbiology, Sri Ramakrishna College of Arts and Science for women, Coimbatore, Tamilnadu, India

Abstract: *Advances in nanotechnology are increasingly utilized in the life sciences. Designs and structures at the nanometre scale are being applied to fields such as pharmaceuticals, biotechnology and tissue engineering. Biomaterial with antimicrobial properties and tissue regeneration Nanotechnology employment in the field of medicine with particular potential and new technologies improves the drug delivery along with novel methods of diagnosis. Clinical applications of nanotechnology are already occurring and will increase in frequency as current research develops into practice. An important development in treatment methods has been the use of nanotechnology for drug delivery. Nanoparticles can be used to transport drugs to target sites, which provide the advantage of reducing accumulation in healthy tissues.*

1. Introduction

Nanotechnology can be defined as the technology that emphasizes on the design, characterization, production and application of structures, devices and systems that present unique physical, chemical and biological properties by controlling size and shape of nanomaterials. Over the past decade, nanotechnology has been used in many different fields of science as an innovative tool for technological evolution. Nanotechnology is a field of technology that brings in close collaboration many scientists such as physicists, chemists, mathematicians, biologists, doctors and engineers in order to expand the research mentality across the traditional disciplinary boundaries. [1]. The orientation of nanotechnology in life sciences has led to the evolution of nanomedicine that is considered as a sub discipline contained within nanotechnology and Nanoscience (1). Hydroxyapatite (Hap) nanocrystals have good bioactivity, biocompatibility, osteoconductivity and also resembles the inorganic constituent properties of calcified tissues which are widely used for biomedical applications. Microbial infection India perhaps the largest producer of medicinal herbs and is called Botanical Garden of the World. Medicinal herbs have been in use for thousands of years, in one form (or) another, under the indigenous systems of medicine like Ayurveda, Sidha and Unani. *Plumeria alba* Linn. is a fast-growing, medium size tree, that is botanically belongs to family *Apocynaceae*. The white flowers bearing five petals and have fragrance. The vernacular names of the plant is Chameli (Hindi), Perungalli (Tamil), Khairchampa (Marathi), Frangipani, (English), Veyyivarahal (Telgu), Dalanaphula and (Benghal) (Nandkarni, 1954). It contains primarily deciduous shrubs and small trees.[2] The flowers are native to Central America, Mexico, the Caribbean, and South America as far south as Brazil but can be grown in tropical and sub-tropical regions.[3] *Plumeria alba* is a small laticiferous tree or shrub, a native of tropical America. It is 4.5 m high, occasionally grown in the gardens. The plant is mainly grown for its ornamental and fragrant flowers. Leaves lanceolate to oblanceolate, flowers white, fragrant in corymbose fascicles.[4] The fruit is edible; latex is applied to ulcers, herpes and scabies and seeds possess hemostatic properties. Moreover, its bark is bruised and applied as plaster over hard tumors.[5] Whereas the latter taxon finds

use as purgative, cardiotoxic, diuretic, and hypotensive.[6] Methanolic extract showed antimicrobial activity against *Bacillus anthracis*, *Pseudomonas aeruginosa*. [7]The plant is reported to contain amyriacetate, mixture of amyriins, β -sitosterol, scopotetin, the iridoids isoplumericin, plumieride, plumieride coumerate, and plumieride coumerate glucoside.[8, 9]

Different part of the *P. alba* was believed, have been useful in a variety of diseases, namely, the diseases of Malaria, Leprosy, Rheumatism, and abdominal tumors. The milky sap of the stem and leaf is applied to skin diseases such as herpes, scabies, and ulcers.[10, 11] Its bark is used as plaster over hard tumors, the seeds in hemostasis while the latex is used as purgative, cardiotoxic, diuretic, and hypotensive.[9]

In this present research work the Hydroxyapatite nanoparticles is synthesized using the latex of *Plumeria alba* and characterized by UV-Visible spectrophotometer by the formation of surface plasmon peak. The developed fruit nanoparticles were coated on cotton gauze by dipping technique. The bonds present in the nanoparticle coated gauze were confirmed by fourier transform infrared spectroscopy and the morphology were determined by scanning electron microscope. The nanoparticle coated gauze was subjected to antimicrobial activity against wound causing pathogens for evaluating its biomedical application

2. Materials and Method

2.1 Sample Preparation

Plumeria alba latex were collected and centrifuged at 5000 rpm for 10 minutes at 4°C. From the three layers middle portion containing serum was separated and stored for further use

Phytochemical analysis of *Plumeria alba* latex serum:

The serum extract of *Plumeria alba* latex were used for qualitative phytochemical screening of alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, saponins, resins and cardiac glycosides following the standard procedure (12).

Production of hydroxyapatite nanoparticle- wet chemical precipitation method:

Serum with calcium chloride and serum with disodium hydrogen phosphate were prepared and the pH of both was maintained at 10. Calciumchloride containing mixture was kept in magnetic stirrer and disodium hydrogen phosphate was added drop by drop at 60°C for 1 hour and incubated at room temperature for 24 hours. The gelatinous white precipitate obtained was centrifuged at 2000rpm for 10 minutes to remove the byproducts. The pellet was dried in water bath at 60°C and made into fine powder (13).

Characterization of hydroxyapatite nanoparticle uv-visible spectrophotometer:

The developed hydroxyapatite nanoparticle was confirmed by UV-VIS spectrum of the reaction medium at 24 hours' time interval by drawing 1 cm³ of the samples and their absorbance was recorded at 200-700 nm using UV-VIS spectrophotometer (14). Formation of Plasmon peak was observed and recorded.

Chemical characterization by Fourier transformed infrared spectroscopy:

The developed nanoparticle coated gauze was characterized for interpreting the presence of chemical bonds and functional groups by FTIR analysis (SHIMADZU- Single reflection ATR accessory)., the sample was irradiated with infrared radiation from an infrared source, and absorption of the radiation stimulates vibrational motions by depositing quanta of energy into vibrational modes (15). The changes in the vibration motion gave rise to bands in the vibrational spectrum; each spectral band was characterized by its frequency and amplitude.

Development of cotton gauze with hydroxyapatite nanoparticle: (Mekala *et al.*, 2019)

Destarched and air-dried cotton gauze were coated with nanoparticle by dipping the hydroxyapatite nanoparticles with isopropanol in the ratio of 1:5 for 24hours (16). Consequently control gauze was dipped in control hydroxyapatite nanoparticle (produced from distilled water) in the same ratio with isopropanol.

Physical characterization by scanning electron microscope:

Scanning Electron Microscope uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. Scanning Electron Microscope was performed to study shape, size and surface area of the developed hydroxyapatite nanoparticle coated on cotton gauze. The cotton gauze was visualized under Scanning Electron Microscope (17).

Antimicrobial sensitivity test for developed hydroxyapatite nanoparticle coated cotton gauze:

The antibacterial susceptibility test were carried out for the developed hydroxyapatite nanoparticle coated gauze against the major wound causing pathogens like *E.coli*, *Klebsiella sp*, *Bacillus sp*, *Pseudomonas sp*, *Staphylococcus sp*. Test organisms were swabbed on Muller-hinton plates The developed hydroxyapatite coated gauze were placed in centre of the plate and incubated at 37°C for 24 hours (18). The zone of inhibition was observed and recorded.

3. Result and Discussion**Sample Preparation**

The serum was obtained by adding latex and distilled water in the ratio of 1:1. After centrifugation the serum was collected and stored at 4°C for future use.

Phytochemical analysis of *Plumeria alba* serum

Phytochemical compounds present in *Plumeria alba* serum were analysed by standard procedures and their results were tabulated in Table 1. The phytochemical screening of the latex serum revealed the presence of Alkaloids, Phenols, Flavonoids, Tannins, Saponins, Glycosides, Proteins and absence of Terpenes and Resins. Similarly Sibi G *et al.*, 2012 reported that phytochemical analysis of *Plumeria alba* bark reveals the presence of Tannins, alkaloids, phenol and Flavanoids.

Table 1: Phytochemical constituents of *Plumeria alba* latex serum

S.NO	PHYTOCHEMICALS	LATEXSERUM
4.3.1	Alkaloids	+
4.3.2	Phenols	+
4.3.3	Flavonoids	+
4.3.4	Terpenes	=
4.3.5	Tannins	+
4.3.6	Saponins	+
4.3.7	Resins	=
4.3.8	Glycosides	+
4.3.9	Proteins	+

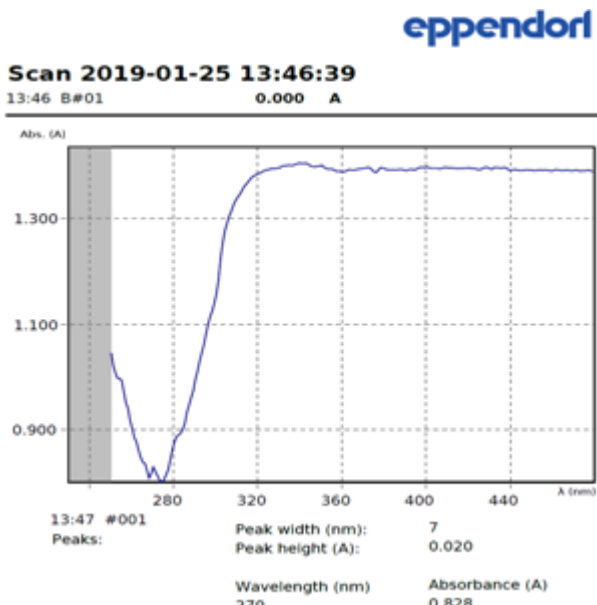
Presence (+) Absence (-)

Production of hydroxyapatite nanoparticle using *Plumeria alba* latex serum:

The hydroxyapatite nanoparticle was developed using the latex serum of *Plumeria alba* and control was developed using distilled water. The powder obtained from wet chemical precipitation method was white in colour and stored at room temperature for future use.

Characterisation of produced hydroxyapatite nanoparticle by UV-visible spectrophotometer:

Graph 1 represents the hydroxyapatite nanoparticle produced from *Plumeria alba* latex serum and Graph 2 represents the hydroxyapatite nanoparticle produced from distilled water (control). Formation of Plasmon peak at 286nm for latex serum and 270 nm for control confirms the presence of hydroxyapatite nanoparticle. Similarly Marcos *et al.*, 2013 reported that hydroxyapatite nanoparticle production was confirmed by high absorbance peak at 270nm.



Graph 1: UV-Visible Spectrophotometer Analysis for Pure Hydroxyapatite

Chemical characterization by Fourier transformed infrared spectroscopy

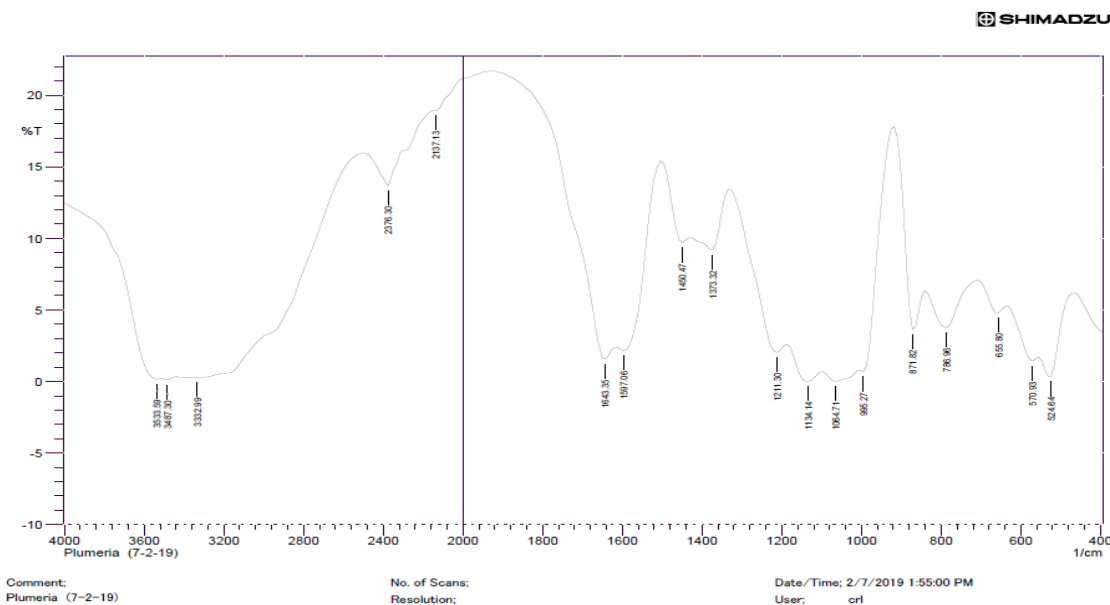
Functional groups associated with hydroxyapatite were identified by FTIR spectroscopy. The ion stretching vibration around 3568 cm⁻¹ confirms the presence of a hydroxyl group. The formation of produced hydroxyapatite nanoparticle from *Plumeria alba* latex serum was confirmed by analysing the absorption spectra of the functional groups and comparing its results with Gopiet *et al.*, 2013. Similarly, the other stretching vibrations for carbonyl and phosphate groups were also observed as reported earlier Aruneshan *et al.*, 2013. Similarly, the stretching vibration for hydroxyl group was confirmed between the range 3643 cm⁻¹

Table 2: FTIR analysis of developed hydroxyapatite nanoparticle

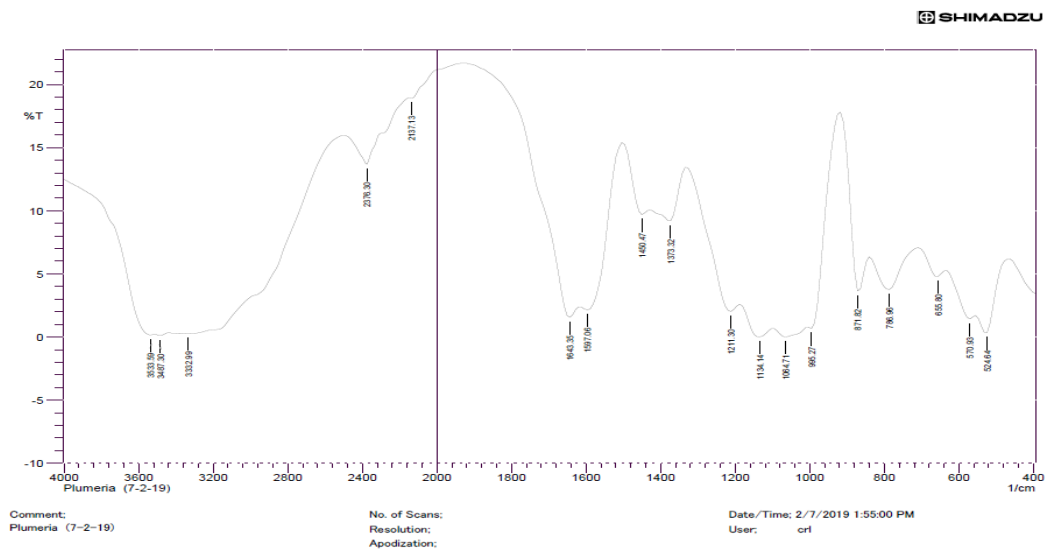
S. No	Peaks of Control	Peaks of <i>Plumeria alba</i>	Functional Groups
1	3541.31	3533.59	OH ⁻ (Hydroxyl group)
2	1442.75, 871.82	1450.47, 871.82	CO ₃ ²⁻ (Carbonate group)
3	1064.71, 570.93	1064.71, 570.93	PO ₄ ³⁻ (Phosphate group)



Graph 2: UV Visible Spectrophotometer Analysis of *Plumeria Alba* Nanoparticle



Graph 3: FTIR Analysis for Control Hydroxyapatite Nanoparticle



Graph 4: FTIR Analysis for *Plumeria alba* Hydroxyapatite Nanoparticle

Development of cotton gauze with hydroxyapatite nanoparticle:

The cotton gauze coated by dipping method with hydroxyapatite nanoparticle produced from latex serum of *Plumeria alba* and control hydroxyapatite nanoparticle produced from sterile distilled water was dried and subjected to characterization.

Development of hydroxyapatite nanoparticle coated gauze by scanning electron microscope:

The morphological appearance of hydroxyapatite nanoparticle incorporated on cotton gauze was observed by scanning electron microscope. The plate of SEM images with 200X, 750X, 2000X, 5000X represents the hydroxyapatite nanoparticles were aggregated. In the micrographs it was observed that the developed hydroxyapatite nanoparticle were in size ranging from 5-100 μ m.

Antimicrobial sensitivity test for developed hydroxyapatite nanoparticle coated cotton gauze:

The antibacterial effectiveness was performed with 5 strains. Zone of inhibition in the plates represents the antibacterial activity of the developed nanoparticle coated on cotton gauze against the test pathogens namely *Escherichia sp.*, *Pseudomonas sp.*, *Staphylococcus sp.*, *Klebsiella sp.*, *Bacillus sp.* The maximum zone of inhibition was found for *Klebsiella* of 22mm and for *Escherichia sp.*, *Pseudomonas sp.*, *Bacillus sp.* was observed at 19mm. The antifungal activity was performed with *Candida sp.* the zone of inhibition in the plate represents the effectiveness of the coated cotton gauze against the test pathogen. The zone of inhibition was measured to be 23mm. Similarly Ragabet *et al.*, 2014 reported that the *Plumeria rubra* stem bark showed inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*.

SEM Analysis for Pure Hydroxyapatite Coated Gauze and *Plumeria Alba* Nanoparticlecoated Gauze under 200X, 750X, 2000X, 5000X

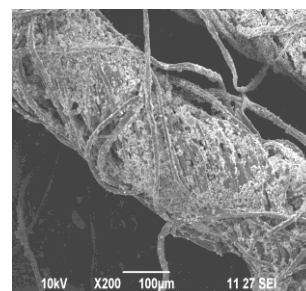


Plate 7: Control (200X)

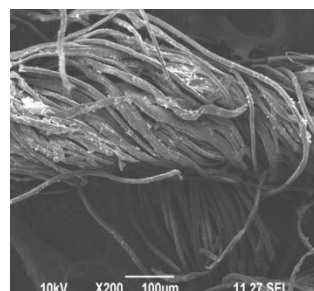


Plate 8: *Plumeria alba* (200X)

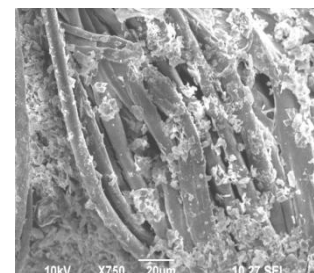


Plate 9: Control (750X)

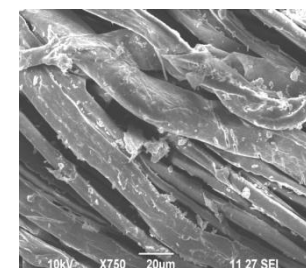


Plate 10: *Plumeria alba* (750X)

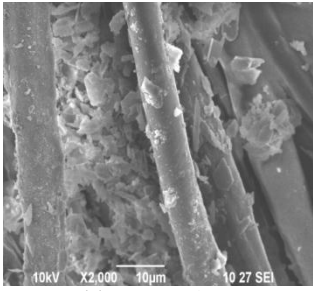


Plate 11: Control (2000X)

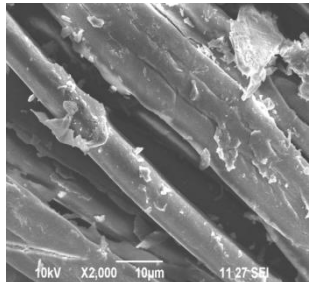


Plate 12: *Plumeria alba* (2000X)

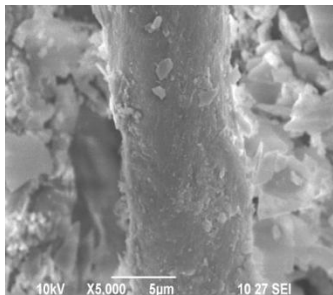


Plate 13: Control (5000X)

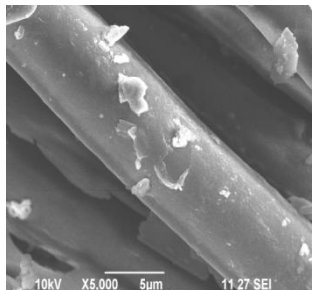


Plate 14: *Plumeria alba* (5000X)

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