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Aegle Marmoles Biosynthesized Silver Nanoparticle-loaded Hydrogel for Topical Application for Wound Dressing

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ABSTRACT

Nanotechnology has gained prominence in various fields such as medicine, electronics, waste treatment and defence sectors. *Aegle marmelos*, commonly known as Bael, is used in ancient Siddha and Ayurveda medicines from time immemorial and is considered as one of the most sacred plants in India. An attempt has been made in this research work to synthesize silver nanoparticles with the aid of a novel, non-toxic, eco-friendly and biological method using *Aegle marmelos* leaf extract for the application as a topical ointment to prevent infection against burns and wounds. Hydrogel loaded with metal nanoparticle have great importance for many applications in the biomedical and biotechnological field. The present study is aimed at the biosynthesis of silver nanoparticle-based hydrogel of *Aegle marmelos* (Mahavilvam) extract for topical delivery in wound dressing. Further, the characterization study was carried out on biosynthesised silver nanoparticle-loaded hydrogel by using UV-Visible Spectrophotometer, Fourier Transform Infrared Spectroscopy and SEM analysis. The antimicrobial resistance against *Escherichia coli* and *Staphylococcus aureus* was measured.

Keywords: Silver nanoparticles; Hydrogel; *Aegle marmelos*.

1. INTRODUCTION

Nanoscience is a boon and can bring unanticipated and unprecedented transformation in diverse areas of research and application (Roopa *et al*. 2013). Nanotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. New applications of nanoparticles and Nanomaterials are emerging rapidly (Jahn *et al*. 1999). Nanotechnology in recent years has demonstrated its role in a cosmetic, pharmaceuticals, electronics, paints and construction materials. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanoparticles show unique electrical, light emitting and catalytic properties as compared to conventional bulk materials. Synthesis of nanoparticles with different compositions, sizes, shapes and controlled disparities is an important area of research. The biological route of synthesizing nanoparticles has many advantages, such as stable production of nanoparticles with controlled sizes and shapes, the lack of subsequent complex chemical synthesis, the lack of toxic contaminants and the ability for rapid synthesis using numerous medicinal plants and microorganisms.

About 5000 years ago, silver was used to store food by Romans, Persians, Egyptians and Greeks (Dipankar *et al*. 2012). The age-old application of silver in the making of utensils for drinking water and eating was probably due to its antibacterial nature. Owing to its antibacterial, antiviral, antifungal and antioxidant nature, silver nanoparticles offer many advantages as drug carriers, including adjustable size and shape and enhanced stability of surface-bound nucleic acid. The silver nanoparticles explored many possible routes to synthesize and stable nanoparticles for different applications. The natural compound from plants (phytochemical) and microorganisms (secondary metabolite) have been extensively used to reduce silver ions to metallic nanoparticles. In recent years, nanoparticle synthesis using plant sources is gaining more interest, by using various parts of the plant. Among the various noble metals, silver is preferred owing to its antibacterial catalytic properties and non-toxicity towards humans in comparison to other metals (*Rai et al.* 2009). The silver nanoparticles are having a high surface area. Of all the metals, silver nanoparticles have gained tremendous interest due to their increasing commercial demand day by day. Silver nanoparticles have wide applications owing to their unique properties such as chemical stability, good conductivity and catalytic activity which can be incorporated into composite fibre, cryogenic superconducting material, cosmetic products, food industry and electronic components (Seamed *et al.* 2014. Chan *et al.* 2001). Studies have shown that the morphology, stability and properties (physical, chemical and biological) of the nanomaterials are strongly influenced by experimental conditions such as concentration of precursors, temperature, nature of solvent, reducing agent, stability and capping agent. The plant extract contains metabolites such as polyphenols flavonoids, proteins, terpenoids and tannins (Raghunathan *et al.* 2011). These metabolites not only act as reducing agents for metal ions but remain on the metal nanoparticles as capping agents, which helps to minimize the agglomeration of nanoparticles. Thus, it is possible to control the morphology and protect/stabilize the nanoparticles to improve their biological properties. Further, they offer non-pathogens, low or nil toxicity and are easy to adopt (Hauns *et al.* 2007). Hence, the use of these medicinal plants has gained much importance, soon after the first reports by Gardea-Torresday *et al.* on the formation of silver and gold nanoparticles by using living plants. The synthetic procedure using plant extract exemplifies a green approach. Currently, nanoparticle synthesis using plant sources are gaining more interest - specifically by using various parts of the plant such as leaf, tubers, bark and buds. Many reported studies were related to the biological synthesis of silver nanoparticles especially using medicinal plants (Krishna *et al.* 2010).

Fig. 1: *Aegle marmelos*

In this study, *Aegle marmelos*-synthesised silver nanoparticles is utilized as a therapeutic agent for wound dressing through hydrogel composition. *Aegle marmelos* (linn) correa commonly known as bael, belongs to the family rutaceae (Gupta *et al.*, 2004). It is distributed in the deciduous forests of India. It is found almost in all the states of India (Hankhar *et al.*, 2001). The therapeutic value of the plant has been referred by almost all the ancient ayurvedic treatise like Siddha, Unani, Sushruta Samhita and Charaka Samhita, etc. Bael tree is a medium-sized deciduous tree with unusual branches surrounded by aromatic trifoliate leave (Padmanav *et al.*, 2014). In the last five decades this plant have been extensively studied by advanced scientific techniques and reports reveal its various medicinal properties such as anticancer, antibacterial, antifungal, anti-diabetic, antioxidant, hepatoprotective, hemocytic, larvicidal and anti-inflammatory (Dinesh *et al.* 2011). A hydrogel, is a three-dimensional polymeric network that can be contained in water or body fluids (Francis *et al.* 2004). Hydrogel have been widely used in biological applications such as their marked similarity of human tissue, excellent biocompatibility and controllable properties (Jabbari *et al.* 2000).

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation of Aqueous Extract

Fig. 2: Preparation of aqueous extract

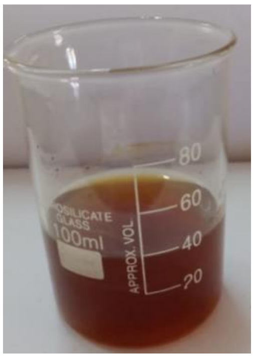

Fig. 3: Biosynthesis of silver nanoparticles

The Fresh leaves of *Aegle marmelos* (Mahavilvam) were collected from Injimedu, Thiruvanamalai district, Tamil Nadu, India, in sterile bags and washed thoroughly to remove dust and sand particles, followed by washing using distilled water. The aqueous extract was prepared from 5 g of fresh leaves with 100 ml of de-ionized water by boiling at 30 ºC for 20 minutes in a hot plate. After boiling the extracts were brought to room temperature and were cooled. Then the extracts were filtered using Whatman No. 1 filter paper and were stored in sterile air-tight container for further use.

2.2 Biosynthesis of Silver Nanoparticles

To synthesise silver nanoparticles, 0.1 M of silver nitrate solution were prepared by dissolving 0.01 g $AgNO_3$ to 1 litre of distilled water. The silver nitrate solution and plant aqueous extract were added in 1:1 ratio and incubated by keeping in a dark environment for 2 h undisturbed. The color change from yellow to dark brown indicates the synthesis of silver nanoparticles and it confirms that the plant extract acts as a capping and reducing agent.

Fig. 4: Carbopol 940

2.3 Preparation of Hydrogel

The hydrogel was prepared by dissolving 1 g of Carbopol 940 in 100 ml of distilled water under continuous stirring and allowed to get swollen for 24 h. Further, 12 g glycerine was added and neutralized with triethylamine, till it turns to appear transparent and then was stored in a freezer.

2.4 Incorporation of Silver Nanoparticles with Hydrogel

The final formulation was prepared by incorporating silver nanoparticles with hydrogel; the aqueous extract-synthesized silver nanoparticle solution was mixed with hydrogel in a slow mixing process (50 rpm) for 5 min.

2.5 Characterization of Biosynthesized Silver Nanoparticles Loaded Hydrogel

The aqueous extract-synthesised silver nanoparticles were characterized using UV-Visible Spectrophotometer, FT-IR and SEM analysis to confirm the silver nanoparticles getting loaded into the hydrogel constituents.

2.6 Hydrogel Testing

2.6.1 Physical test

The silver nanoparticle-loaded hydrogel formulations were tested for colour, homogeneity and consistency. The pH was evaluated by using pH meter to make sure the gel was stable at a pH of 5.5 and was found to be alkaline in nature. The cream color solution appeared. The spreadability test was performed using sterile grease-free glass slide; the silver nanoparticle-loaded hydrogel gel was placed between two glass slides and the spreading consistency of the hydrogel were measured using the formula:

S = M x L / T

where, S - spreadability, M - weight tied to the upper slide, L - length of the glass slide and T - time taken to separate the slide completely from each other.

2.6.2 Skin Irritation Test

The test for skin irritation was performed by placing the silver nanoparticle-loaded hydrogel on the informed volunteers. 5 volunteers were selected and 1 g of formulated gel was applied on the back of their palm. The volunteers were observed for lesions/irritation.

2.6.3 Release of Silver Nanoparticles from Hydrogel gel - Drug content release analysis

About 1 g of hydrogel was dissolved in 5 ml of water and centrifuged at 2000 rpm for 30 minutes and the supernatant was collected and analysed by absorbance OD value using UV-Visible Spectrophotometer.

Fig. 5: Preparation of Hydrogel in aqueous medium

Fig. 6: Hydrogel loaded with Silver Nanoparticles

2.6.4 Determination of Antimicrobial activity for Silver Nanoparticle-loaded Hydrogel

The antimicrobial activity of the silver nanoparticle-loaded hydrogel was tested with *Escherichia coli* and *Staphylococcus aureus* by Agar well diffusion technique. The agar plates were prepared and allowed to get solidified and wells were bored using a sterile well borer; a definite volume of hydrogel was loaded into wells. The plates were incubated at 37 °C for 24 h. The growth was examined after incubation and the diameter of the inhibition zone was measured.

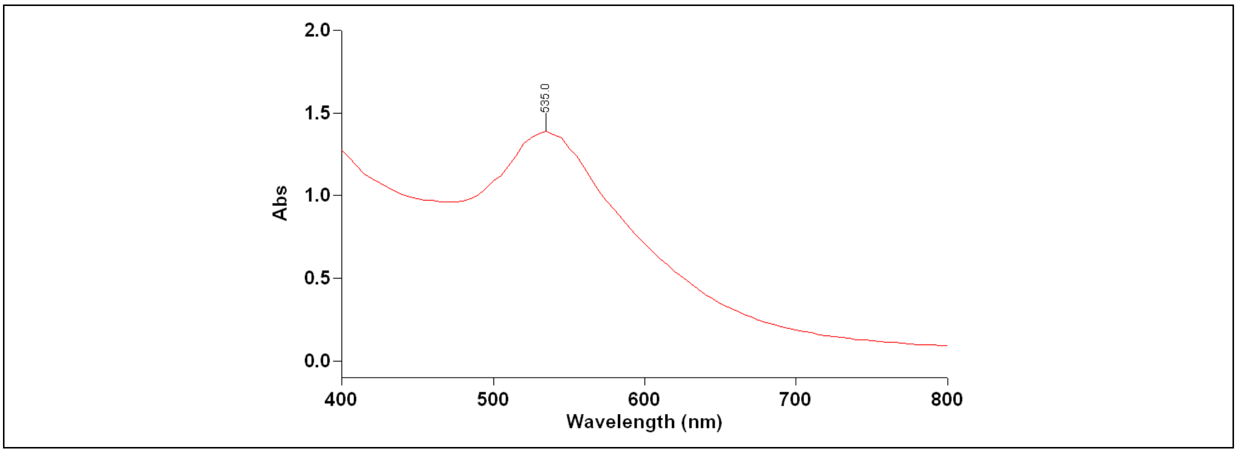

Fig. 7: UV-Visible Spectrum of *Aegle marmelos* Biosynthesised Silver nanoparticle-loaded Hydrogel

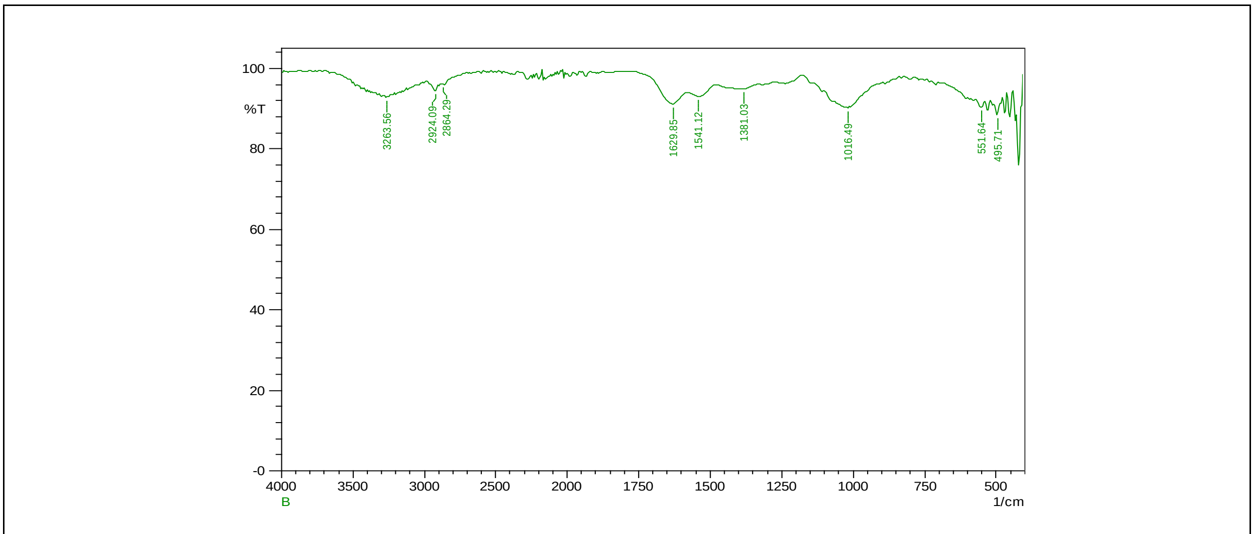

Fig. 8: FT-IR spectrum of *Aegle marmelos* Biosynthesised Silver Nanoparticle-loaded Hydrogel

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles and Preparation of Silver Nanoparticle-loaded Hydrogel

The *Aegle marmelos* aqueous extract was mixed with 0.1 M of silver nitrate solution, followed by incubation in the dark; the color change of the solution was visualized to confirm the synthesis of silver nanoparticles. Then, the hydrogel was prepared using Carbopol 940 grade with the aqueous medium and neutralized using triethylamine. The silver nanoparticle-loaded hydrogel was prepared by the slow mechanical mixing process.

3.2 Characterization Study of Silver Nanoparticle-loaded Hydrogel

Characterization done using UV Spectrophotometer absorbance band form in the range of 535 nm indicates the presence of silver nanoparticles with hydrogel. FT-IR spectrum was analysed for the presence of functional groups at the surface of the silver nanoparticle-loaded hydrogel. The analysis showed the presence of peaks at 3263.56 cm^{-1}, 2924.09 cm^{-1}, 2664.29 cm^{-1}, 1629.85 cm^{-1}, 1381.03 cm^{-1}, 1016.49 cm^{-1} and 495.71 cm^{-1} corresponding to the functional groups such as O-H stretch, alkyl C-H stretch, carboxylic acid O-H stretch, aromatic C=C bending, C-H bending, C-O stretching and ester C-O stretch. The stretches confirm the stabilization of silver nanoparticle-loaded hydrogels capped by the aqueous extract of the *Aegle marmelos*. Then the hydrogel was examined using SEM and the diameters of silver nanoparticles were found to be in the range of 80 to 100 nm; they appeared in spherical form.

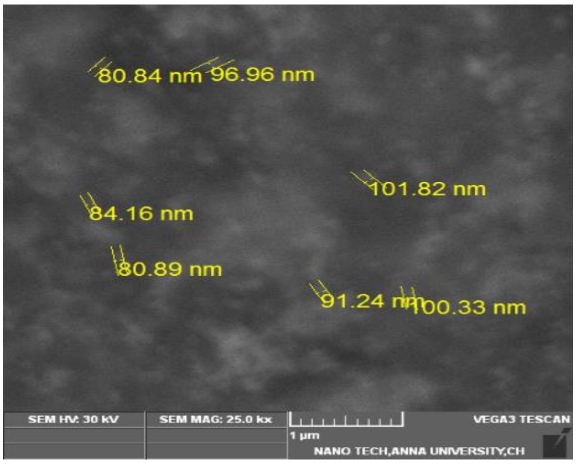

Fig. 9: SEM Image of *Aegle marmelos* Biosynthesised Silver Nanoparticle-loaded Hydrogel

3.3 Physical Testing of Hydrogel

The hydrogel loaded with silver nanoparticles was subjected to physical tests and the results are presented in Table 1.

Table 1: Physical tests carried out for Silver Nanoparticle-loaded Hydrogel

S. No.	Particulars	Inference
1.	Color appearance	Creamy white; gel-like in nature
2.	pH	Alkaline 6.2
3.	Spreadability	67 g.cm-s^{-1}
4.	Homogeneity	Very good consistency
5.	Skin irritation	No irritation and no lesion formation
6.	Drug release analysis	80.3 %

Table 2. Antimicrobial Resistance of Biosynthesised Silver Nanoparticle-loaded Hydrogel

S. No.	Microorganism	Zone of Inhibition in cm
1.	*Escherichia coli*	2.5 cm
2.	*Staphylococcus aureus*	1.8 cm

3.4 Antimicrobial Activity

The antimicrobial activity of the hydrogel loaded with silver nanoparticles was analysed by Agar well diffusion method; after 24 h of incubation, the plates were observed with clear zone around the wells and the zone of inhibition was measured and presented in Table 2.

4. CONCLUSION

Biosynthesis of silver nanoparticles using *Aegle marmelos* (Mahavilvam) aqueous extract was demonstrated. With their unique chemical and physical properties, silver nanoparticles are a definite alternative for the development of novel therapeutic agents. The plant Mahavilvam (worshipped as the presence of Lord Shiva in India) has been utilized as a folklore medicine in ancient days for the treatment of various health disorders. The biosynthesised silver nanoparticles were loaded with hydrogel for the topical application for the skin burns to treat. Future study is required to understand the healing of skin burns by using the biosynthesised silver nanoparticle-loaded hydrogel in order to achieve a better topical aliment for skin-related disorders.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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