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Microencapsulation of *Momordica cymbalaria* (Athalakkai) fruit nanoparticle coated on cotton gauze for biomedical application

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Abstract

Background: Nanotechnology is an inception technology to create new products with enormous potential in a wide range of applications. The green method of nanoparticle synthesis involves the use of plants and plant extracts which are easy to handle, safe and cost effective. *Momordica cymbalaria* belongs to *Cucurbitaceae* family is a versatile nutritious plant with enormous medicinal values for long time for the treatment of diabetes, rheumatism, ulcer, skin diseases and diarrhea. The fruit has lot of nutrients like vitamin, calcium, potassium, sodium, antioxidants, flavanoids, quercetin, saponin, cucurbitacins, momordicine glycosides etc. The antioxidant activity in fruits is notable because of its radical scavenging activity. Microencapsulation is a process in which tiny particles like nanoparticle were surrounded by a polymer coating to give small capsules of many useful properties.
Objective: The main objective of present research work focus on synthesis of silver nanoparticles using the fruit extract of *Momordica cymbalaria* and phytochemical analysis was studied. The developed nanoparticles were microencapsulated using gum acacia polymer, microencapsulation improves the stability of particles by preventing ingredient concentration and degradation and coated on cotton gauze.
Results: The developed fruit nanoparticle production was characterized by the formation of plasmon peak by UV-Visible spectrophotometer. The interaction of functional group and bonding were confirmed by fourier transform infrared spectroscopy. The morphological characterization which concludes the developed nanoparticle bonded with cotton fabric were analysed by SEM.
Conclusion: The developed microencapsulated *Momordica cymbalaria* fruit nanoparticle coated cotton gauze was subjected to antimicrobial activity and it has potent biomedical application.Since it is biocompatible and eco-friendly.

Key words: *Momordica cymbalaria*, Silver nanoparticle, UV-Vis spectrophotometer, Microencapsulation, wound healing activity, FTIR and SEM.

INTRODUCTION

The great innovations are foreseen in nanoscience lie in the fields of pharmaceutics, cosmetics, food and feed, chemical engineering, biomedical science and environmental science. The biomimetic approaches for the growth of advanced nanomaterials make use of plant extract as possible environment friendly process. Among various metal nanoparticles, silver nanoparticles have remarkable attention due to their in-resistible physiochemical properties Silver nanoparticles have been widely used as a novel therapeutic agent extending its use as antibacterial, antifungal, antiviral and anti-inflammatory agent (Kalishwarlal *et al.*, 2008). Antioxidants and phenolic compounds of Caper leaves extract exhibit various medicinal properties (Bhoyar MS *et al.*, 2011). *Momordica cymbalaria* belongs to *Cucurbitaceae* family which is relative of the bitter melon plant. The plant is a perennial herb with slender, branched and striate stem. The plant has large tuberous root stock; flowers are white to yellow in color. The plant has also been named *Luffa tuberosa* or *Momordica tuberosa*. The aqueous extract of *Momordica charantia* was orally administered to overweight rats to assess the effect of the extract on body weight of rats (Farhat Bano *et al.*, 2011). The antioxidant activity in fruits is notable since fruits are rich in compounds that have an important role in free radical-scavenging activity (Sabaianah Bachok et al., 2014). The reason for selecting plant for biosynthesis is because they contain reducing agents such as citric acid, ascorbic acids, flavanoids, reductases, dehydrogenases and extracellular electron shuttlers that may play an important role in biosynthesis of metal nanoparticles (Sunil pandey *et al.*, 2012. The main objective of the present study is

- To prepare extract from the fruit of *Momordica cymbalaria*
- To synthesize silver nanoparticle using fruit extract (*Momordica cymbalaria)*
- To confirm the developed nanoparticle production by reduction of Ag^+ ions using UV-VIS spectrophotometer
- To develop the cotton gauze with fruit nanoparticle as a core material and gum acacia as a coating material by microencapsulation technique
- To determine the size of the nanoparticle coated gauze by SEM-Physical analysis
- To determine the functional groups and bonds of nanoparticle coated gauze by FTIR spectroscopy-Chemical analysis
- To evaluate the antimicrobial activity of the coated cotton gauze

2. MATERIALS AND METHOD

2.1 Collection of Samples

The fruit of *Momordica cymbalaria* were collected from Gandhi park market, Coimbatore. The micro organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida sp* were collected from Sri Ramakrishna Hospitals Coimbatore for evaluating its anti microbial activity. The gum acacia powder was collected from urban platter store, Mumbai.

2.2 Preparation of *Momordica cymbalaria* Fruit Extract

The fruit of *Momordica cymbalaria* was taken and sliced into small pieces. To the 10 grams of fruit 100 ml of distilled water was added and kept in a water bath at 60˚C for 60 minutes. The mixture was filtered using Whatmann No.1 filter paper and the extract was obtained.

2.3 Phytochemical Analysis of *Momordica cymbalaria* Fruit Extract

2.3.1 Test for alkaloids: (*Wagner's* test)

The aqueous extract of fruit was taken and treated with wagner's reagent (Iodine potassium iodide solution). The formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for tannins: (Ferric chloride test)

The aqueous extract of fruit was taken and treated with ferric chloride solution. The formation of mild green color indicates the presence of condensed tannins.

2.3.3 Test for flavanoids: (Zinc hydrochloride test)

The aqueous extract of fruit was taken and treated with zinc dust and concentrated hydrochloric acid. The brisk effervescence with a muddy color after few minutes indicates the presence of flavanoids.

2.3.4 Test for steroids: (*Salwoski* test)

The aqueous extract of fruit was taken and treated with few drops of concentrated sulphuric acid, which leads to the formation of milky color with cloudy appearance indicates the absence of steroids.

2.3.5 Test for saponin: (Froth formation test)

The aqueous extract of fruit was taken and treated with water and shaked frequently, formation of a stable froth indicates the presence of saponins.

2.3.6 Test for cardiac glycosides: (*Baljet's* test)

The aqueous extract of fruit was taken and treated with picric acid; the formation of orange colour indicates the presence of cardiac glycosides.

2.3.7 Test for triterpenoids: (*Salwoski* test)

The aqueous extract of fruit was taken and treated with few drops of concentrated sulphuric acid, which leads to the formation of milky color with cloudy appearance indicates the absence of triterpenoids.

2.3.8 Test for proteins: (Coagulation test)

The aqueous extract of fruit was taken and heated, the formation of coagulate indicates the presence of proteins.

2.4 Synthesis of Silver Nanoparticles Using *Momordica cymbalaria* Fruit Extract

To the 1 ml of fruit extract 9 ml of 1mM silver nitrate was added. The mixture was kept in a magnetic stirrer at 60˚C for 20 minutes and then incubated under dark condition for 24-48 hours. The color change was visually observed.

2.5 Characterization of Developed *Momordica cymbalaria* Fruit Nanoparticle By UV-VIS Spectrophotometer

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 24 hours time interval by drawing $1cm^3$ of the samples and their absorbance was recorded at 300-600 nm using UV-VIS spectrophotometer. Formation of Plasmon peak was observed and recorded.

2.6 Development Of *Momordica cymbalaria* Fruit Nanoparticle Cotton Gauze By Micro-Encapsulation Using Gum Acacia Polymer

The cotton gauze was developed by micro encapsulation method. Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules of many useful properties. Coacervation phase separation method was adopted in the microencapsulation technique. The fruit nanoparticle was used as a core material and the gum acacia (natural polymer) was used as a wall material for coating. 10 grams of gum acacia powder was allowed to swell for 15 minutes in 100 ml of hot water. Further 50 ml of hot water was added and continuously stirred for 15 minutes at 40-50˚C. To the mixture 2.5 ml of developed fruit nanoparticle were added under stirring condition. Stirring was continued for another 15 minutes and then 10 ml of 20% sodium sulphate and 6 gram of citric acid was added. The mixture obtained was kept in freezing condition for 30 minutes to develop microcapsules. The cotton gauze was immersed in a microcapsule solution for 24 hours under dark condition. The immersed gauze was then padded and cured.

2.7 Characterization of *Momordica cymbalaria* Fruit Nanoparticle Developed Gauze

2.7.1(A) Physical Characterization of Developed Fruit Nanoparticle Coated Gauze By Scanning Electron Microscope

SEM study was performed to study shape, size and surface area of the developed fruit nanoparticle coated on cotton gauze. The cotton gauze of 1-2 cm were taken and visualized under SEM.

2.7.1(B) Thickness of Developed Fruit Nanoparticle Coated Gauze (ASTM D 1777-96)

The thickness of developed fruit nanoparticle coated cotton gauze was determined by ASTM D 1777-96 at South India Textile Research Association.

2.7.2 Chemical Characterization of Developed Fruit Nanoparticle Coated Gauze By Fourier Transform Infra-Red Spectroscopy

The developed nanoparticle coated cotton gauze was characterized for interpreting the presence of chemical bonds by FTIR analysis (SHIMADZU- Single reflection ATR accessory). Fourier-transform infrared spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. The distinction of an individual spectrum which are determined by the chemical structure of each component and the degree to which each component contributes to the spectrum was directly related to the concentrations of the components of the sample. A spectrum was recorded at 4000 cm^{-1} to 750 cm^{-1}.

2.8. Anti-Microbial Activity of Developed Fruit Nanoparticle Coated Gauze

The **antibacterial susceptibility** tests were carried out for the developed fruit nanoparticle coated gauze against major wound causing pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The nutrient agar plates were prepared and the test organisms were swabbed on to it. The developed fruit nanoparticle coated gauze were cut into 3x3 cm (length x breadth) and placed in the centre of a plate and incubated at 37˚C for 24 hours. The zone of inhibition was observed and recorded.

The **antifungal susceptibility** tests were carried out for the developed fruit nanoparticle coated gauze against *Candida sp.* The potato dextrose agar plate was prepared and the test organisms were swabbed on to it. The developed fruit nanoparticle coated gauze were cut into 3x3 cm (length x breadth) and placed in the centre of a plate and incubated at room temperature. The zone of inhibition was observed and recorded.

3. RESULTS AND DISCUSSION

3.1 Collection of Samples

The fruits of *Momordica cymbalaria* were collected, cleaned and stored. The micro organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida sp* were collected and sub cultured on to a sterile medium for evaluating its anti microbial activity. The gum acacia powder was collected and stored in a refrigerator for future use.

3.2 Preparation of *Momordica cymbalaria* Fruit Extract

The fruits of *Momordica cymbalaria* were taken and extracted. The prepared extracts were stored in a refrigerator for future applications.

3.3 Phytochemical Analysis of *Momordica cymbalaria* Fruit Extract

Different phytochemical components were present in the aqueous fruit extract of *Momordica cymbalaria* were analysed by using the standard procedures and the results were tabulated. Table 1 represents the preliminary phytochemical constituents of aqueous fruit extract of *Momordica cymbalaria.* The phytochemical screening of the aqueous extract revealed the presence of Alkaloids, Tannins, Flavonoids, Steroids, Saponins and Triterpenoids and absence of Proteins and Cardiac glycosides. Jonathan Yisa. 2009 reported that the phytochemical screening of *Nymphaea lotus* reveals the presence of alkaloids, saponins, and tannins. Beulah Kolluru *et al.,* 2017 reported the significant amount of phytochemicals present in the *Momordica cymbalaria* plant. Ghoshal *et al.,* 2013 reported that the pollens of Momordica charantia showed the presence of saponins along with flavonoids, carbohydrates and tannins.

3.4 Synthesis of Silver Nanoparticles Using *Momordica cymbalaria* Fruit Extract

The formation of silver nanoparticle in the solution of 1mM $AgNO_3$ and *Momordica cymbalaria* fruit extract was confirmed by change in color to dark brown. Plate 1 represents the synthesis of silver nanoparticle using fruit extract of *Momordica cymbalaria.* Natesan Geetha *et al.,* 2014 reported that the colourless silver nitrate solution is changed from pale yellow to ruby red and finally dark brown colour.

3.5 Characterization of Developed *Momordica cymbalaria* Fruit Nanoparticle By UV-VIS Spectrophotometer

The Figure 1 represents the silver nanoparticle synthesized by fruit extract of *Momordica cymbalaria*. The reduction of silver ions into silver nanoparticles during exposure to fruit extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The optical property of AgNPs was determined by UV-Visible spectrophotometer. After the addition of Silver nitrate to the fruit extract, the spectrum was taken at 250 nm to 550 nm. The AgNPs has sharp absorbance with highest peak at 420 nm. Dhivya and Rajasimman. 2015 reported that the AgNPs has sharp absorbance with highest peak at 422 nm and progressively decreased while nanometer increased. Raghda *et al.,* 2017 reported that the bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots 3 ml and measuring UV–Vis spectra of the solution at 200–800 nm using a 3–5 mm quartz cuvette.

3.6 Development of *Momordica cymbalaria* Fruit Nanoparticle Cotton Gauze By Micro-Encapsulation Using Gum Acacia Polymer

The encapsulation of gum acacia with the fruit nanoparticle was done by micro encapsulation (Coacervation phase separation). The gum acacia in a liquid phase serves as a wall material and the fruit nanoparticle solution serves as a core material. Sathianarayanan *et al.,* 2010 reported that the microencapsulating fabrics were efficient when compared to other direct application coating methods. Plate 6 represents the microencapsulation of developed fruit nanoparticle in an oil immersion objective. Plate 5 represents the microencapsulated gauze. Badee et al., 2012 reported that the micro encapsulation of peppermint oil by Spray drying quickly removes water by vaporization from oil-in-water emulsions enabling high retention of volatiles.

3.7 Characterization of *Momordica cymbalaria* Fruit Nanoparticle Developed Gauze

3.7.1(A) Physical Characterization of Developed Fruit Nanoparticle Coated Gauze By Scanning Electron Microscope

The morphological appearance of developed fruit nanoparticle incorporated on cotton gauze was observed by Scanning Electron Microscope. The plate of SEM images with 500X, 1000X, 2000X and 5000X magnification represents that the developed nanoparticles are aggregated. In the micrographs it was observed that the nanoparticles were in the size ranging from 5-50 µm with a variety of morphology in structure. Swamy *et al.,* 2015 reported that the presence of spherical shaped silver nanoparticles of *Momordica cymbalaria* with an average particle size of 15.5 nm. Similarly Krithiga *et al.,* 2015 reported that the silver nanoparticles of *Momordica charantia* were found to be spherical in shape and were aggregated and the aggregation may be due to secondary metabolites in the leaf extracts. Singh *et al.,* 2011 reported that the production of AgNPs biologically from non pathogenic fungus was found to be 30 to 50nm in size.

3.7.1(B) Thickness of Developed Fruit Nanoparticle Coated Gauze (ASTM D 1777-96)

The thickness of developed fruit nanoparticle coated cotton gauze was determined as **0.40 mm** by ASTM D 1777-96 and tabulated in table 2.

3.7.2 Chemical Characterization of Developed Fruit Nanoparticle Coated Gauze By Fourier Transform Infra-Red Spectroscopy

FTIR analysis represents biomolecules and functional groups present in the *Momordica cymbalaria* fruit nanoparticle. The strong and broad peaks at 3387.00 cm^{-1}, 3356.14 cm^{-1} represents the presence (OH) alcoholic compound is mainly responsible for bioreduction of Ag ions. The narrow (over ~ 500 cm-1) often looks like distorted baseline peaks at 3028.24 cm^{-1}, 2866.22 $cm^{-}1$ represents (N-H) 3˚Amine functional group respectively. The strong and multi-banded absorption peak at around 2754.35 cm^{-1}, 2684.91 cm^{-1} represents (C-H) SP^3 carbon. The peaks at 2596.19 cm^{-1}, 2534.46 cm^{-1}, and 2353.16 cm^{-1} represent the presence of (COOH) carboxylic acid respectively. The strong and broad peaks at 2110.12 cm^{-1}, 2017.54 $cm^{-}1$ represents the presence of alkyne group. The sharp, narrow and strong absorption peak at 1951.96 cm^{-1} ($-NH_2$) primary amine, 1732.08 cm^{-1}(C=O) ketone and 1365.60 cm^{-1}, 1219.01 cm^{-1}, 1080.14 cm^{-1} represents the presence of (CHO) aldehyde group. The reduction of silver ions to silver nanoparticles was confirmed from the peak value of *Momordica cymbalaria* fruit extract at 1643.35 cm^{-1} confirms the presence of (C=C) **Momordicin** compound. The other several short peaks represent the presence of phytoconstituents of fruit such as steroids, terpenoids, tannins, sterols, saponins and flavonoids respectively. The wave number, functional group and the description of peaks were tabulated in table 3. The reduction of silver ions to silver nanoparticles was confirmed from the peak value of Bitter gourd extract (1630 cm-1 to 1529 cm-1) were recorded by Dhivya and Rajasimman. 2015. Krithiga *et al.,* 2015 reported that the FTIR spectra of silver nanoparticles synthesized at room temperature showed a strong band at 3292.3 cm-1 which corresponds to O-H Stretching which was due to the presence of double distilled water .The band at 1636.1 cm-1 reveals the presence of C=C group which was due to the presence of Momordicin compound. Table 3 represents the FTIR analysis of fruit

nanoparticle coated gauze. The wave number with their corresponding functional groups and peaks were described. The alcohol group, amine and the carboxylic acid are recorded as more, while alcohol and carboxylic acid group have strong and broad peaks repectively. The ketonic and adehyde group were recorded with lower frequencies and they are described as narrow and sharp peaks. The specific aromatic ring compound called Momordicin (C=C) which is specific to *Momordica* sp was recorded and tabulated.

3.8. Anti-Microbial Activity of the Developed Fruit Nanoparticle Coated Gauze

The **antibacterial activity** was performed with two different strains. Zone of inhibition in the plate represents the developed fruit nanoparticle coated gauze have the antibacterial activity against test pathogens namely *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The zone of inhibition was measured as 34±0.8 mm for *Staphylococcus aureus* and 36±0.7 mm for *Pseudomonas aeruginosa.* The **antifungal activity** was performed with *Candida* sp. Zone of inhibition in the plate represents the developed fruit nanoparticle coated gauze have the antifungal activity against the test pathogen *Candida* sp. The zone of inhibition was measured as 33±1.3 mm and the results were tabulted in table 4 and 5. Similarly Swamy *et al.,* 2007 reported that *M .cymbalaria* fruit extracts may be useful as a broad-spectrum antimicrobial agent for its potent activity against pathogens. Abdulmoneim *et al.,* 2011 reported that the In-vitro antimicrobial activity of *Moringa oleifera* extract showed that the aqueous and methanol extracts have substantial inhibitory effects against the four tested bacterial strains. *S. aureus* and *Aspergillus falvus* were most susceptible to chloroform extract of *M. cymbalaria* were reported by Shrivardhan *et al.,* 2013.

Table 1: Phytochemical constituents of *Momordica cymbalaria* fruit extract

S.No	Phytoconstituents	Aqueous Extract
1	Alkaloids	+
2	Tannins	+
3	Flavanoids	+
4	Steroids	+
5	Saponin	+
6	Cardiac glycosides	-
7	Triterpenoids	+
8	Proteins	-

Table 2: Thickness of microencapsulated fruit nanoparticle coated cotton gauze

S. No	**Sample**	**Control**	**Developed fruit nanoparticle coated cotton gauze**
1	Thickness	0.30 mm	0.40 mm

Table 3: FTIR analysis of developed *Momordica cymbalaria* fruit nanoparticle coated gauze

S. No	**Peaks**	**Functional Group**	**Description**
1	3387.00 cm^{-1}, 3356.14 cm^{-1}	O-H (ALCOHOL)	Strong and broad
2	3028.24 cm^{-1}, 2866.22 cm^{-1}	N-H (3˚AMINE)	Narrow (over ~ 500 cm-1), often looks like distorted baseline
3	2754.35 cm^{-1}, 2684.91 cm^{-1}	C-H (SP^3 CARBON)	Strong and multi-banded
4	2596.19 cm^{-1}, 2534.46 cm^{-1}, 2353.16 cm^{-1}	COOH (CARBOXYLIC ACID)	Very strong and broad
5	2110.12 cm^{-1}, 2017.54 cm^{-1}	C $\underline{\underline{C}}$ (ALKYNE)	Medium intensity for terminal alkynes, very weak for internal
6	1951.96 cm^{-1}	$-NH_2$ (1˚ & 2˚AMINE)	Lower frequency for amides
7	1732.08 cm^{-1}	C=O (KETONE)	Very strong and narrow
8	1643.35 cm^{-1}	C=C (MOMORDICIN)	Aromatic ring specific for *Momordica sp.*
9	1365.60 cm^{-1}, 1219.01 cm^{-1}, 1080.14 cm^{-1}	CHO (ALDEHYDE)	Usually sharp, narrow and strong

Table 4: Antibacterial activity of developed *Momordica cymbalaria* fruit nanoparticle coated gauze

S.No	**Organism**	**Zone of Inhibition**
1	*Staphylococcus aureus*	34±0.8 mm
2	*Pseudomonas aeruginosa*	36±0.7 mm

Table 5: Antifungal activity of developed Momordica cymbalaria fruit nanoparticle coated gauze

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S.No	Organism	Zone of Inhibition
1	*Candida* sp	33±1.3

GRAPHS

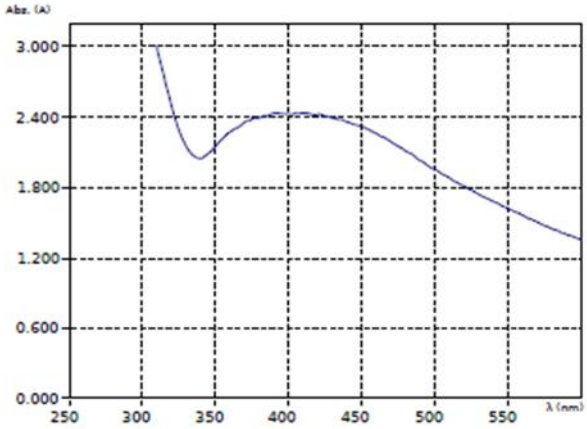

Figure 1: UV- VIS spectroscopy for silver nanoparticle developed from fruit extract of *Momordica cymbalaria*

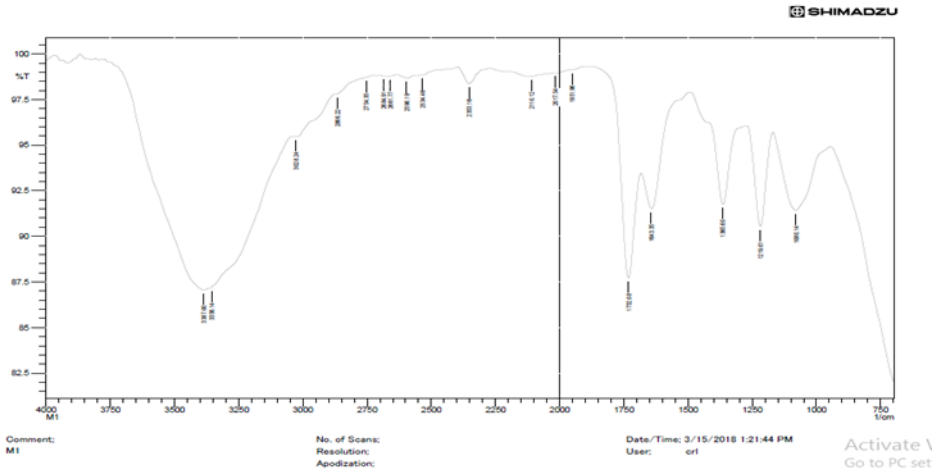

Figure 2: FTIR analysis of developed *Momordica cymbalaria* fruit nanoparticle coated gauze

PLATES

Plate 1: Synthesis of Silver Nanoparticle (A) Fruit Extract of *MOMORDICA CYMBALARIA*, (B) Developed Fruit Nanoparticle, (C) Silver Nitrate Solution

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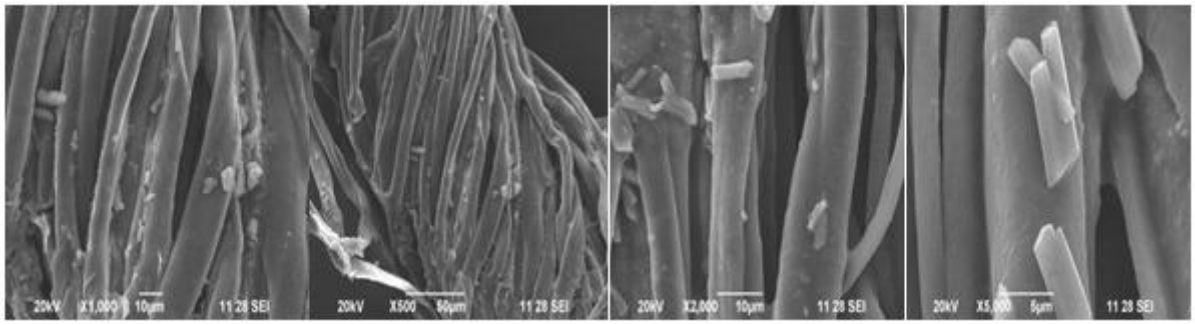

Plate 2: Sem Analysis of Developed Fruit Nanoparticle Coated Gauze Under 1000 X, 500 X, 2000X, 5000X

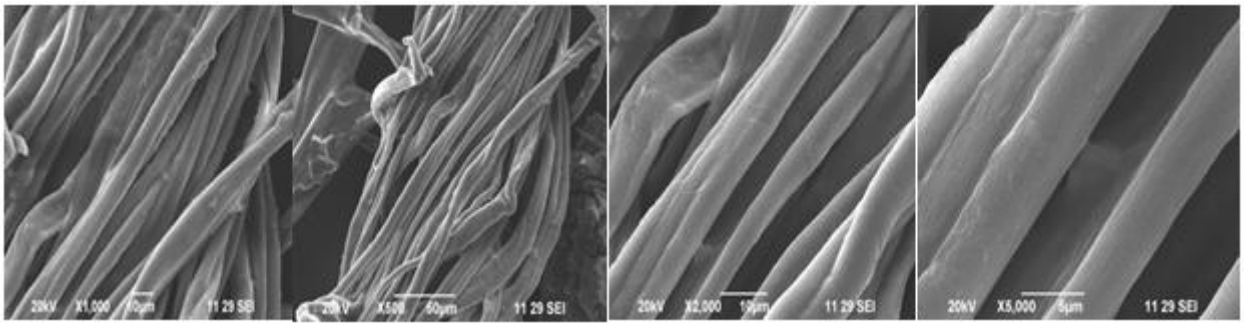

Plate 3: Sem Analysis of Control Cotton Gauze Under 1000 X, 500 X, 2000X, 5000X

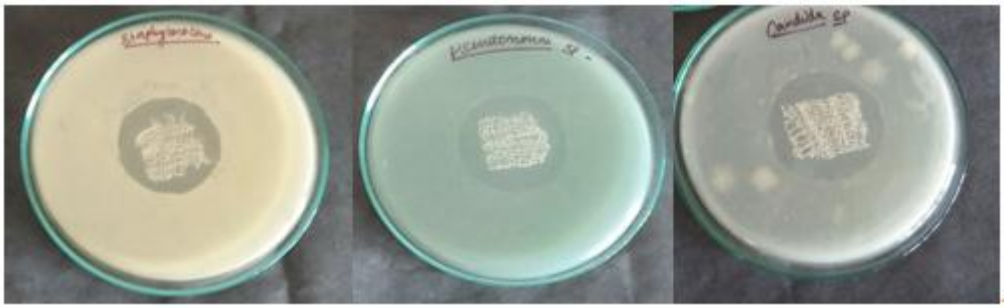

Plate 4: Antimicrobial Activity of Developed Fruit Nanoparticle Against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida* SP

4. SUMMARY AND CONCLUSION

Green synthesis of nanoparticles is safe and environment friendly. The present work concerned with the synthesis of silver nanoparticles using *Momordica cymbalaria* (Athalakkai) fruit extract. The *Momordica cymbalaria* fruit were extracted and nanoparticle was synthesized using 1mM silver nitrate solution. The developed nanoparticle solution was characterized by UV-Vis spectrophotometer. The developed fruit nanoparticle was coated on cotton gauze by microencapsulation method. The biomolecules and functional groups present in the coated gauze were determined by FTIR and the morphology and surface area by SEM. The developed fruit nanoparticle exhibits antimicrobial activity against the test pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida* sp. Thus the microencapsulated *Momordica cymbalaria* fruit nanoparticle coated on cotton fabric has potent biomedical application for wound dressing. Since it is biocompatible and pollutant free.

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