SYNTHESIS OF SELENIUM NANOPARTICLES FROM SERICIN & BANANA SAP & FORMULATION OF WOUND HEALING GAUZE

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Abstract

In this study selenium nanoparticles synthesized by a completely environmentally safe and facile process using sericin protein from silkworm cocoon aqueous extracts and banana stem sap. Phytochemical screening of the banana sap extracts showed the presence of alkaloids, flavanoids, terpinoids, carbohydrates, saponin, steroids and tannins. The synthesized SeNPs was characterized by UV, FTIR and protein test were confirmed by biuret test. This green synthesis indicate that it is efficient method in chemical method. The antioxidant properties of SeNPs from sericin and banana sap were determined by DPPH assay H_2O_2 assay. In anti inflammatory activity showed a albumin denaturation and membranes stabilization of SeNPs from sericin and banana sap also possess antimicrobial activity. The study suggest that the green synthesis SeNPs also possess antioxidant, antiinflammatory and potent antibacterial activity. The bacterial organisms used to gram positive bacteria staphylococcus aureus and micrococcus luteus and gram negative bacteria E.coli and salmonella typhi. The gram positive control or chloramphenicol drug is used.

Key words: Selenium nanoparticles, sericin, banana sap, antioxidant, antimicrobial activity.

1. Introduction

1.1. Sericin

Silk fiber is an essential component of the textile or sericulture industry is mainly produced by a number of silkworms belonging to the families Bombycidae, Lasiocampidae and saturniidae, which include a few spiders. The most commonly used silk is form silk cocoons made by the silkworm *Bombyx mori*. Silk sericin are mainly composed of two types of protein, fibroin and sericin. As an adhesive substance, sericin surrounds the exterior of fibroin, which lies at the center of silk fibers. Sericin is detached from fibroin by the silk industry in order to improve the smoothness, luster, lightness and dye ability of the fibers. As sericin is a major component of raw silk, it has been estimated that out of 4.0 lakh tons of dry cocoons produced world wide, around 50,000 tons of sericin are usually discorded as waste in sewage, representing an environmental hazard. It is reported that sericin as a number of useful applications, starting from useful food elements to application in the cosmetics and medical fields. Sericin has been extracted using a variety of methods such as hot water, acidic, citric acid solution, urea and sodium carbonate solution; however, the degumming is the method most commonly adopted by industries. The different extraction methods of silk might result in varying molecular weights, aminoacid content, confirmation, zeta powder and particle size as well as different physicochemical properties and biological activities of sericin. Sericin contains eighteen

Amino acids including positively and negatively charged, aromatic, polar and non polar amino acids. Moreover, this properties of sericin have made it useful for a variety of pur poses and particularly in food sectar industries. Sericin protein polar chemical groups possess antioxidant, antibacterial and anti-tyrosinase properties along with anti inflammatory, anti tumor activity. A variety of applications of sericin, including in cosmetics, pharmaceuticals, food and textiles.

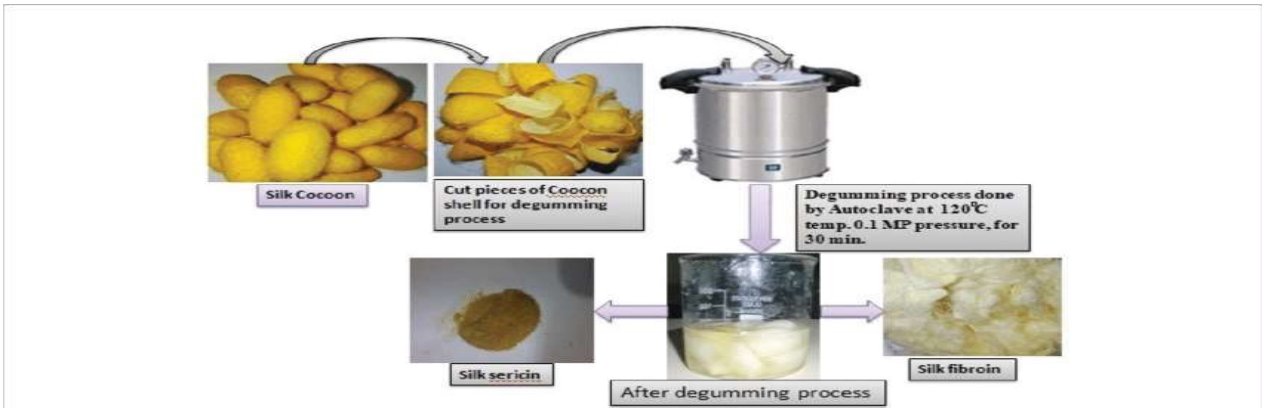


Fig1. Sericin fromcocoons

1.2. Banana sap

Crack mending is a complex and a dynamic process of replacing devitalized and missing cellular structures and kerchief layers. Infections, surgical interventions, and drugs can affect in a different rates of mending. The Ambonese banana (*Musa paradisiacavar*. sapientum) is a plant kingdom that's generally set up in Indonesia, especially in areas with a lot of sun. Empirically, Ambonese bananas are extensively used to treat Conditions analogous as uterine bleeding, intestinal ulceration, hemorrhoids, funk spell, observance and throat lump, dysentery, colon bleeding and diarrhea. The shops with a high lectin attention can be used for crack mending either through coagulation or by blood clot conformation. The administration of banana stem tire extract showed ulcer mending as indicated by increased situations of hydroxyproline, hexuronic acid, hexosamine, superoxide dismutase and dropped glutathione in granulation kerchief and lipid peroxidation, compared to the control group. The result of- seditious and analgesic tests showed that banana tire with over to 100 attention was fairly non- poisonous to fibroblast cells, and had effectiveness as an anti-inflammatory and analgesic. Banana trees contains various mixes that truly salutary for medicine. They're with high contents of allantoins and tannins are constantly used for treating injuries, laryngitis, bleeding, and urinary tract infections. The roots of these plant are truly useful for relieving dental pains, ulcers and intestinal inflammation. Banana tire contains saponins, Anthraquinone, and tannins that can serve as an antibiotics and as a pain relief agents. In addition, banana tire also contains lectin, which serves to stimulate skin cell growth.

1.3. Nanoparticle

There has been tremendous increase in nanotechnology in the past decade due to its application in chemistry, medical and biotechnology. Progress in this field has opened new horizons in nano science, particularly in gene delivery, drug delivery nano medicine, Biosensing, etc..One of the unique properties that make Nano-Sized particles so interesting is their high surface to volume ratio. This feature of nanoparticle, that enable more reactive than the bulkmaterial as atoms on the surface tend to more active than those at the center. These nanoparticles of synthesized using chemical, physical or biological methods. The nano particles obtained via the green synthesis method are found to be non toxic, cost effective and biodegradable in nature.

2. Methodology

2.1. Sample collection

Banana stem was collected from Rasipuram namakkal district. The stem where washed with distilled water to eliminate the unwanted dust and sand.

2.2. Synthesis of sericin from silk cocoon

Sericin was extracted by using water under pressure in an autoclave (121 °C for 30 min, and a liquor ratio 1:30 (w/v)), and dehydrated by freeze-drying. Approximately 15 g of Bombyx mori cocoons were weighed into a 2000 mL Erlenmeyer flask, added with 1000 mL of ultrapure water, and autoclaved at 120 °C during 60 min. After autoclaving, sericin was extracted. The excess cocoon mass was then withdrawn and the resulting solution was vacuum-filtered through a 0.45 μm pore dia- meter filter using a Millipore filtration device. The filtered solution was then distributed into several glass flasks and duly frozen at −86 °C prior to lyophilization. After lyophilization, the crude extract was duly grinded. The resulting crude sericin extract powder is used for further analysis.

2.3. Synthesis of banana sap

The general procedure of Banana SAP isolation includes preparation, extraction, and recovery. The preparation mostly consists of washing, cleaning, the separation of animal parts, and size reduction by cutting or mincing the samples for facilitating the following pretreatment of the samples. After the preparation, a mild chemical pretreatment is performed to increase the efficacy of the extraction and remove non-Banana SAPous substances. Generally, depending on the raw materials and the extraction method, different pretreatments can be performed (alkaline or acid treatment). Pretreatment is used with a diluted acid or base to break down the crosslinked Banana SAP before the extraction because of crosslinked Banana SAP in the connective tissue of animals. The alkaline pretreatment is mostly performed by using sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$).

2.4. Synthesis of selenium nanoparticle

Take 4gm of sodium selenate is dissolved in 100ml of distilled water. To take 60ml of sodium selenate solution and mixed each 20 ml of Sericin solution and banana sap. After takes place in shaker at 24 hours and get reddish brown colour solution. After centrifuge to takes place the UV-IR.

2.5. Phytochemical analysis

The phytochemical analysis of the banana sap was carried out using standard chemical procedure.

2.6. Biological activity

2.6.1. Antioxidant Activity

1. DPPH RADICAL SCAVENGING ACTIVITY

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with hydrogen donor changes to yellow color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

a) **Reagents**
2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 µM): 22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 µM DPPH solution.

b) **Preparation of test solutions**: 21 mg each of the extracts was dissolved in distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations.

c) **Preparation of standard solutions:**10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 0.95 ml of Dimethyl sulfoxide (DMSO) to get 10.5 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

PROCEDURE

The assay was carried out in a 96 well microtiter plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtiter plate. The final concentration of the test and standard solutions used were 250, 200, 150, 100, and 50µg/ml. The plates were incubated at 37^{o} C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

H_2O_2 RADICAL SCAVENGING POWER ASSAY

PREPARATION OF TEST AND STANDARD SOLUTIONS

Weighed accurately 2 mg of each extracts and the standard, ascorbic acid and dissolved in 2 ml of DMSO. Then take 0.5 of above solution then make up to 2.5 ml with phosphate buffer (0.2 M, pH 6.6) .The lower dilutions were made serially with DMSO.

PROCEDURE

A method developed by Oyaizu, 1986 for reducing power test was used. The above sample was spiked with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then kept in a 50°C water-bath for 20 min. The resulting solution was then cooled rapidly, spiked with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was then mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance at 700 nm was then detected after reaction for 10 min. The higher the absorbance represents the stronger the reducing power.

2.6.2. Anti-inflammatory Activity

Membrane stabilization test

- **Preparation of red blood cells (RBCs) suspension**

  Fresh whole human blood (10ml) is collected and transferred to the centrifuge tubes. The tubes are centrifuged at 3000 rpm for 10min and are washed three times with equal volume of normal saline. The volume of blood is measured and re constituted as 10% v/v suspension with normal saline (Sadique et al., 1989; Saket et al., 2010)

- **Anti- inflammatory properties: Inhibition of albumin denaturation**

  Denaturation of proteins will be well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of different solvent plant extract protein denaturation will studied. Repeated the experiments three times for each replicates, According to Duncan's Multiple Range Test (DMRT), the values are to be followed by different subscripts and checked for significant difference at $P<0.05$, SE-standard error of the mean.

Antifungal assay

Totally two fungal strains were used throughout investigation. All the fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young fungal broth cultures were prepared before the screening procedure.

2.6.3. Antifungal activity

Antifungal activity was measured using methods of well diffusion plates on agar. To test the antifungal activity, the fractions of different concentration of plant extract were dissolved in 70% ethanol. 20 mL of Sabouraud Dextrose Agar was poured into each 15 cm Petri dish. *C. albicans* and *Aspergillus terreus* were grown in sabouraud dextrose broth at 27° C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with sabouraud dextrose broth. Then, Wells were cut and 20 µl of the different concentration of test drug were placed on agar to load 10 and 15 µL of each spice sample (1 mg/mL). 100 units of Fluconazole, obtained from a local pharmacy, were used as a positive control. Inhibition zones were determined after incubation at 27° C for 48 hrs.

2.6.4. Antibacterial assay

i. Preparation of inoculums

Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37^{o}C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0 X 10^{6} colony forming units (CFU/ml) for bacteria.

ii. PREPARATION OF STERILE SWABS

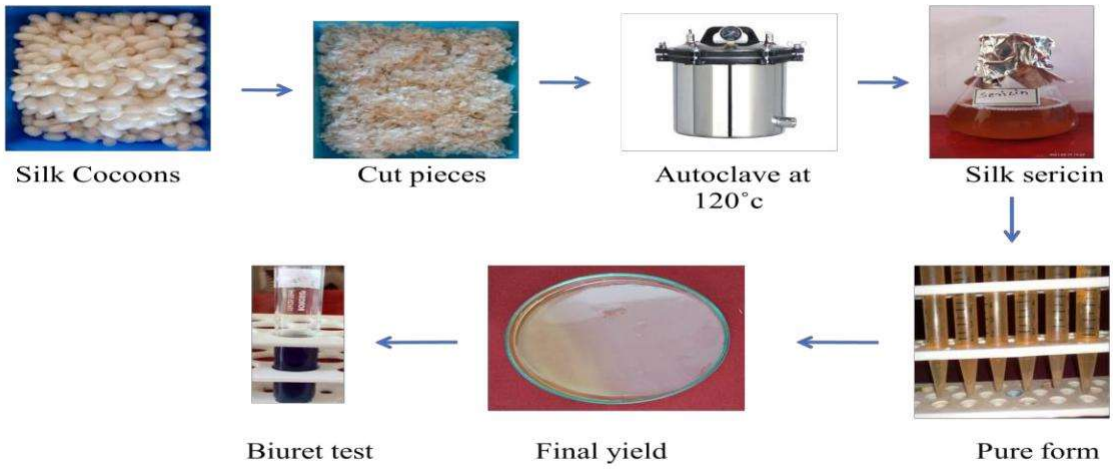
Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes, papers, or tins etc.

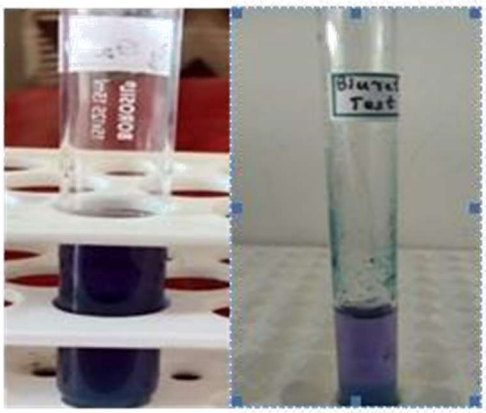
iii. STERILIZATION OF FORCEPS

Forceps can be sterilized by dipping in alcohol and burning off the alcohol.

3. RESULTS AND DISCUSSION

i. Extraction, purification, isolation and characterization of sericin from silk waste cocoons





Biuret confirmation test for Sericin protein

Extraction, purification, isolation and characterization of sericin from silk waste cocoons

ii.EXTRACTION, PURIFICATION AND ISOLATION OF BANANA SAP

Fig 4: Banana spseudo stem

Fig 5: Banana sap

iii.Synthesis Of Selenium Nano Particles

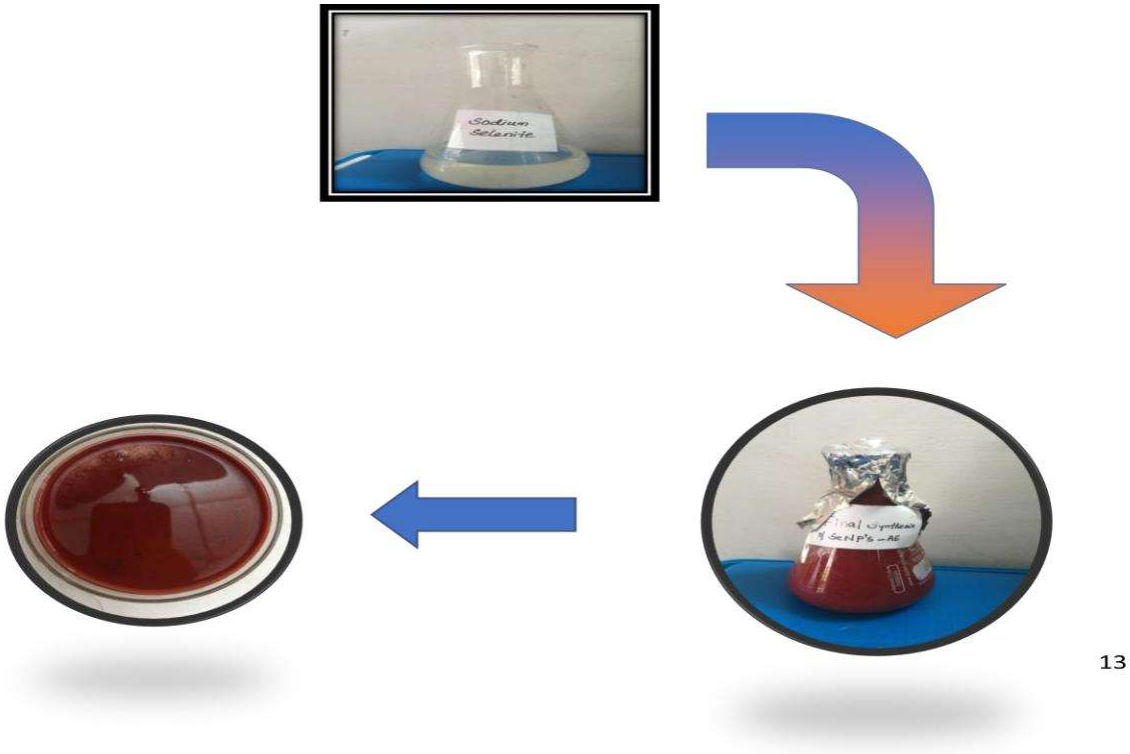


Fig 6: synthesis of selenium nano particles

UV SPECTRAL ANALYSIS OF SEIENIUM NANOPARTICLE

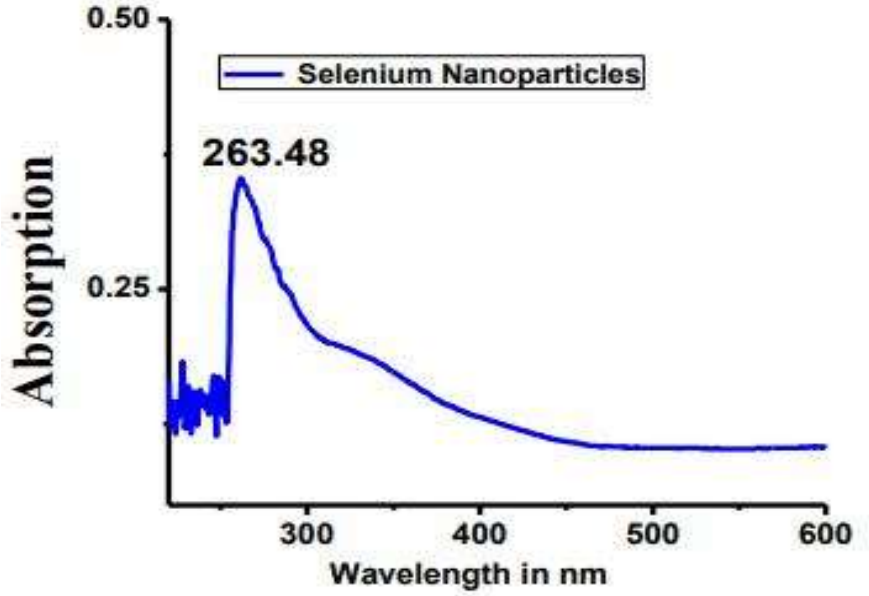


Fig 7: UV spectral analysis of Selenium nanoparticle

FT-IR SPECTRAL ANALYSIS OF SERICIN AND BANANA SAP

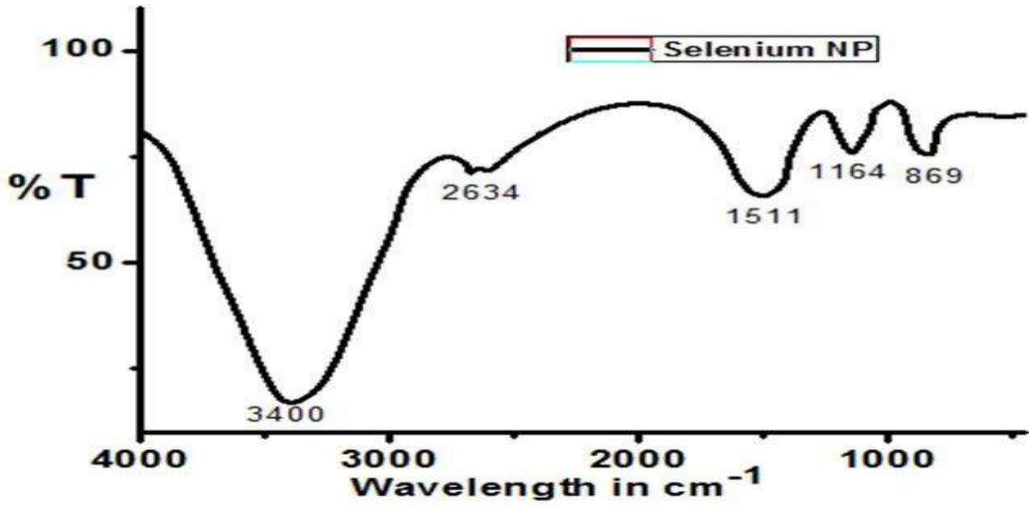


Fig 8: FT-IR spectral analysis of sericin and banana sap

4.QUALITATIVE EVALUVATION OF PHYTOCHEMICAL CONSTITUENTS OF DIFFERENT EXTRACTS OF BANANA SAP

TABLE-1

S.NO	COMPONENTS	AQUEOUS	METHANOL	CHLOROFORM
1	Alkaloid	++	++	ND
2	Flavonoid	++	+++	ND
3	Triterpenoid	++	+++	+
4	Carbohydrates	++	+++	ND
5	Saponin	+++	+++	ND
6	Steroids	+	+++	+
7	Amino acid	ND	ND	ND
8	Tannin	+++	+++	+
9	Gums & Mucilage	ND	ND	ND
10	Chlorogenic compound	+	++	ND

4.1 ANTIOXIDANT AND FREE RADICAL SCAVENGING ANALYSIS OF SERICIN, BANANA SAP AND SeNPS OF SERICIN- BANANA SAP

TABLE 2: DPPH Radical scavenging activity of sericin, banana sap and SeNPS of serin-banana sap

Group	Concentration (µg/ml)	% of Inhibition			IC_{50} VALUE		
		Sericin	Banana SAP	SeNPS of Sericin-Banana SAP	Sericin (µg/ml)	Banana SAP (µg/ml)	SeNPS (µg/ml)
Sericin, Banana SAP and SeNPS of Sericin - Banana SAP	100	80	74	92	22.12	27.36	36.4
	80	75	62	80			
	60	62	50	68			
	40	49	37	56			
	20	43	30	47			
Ascorbic Acid	100	94			58.93(µg/ml)		
	80	83					
	60	72					
	40	58					
	20	50					

4.2 DPPH RADICAL SCAVENGING ACTIVITY OF SERICIN, BANANA SAP AND SeNPS OF SERIN-BANANA SAP

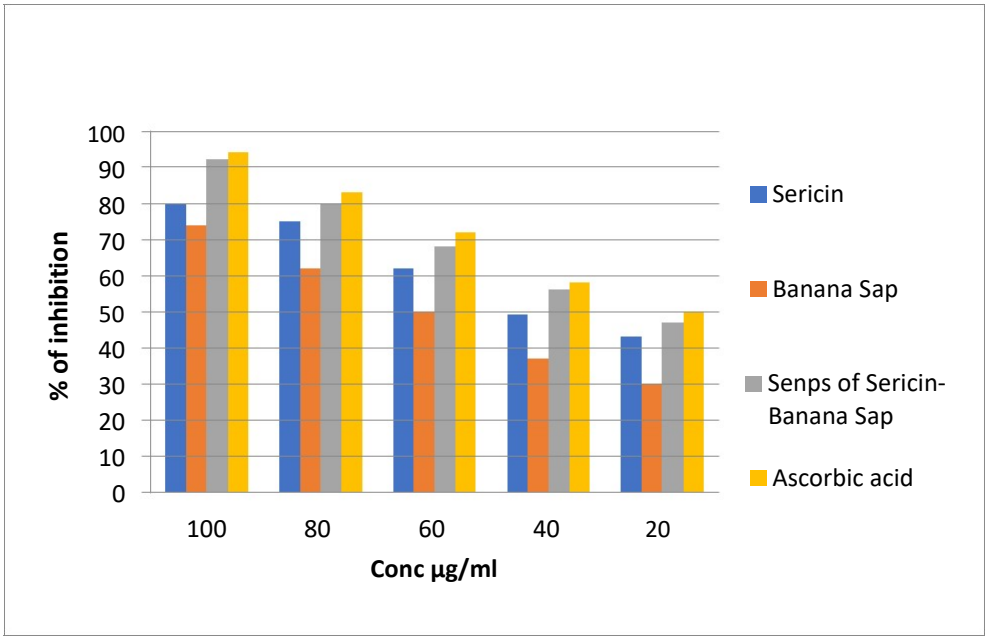


Fig 9: DPPH radical scavenging activity of sericin, banana sap and senps of sericin-banana sap

4.3 H_2O_2 RADICAL SCAVENGING ACTIVITY OF SERICIN, BANANA SAP AND SeNPS OF SERIN-BANANA SAP

TABLE 3: H_2O_2 Radical scavenging activity of sericin, banana sap and senps of sericin-banana sap

Group	Concentration (µg/Ml)	% Of Inhibition			Ic_{50} Value		
		Sericin	Banana SAP	SeNPS Of Sericin-Banana SAP	Sericin	Banana SAP	SeNPS Of Sericin-Banana SAP
Sericin, Banana SAP and SeNPS of Sericin - Banana SAP	100	67	60	79	81.66µg/ml	72.81µg/ml	52.42µg/ml
	80	56	48	68			
	60	41	36	59			
	40	28	24	44			
	20	17	12	25			
Ascorbic Acid	100	84			38.78µg/ml		
	80	73					
	60	65					
	40	52					
	20	37					

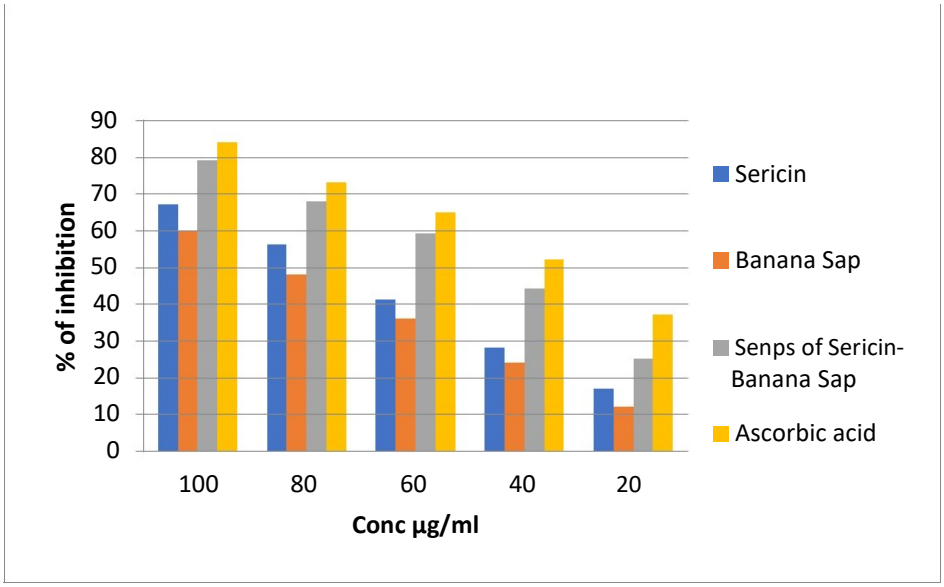


Fig 10: H_2O_2 Radical scavenging activity of sericin, banana sap and senps of sericin -banana sap

4.4 MEMBRANE STABILIZATION ANALYSIS

TABLE 7: Membrane stabilization analysis

Group	Concentration(µg/Ml)	% Of Inhibition		
		Sericin	Banana SAP	SeNPs of Sericin-Banana SAP
Sericin, Banana SAP and SeNPs of Sericin - Banana SAP	100	74.00	67.34	79.63
	80	60.22	51.39	71.41
	60	45.05	38.72	58.77
	40	36.12	24.90	46.18
	20	28.61	15.39	40.33
Aspirin	100	90.03		
	80	82.77		
	60	74.00		
	40	60.52		
	20	46.00		

5.ANTIMICOBIAL ANALYSIS

5.1 DIAGRAMMATIC REPRESENTATION OF WOUND HEALING AND ANTIMICROBIAL PROPERTIES OF SERICIN

GRAM POSITIVE BACTERIA

Staphylococcus aureus

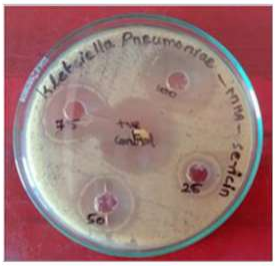
Bacillus subtilis

Enterococcus faecalis

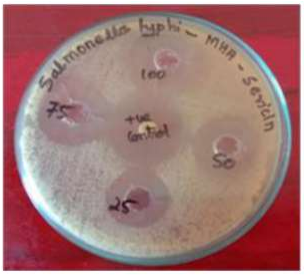
Micrococcus luteus

GRAM NEGATIVE BACTERIA

E.coli



Klebsiella pneumonia

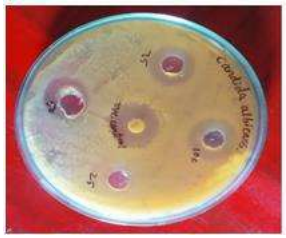


Salmonella typhi

Proteus vulgaris

FUNGAL

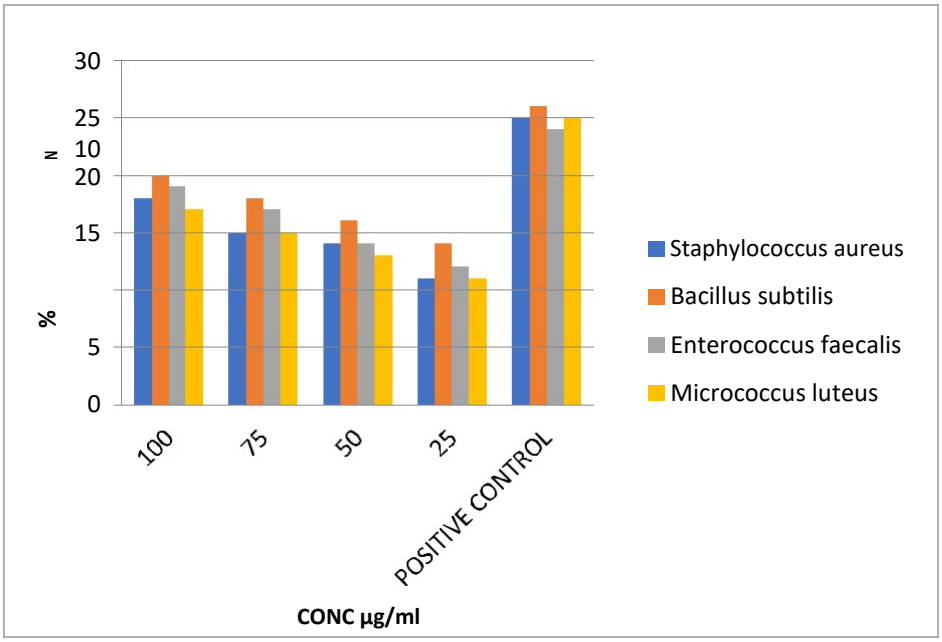
FUNGAL



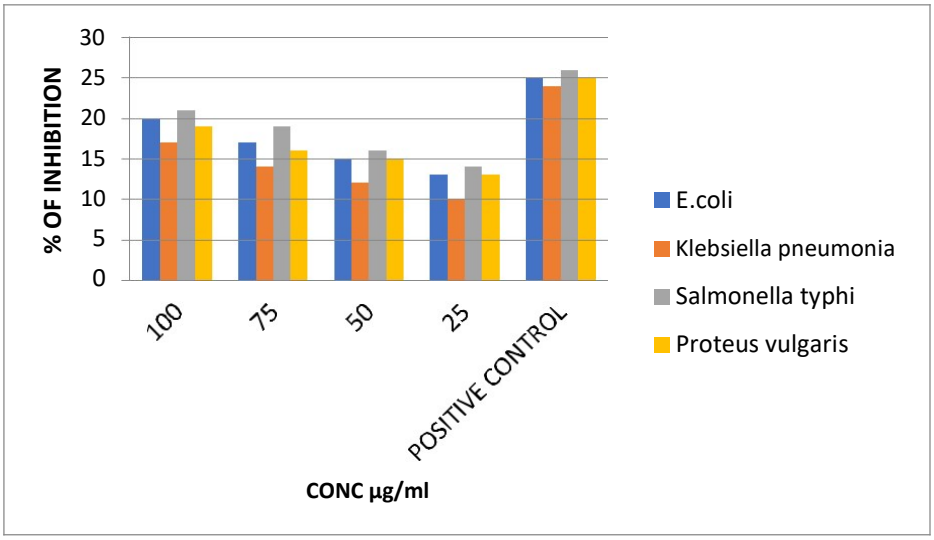
Candida albicans

TABLE 8: WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF SERICIN

S. NO	MICROORGANISMS	ZONE OF INHIBITION (MM)				
		100	**75**	**50**	**25**	**+ VE CONTROL**
GRAM POSITIVE BACTERIA						
1	*Staphylococcus aureus*	18	15	14	11	25
2	*Bacillus subtilis*	20	18	16	14	26
3	*Enterococcus faecalis*	19	17	14	12	24
4	*Micrococcus luteus*	17	15	13	11	25
GRAM NEGATIVE BACTERIA						
5	*E.coli*	20	17	15	13	25
6	*Klebsiella pneumonia*	17	14	12	10	24
7	*Salmonella typhi*	21	19	16	14	26
8	*Proteus vulgaris*	19	16	15	13	25
FUGAL						
10	*Candida albicans*	15	13	12	10	24



Wound healing and antimicrobial activity of sericin -gram positive bacteria



Wound healing and antimicrobial activity of sericin -gram negative bacteria

5.2 WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF BANANA SAP

GRAM POSITIVE BACTERIA

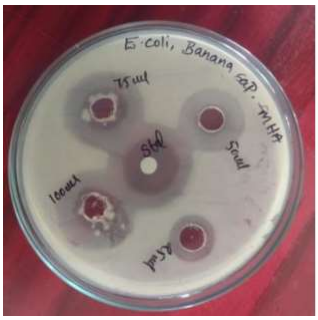
Staphylococcus aureus

Bacillus subtilis

Enterococcus faecalis

Micrococcus luteus

GRAM NEGATIVE BACTERIA



_E.coli

Klebsiella pneumonia

Salmonella typhi

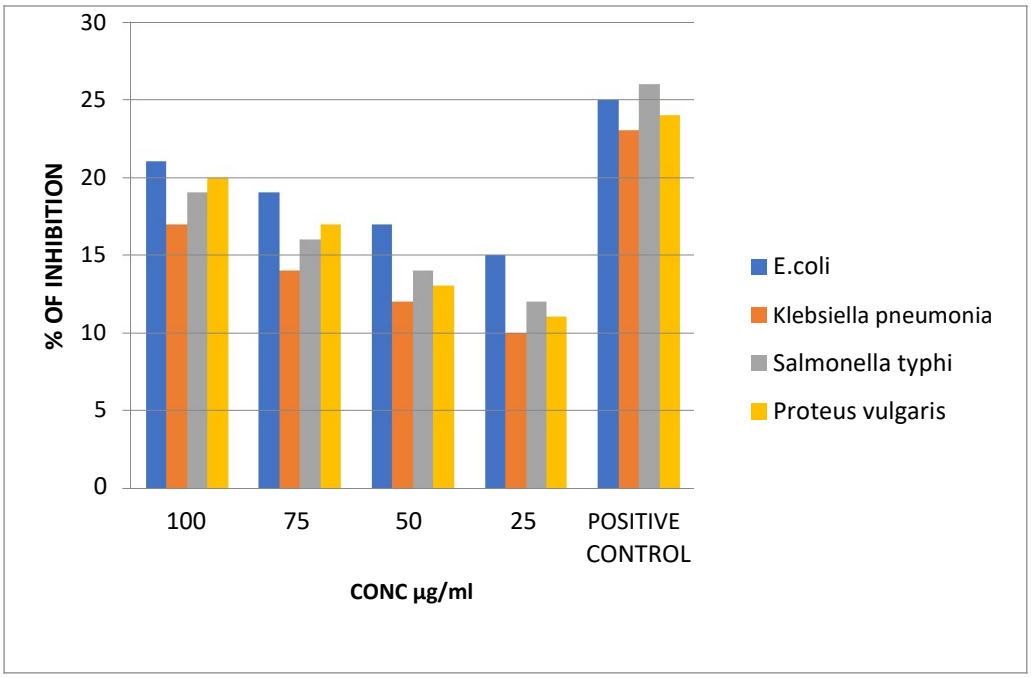
Proteus vulgaris

FUNGAL

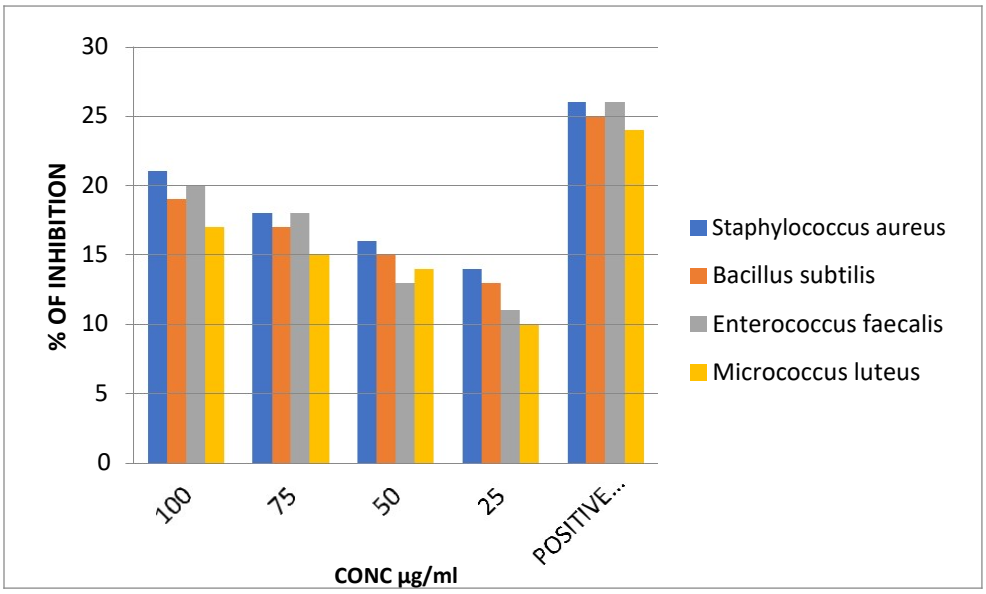
Candida albicans

5.3 WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF BANANA SAP

S. NO	MICROORGANISMS	ZONE OF INHIBITION (MM)				
		100	75	50	25	+ VE CONTROL
GRAM POSITIVE BACTERIA						
1	*Staphylococcus aureus*	21	18	16	14	26
2	*Bacillus subtilis*	19	17	15	13	25
3	*Enterococcus faecalis*	20	18	13	11	26
4	*Micrococcus luteus*	17	15	14	10	24
GRAM NEGATIVE BACTERIA						
5	*E.coli*	21	19	17	15	25
6	*Klebsiella pneumonia*	17	14	12	10	23
7	*Salmonella typhi*	19	16	14	12	26
8	*Proteus vulgaris*	20	17	13	11	24
FUGAL						
9	*Candida albicans*	15	12	11	09	24



Antimicrobial activity of banana sap-gram negative bacteria

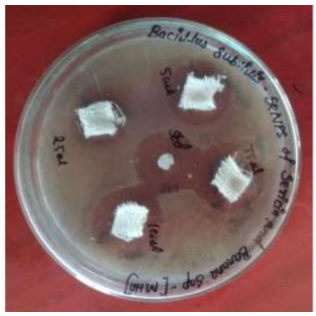


Antimicrobial activity of banana sap -gram positive bacteria

5.4 WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF SeNPS OF SERIN-BANANA SAP:

GARM POSITIVE BACTERIA

Staphylococcus aureus



Bacillus subtilis

Enterococcus faecalis

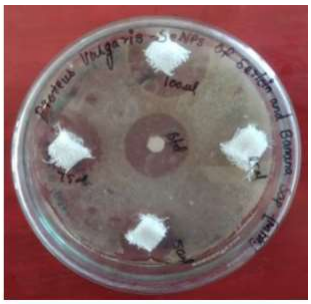
Micrococcus luteus

GRAM NEGATIVE BACTERIA

E.coli

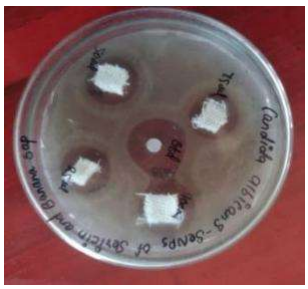
Klebsiella pneumonia

Salmonella typhi



Proteus vulgaris

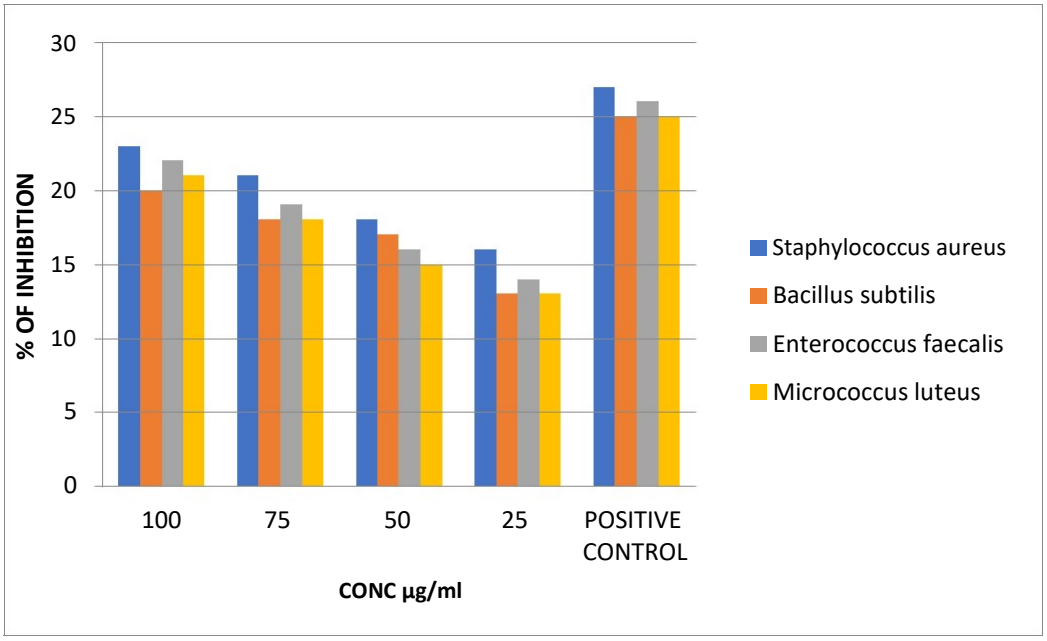
FUNGAL



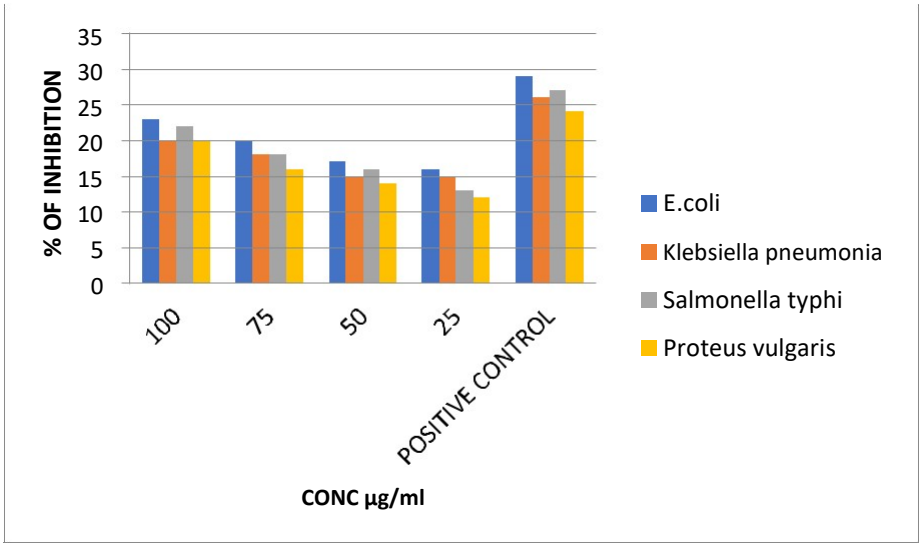
Candida albicans

5.5 WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF SeNPS OF SERIN-BANANA SAP:

S. NO	MICROORGANISMS	ZONE OF INHIBITION (MM)				
		100	75	50	25	+ VE CONTROL
GRAM POSITIVE BACTERIA						
1	*Staphylococcus aureus*	23	21	18	16	27
2	*Bacillus subtilis*	20	18	17	13	25
3	*Enterococcus faecalis*	22	19	16	14	26
4	*Micrococcus luteus*	21	18	15	13	25
GRAM NEGATIVE BACTERIA						
5	*E.coli*	23	20	17	16	29
6	*Klebsiella pneumonia*	20	18	15	15	26
7	*Salmonella typhi*	22	18	16	13	27
8	*Proteus vulgaris*	20	16	14	12	24
FUGAL						
9	*Candida albicans*	17	15	13	12	23



Antimicrobial activity of SeNPs of sericin-banana sap -gram positive bacteria



Antimicrobial activity of SeNPs of sericin banana sap -gram negative bacteri

FINAL PRODUCT

6. CONCULSION

SeNPs of sericin and banana sap is a remarkable output for developing wound dressing that could accelerate healing and could be peeled off without damaging the newly formed skin. In UV-IR study performed to identify the selenium nanoparticle and functional group. The result of the present phytochemical analysis study established the presence of biologically active phytochemicals in the banana sap. Among these solvent extraction, ethanol showed highest amount of phytochemical compounds prosent in it. These data also suggested the banana Sap Contains certain amount of components such as Alkaloids, flavonoids, carbohydrate, tannins and terpenoids. Finally anti-fungal, anti-bacterial, anti-oxidants and anti-inflammatory activity was evaluated.

7.REFERENCE:

[1] Kundu S. C., Dash B. C., Dash R., Kaplan D. L. Natural protective glue protein, sericin bioengineered by silkworms: potential for biomedical and biotechnological applications. Progress in Polymer Science. 2008;33(10):998–1012. doi:10.1016/j.progpolymsci.2008.08.002. [CrossRef] [Google Scholar]

[2] Mondal M., Trivedy K., Kumar S. N. The silk proteins, sericin and fibroin in silkworm, Bombyx mori Linn.,—a review. Caspian Journal of Environmental Sciences. 2007;5(2):63–76. [Google Scholar]

[3] Padol A. R., Jayakumar K., Mohan K., Manochaya S. Natural biomaterial silk and silk proteins: applications in tissue repair. International Journal of Materials and Biomaterials Applications. 2012;2(4):19–24. [Google Scholar]

[4] Joseph B., Raj S. J. Therapeutic applications and properties of silk proteins from Bombyx mori . Frontiers in Life Science. 2012;6(3-4):55–60. doi: 10.1080/21553769.2012.760491. [CrossRef] [Google Scholar]

[5] Gulrajani M. L. Sericin: a bio-molecule of value. Proceedings of the Souvenir 20th Congress of the International Sericulture Commission; 2005; Bangalore, India. pp. 21–29. [Google Scholar]

[6] Molnar J. Nutrition and chronic wounds. Adv wound care., 2014, Vol 3(11): 663-681.

[7] Prihatman K. Pisang :budidaya tanaman pertanian. Kantor Deputi Menegristek Bidang Pendayagunaan dan Pemasyarakatan Ilmu Pengetahuan dan Teknologi, Jakarta, 2000, hal. 1-13.

[8] DJ Deshphande. Handbook of medicinal herbs. Narayana Verlag GmBH, Kanden, Germany, 2006, p. 54-55.

[9] Coopoosamy RM. In-vitro studies on lectin derivatives of aloe excelsa (Berger).Journal of Medicinal Plants Research, 2010, Vol. 4(17): 1738-42.

[10] Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, and Goel RK. Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats.Indian Journal of Experimental Biology, 2009, Vol. 47: 32-40.

[11] Budi HS, Yuliastutik WS, Yuliati, Triranti A. Efek antiinflamasi getah pisang ambon (Musa Paradisiaca Var.Sapientum L.) pada Tikus Putih (Rattus Norvegicus) Galur Wistar.E-journal Departemen Biologi Oral Fakultas Kedokteran Gigi Universitas Airlangga, 2010,Vol 2(2): 1-8.

[12] Budi HS, Yuliastutik WS, Yuliati, Mulyaningtyas R. Efek analgesik getah pisang ambon (Musa Paradisiaca Var. Sapientum L.) pada Mencit (Mus Musculus). E-journal Departemen Biologi Oral Fakultas Kedokteran Gigi Universitas Airlangga, 2010,Vol 2(2): 9-19.

[13] Pelletier and Kenneth R. The best alternative medicine, part I:western herbal medicine.New York:Simon & Schuster, 2002, p 57-60.

[14] Priosoeryanto BP, Putriyanda N, Listyanti AR, Juniantito V, Wientarsih I, Prasetyo BF dan Tiuria R. The effect of ambon banana stem sap on the acceleration of wound healing process in mice (Mus musculus albinus).J. Agric and Rural Devp., 2007, p. 35-49.

[15] M. Singh, S. Singh, S. Prasad, 3(2008). I.S. Gambhir Nanotechnology in medicine and antibacterial effect of silver nanoparticles Dig. J. Nanomater. Bios, pp. 115-122.

[16] G.M. Whitesides 1(2005), Nanoscience, nanotechnology, and chemistry, Small, pp. 172-179.

[17] B. Pelaz, S. Jaber, D.J. De Aberasturi, V. Wulf, T. Aida, J.M. de la Fuente, N.A. Kotov, 6 (2012). The state of nanoparticle-based nanoscience and biotechnology: progress, promises, and challenges ACS Nano, pp. 8468-8483.

[18] C. Jianrong, M. Yuqing, H. Nongyue, W. Xiaohua, L. Sijiao (2004). Nanotechnology and biosensors Biotechnol Adv.22, pp. 505-518

[19] X. Zhang, Q. Guo, D. Cui 9(2009). Recent advances in nanotechnology applied to biosensors Sensors, pp. 1033-1053.

[20] B.D. Yao, Y.F. Chan, N. Wang, 81(2002). Formation of ZnO nanostructures by a simple way of thermal evaporation Appl. Phys. Lett, pp. 757-759.

[21] Y.Yin, R.M. Rioux, C.K. Erdonmez, S. Hughes, G.A. Somorjai, A.P. Alivisatos 304 (2004). Formation of hollow nanocrystals through the nanoscale Kirkendall effect Science, pp. 711-714.

[22] J.V. Barth, G. Costantini, K. Kern (2010). Engineering atomic & molecular nanostructures at surfaces Nanoscience & Technology: A Collection of Reviews from Nature Journals pp.67-75.

[23] J. Virkutyte, R.S. Varma 2(2011). Green synthesis of metal nanoparticles: biodegradable polymers and enzymes in stabilization and surface functionalization Chem. Sci., pp. 837-846

[24] M.N. Nadagouda, R.S. Varma, 10(2008). Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract Green Chem., pp. 859-862

[25] M. Darroudi, Z. Sabouri, R.K. Oskuee, A.K. Zak, H. Kargar, M.H.N.A. Hamid 40 (2014). Green chemistry approach for the synthesis of ZnO nanopowders and their cytotoxic effects Ceram. Int., pp. 4827-4831

[26] S. Iravani 13 (2011). Green synthesis of metal nanoparticles using plants Green Chem., pp. 2638-2650

[27] M. Behravan, A.H. Panahi, A. Naghizadeh, M. Ziaee, R. Mahdavi, A.Mirzapour Facile 124 (2019). Green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity Int. J. Biol. Macromol., pp. 148-154

[28]P. Rajiv, S. Rajeshwari, R. Venkatesh 112(2013). Bio-Fabrication of zinc oxide nanoparticles using leaf extract of Parthenium hysterophorus L. and its size-dependent antifungal activity against plant fungal pathogens Spectrochim. Acta Mol.Biomol.Spectrosc, pp. 384-387

[29] G.R. Navale, M. Thripuranthaka, D.J. Late, S.S. Shinde 3(2015). Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi JSM Nanotechnol Nanomed, p. 1033.