Biological Forum – An International Journal **15(5): 1103-1114(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Wound Healing Gauze Formulation through Synthesis of Selenium Nanoparticles from Sericin and *Wrightia tinctoria* – A Green Approach

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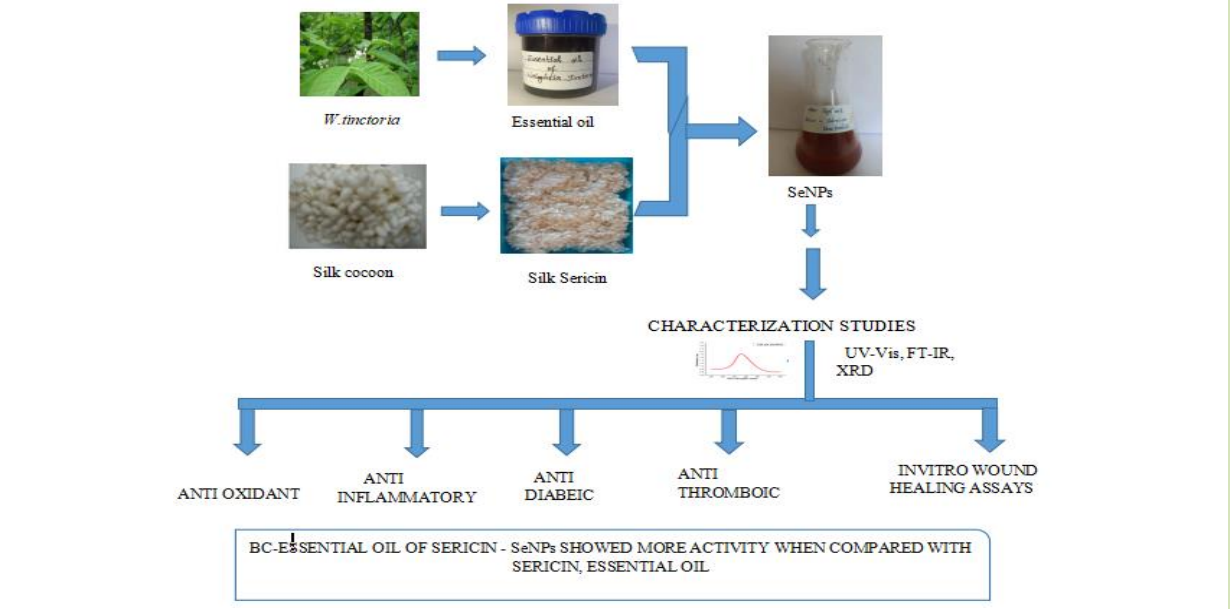
(Received: 08 March 2023; Revised: 17 April 2023; Accepted: 25 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Wound provides a environment for microbial growth which delays healing and cause more prominent acute inflammatory reaction that lead to further tissue damage and injury. An effort is put forth in the present study to develop a bio conjugated selenium nanoparticles (SeNPs) for wound healing which is delayed by infectious microbes. Sericin and essential oil obtained from waste cocoons and *W. tinctoria* bark was utilized for the synthesis of selenium nanoparticle. The structure of Sr-Wt-SeNPs was characterized by UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy and X-ray Diffraction, later investigated for their antioxidant, antidiabetic, antithrombotic, antimicrobial and anti-inflammatory efficacy. The spectral studies showed that the Sr-Wt-SeNPs were formed optimally exhibiting a maximum absorption peak at 266 nm. Sr-Wt-SeNPs showed potent IC_{50} of 34.54 µg/ml and 41.04 µg/ml against DPPH and reducing power assay. The *in vitro* α-amylase and α-glucosidase inhibitory activity of Sr-Wt-SeNPs was 71% & 66% respectively. The antithrombotic activity was also found to be 50.76%. Similarly, the Sr-Wt-SeNPs (100 µg/mL) showed strong antimicrobial property against the selected gram-positive, gram-negative and fungal pathogens. Anti-inflammatory activity with maximum inhibition value for albumin denaturation and membrane stabilization test (100 µg/ml) for Sr-Wt-SeNPs was potent when compared to individual sericin and essential oil. The present study is an attempt to explore the utility and value of Sericin, a waste material as green material and combination of it with essential oil from *W. tinctoria* bark to synthesize pharmacologically active SeNPs.



Graphical abstract.

Keywords: *Wrightia tinctoria*, Sericin, Essential oil, Sr-Wt-SeNPs, antioxidant, anti-diabetic, anti-thrombotic, anti-inflammatory activity.

INTRODUCTION

Wrightia tinctoria, a medium-sized evergreen tree that produces milky-white latex with exceptional therapeutic properties. Plant parts were used as a galactagogue, antipyretic, anti-dysenteric, anti-diarrheal, anti-hemorrhagic, and an antidote for snake poison. It is also used to treat skin conditions, wounds, and skin disorders (Srivastava, 2014; Latimer, 1994) and it also possess anti-microbial activity (Vedhanarayanan *et al.,* 2013). For psoriasis, diarrhea, dysentery, dandruff and the renewal of joint function, many poly-natural products including *W. tinctori*a are availableonthe market (Sundarrajan *et al.,* 2017). Although the phytochemicals such as Terpenoids, steroids, flavonoids, alkaloids, and other compounds from *W. tinctoria* (Selvakumar *et al.,* 2016) have been identified and described in their pure forms, most of them have not yet been tested for pharmacological activity, leaving a knowledge vacuum (Oviya *et al.,* 2015).

Nearly 30% of all cocoon output is made up of silk waste; it is regarded as a waste material and is disposed by the textile industry (Arango *et al.,* 2021). Sericulture therefore endangers the nearest water bodies where waste water is dumped (Siracusa, 2019). Silk fibroin and sericin are two different types of proteins used to make silk fiber. About 20–30% of the total weight of the cocoon is made up of sericin. It is distinguished by the high concentration of serine and18 amino acids, including essential amino acids (Kunz *et al.,* 2016). Nowadays, sericin has become a hot topic in the field of tissue engineering and regenerative medicine. It has been reported that the sericin have more biological applications such as antimicrobial, anticancer, wound healing activity, antihypertensive, neuro-protective, antitum or, anti-diabetic, anti-wrinkle, anti-ageing and antioxidant amongst various others (Suryawanshi *et al.,* 2020; Das *et al.,* 2021; Saha *et al.,* 2019). Green synthesis of nanoparticle using microorganism possesses enormous pharmacological properties, role in biomedical diagnostics, optical imaging, molecular sensing etc (Radhakanta Nag *et al.,* 2022). Selenium nanoparticles (SeNPs) biosynthesized from microorganisms (Tugarova and Kamnev 2017; Ullah *et al*., 2021)and from plant parts like flowers, leaves, peel, fruits and seed extracts have been studied extensively (Pandiyan *et al*., 2022; Visha *et al.,* 2015; Badhusha and Mohideen 2016). The chemical and physical processes need high temperatures, unsafe substances and an acidic pH, which are exceedingly toxic and harmful for biological purposes (Gunti et al., 2019). SeNPs possess antioxidant, antiviral, antitumor, antibacterial, antifungal properties, enzyme inhibitors, cytokine inducers, immunomodulator activities and decreases the risk of tuberculosis in HIV infected patients (Ferro *et al*., 2021). Still now, remarkable antimicrobial activities of SeNP have been evidenced against pathogenic microorganisms (Shakibaie *et al.,* 2015; Filipović *et al.,* 2021).

A living organism ability to replace damaged or broken tissue with freshly formed tissue is referred to as wound repair. Coagulation, inflammation, re-epithelialization, and tissue remodeling are independent but overlapping stages of wound healing. Reactive oxygen species are extremely unstable molecules, play a role in these processes and is now being studied in new ways (Fitzmaurice *et al.,* 2011). Inflammation increases the risk of various syndromes including diabetes (Furman *et al.,* 2019). The use of steroidal and non-steroidal anti-inflammatory medicines is the main stay of current therapy for inflammatory illnesses (Ahmad *et al.,* 2018). The existing drugs may cause serious acute renal failure, cardiovascular events, gastrointestinal ulcers, hypertension and heart failure (Vonkeman and van de Laar 2010; Ghe and Solcan 2021) Still there is a lack of anti-inflammatory drugs and vectors with nil side effects and this provokes the essential and urgent need for developing new molecules for the management of inflammatory related diseases. Medicinal plants bring the main remedy to treat various diseases including inflammation for a long time and these days many drugs have been developed from traditional medicine.

Currently available anti-diabetic drugs have adverse side effect hence effective treatment for DM is important for the medical community (Padhi *et al.,* 2020). Enormous reports show that alternatives traditional medicine is effective for the treatment of diabetes mellitus (DM) also have similar therapeutic efficacy to that of conventional therapeutic agents but without causing significant adverse effects (Salehi *et al*., 2019; Chaudhury *et al.,* 2017).

Over the past decade interdependence of the mechanisms of thrombosis and inflammation has been explored more (Stark and Massberg 2021). Plant materials have the potential to be novel antithrombotic agents which are free from side effects (Vilahur and Badimon 2013; Kim *et al.,* 2016; Fuentes *et al*., 2014). Natural phytochemicals such as flavonoids and polyphenols show effective inhibition on platelet aggregation (Bucki *et al.,* 2003). Therefore, development of antithrombotic agents from medicinal plants has attracted much interest.

Most of the world's population has relied on traditional medicine for thousands of years. The WHO establishes Global Centre for Traditional Medicine (GCTM) during 2022 (World Health Organization 2022). Hence, it is worthwhile to assess the potency of SeNPs synthesized from sericin & essential oil from *W. tinctoria* bark (Sr-Wt-SeNPs) for antioxidant, anti-diabetic, anti-thrombotic antimicrobial and anti-inflammatory activities.

MATERIALS AND METHODS

Plant Material. The plant *Wrightia tinctoria* was collected from Kolli Hills of Namakkal District in Tamil Nadu. The plant was authenticated and its authentication certificate number is No.GRD 21-22/381. After being air dried, the plant barks were homogenized into a fine powder and stored in an airtight bottle. The extracts were thereafter prepared, vacuum-dried and kept in a refrigerator.

Extraction of essential oil from the plant *W. tinctoria.* 150g of fresh *W. tinctoria* bark were cut into small pieces, blended into a paste and steam distilled for

3 hours. Anhydrous sodium sulphate was used to dry the distillate after it had been extracted with diethyl ether (3 × 100ml) and collected in amounts of around 2 liters. After evaporation, the dry ether extract produced 4.5% (0.15% of the sample's fresh weight) of pale-yellow oil which is further purified by steam distillation method (Joshi and Hiremath 2001).

Extraction, Isolation and Confirmation of Sericin from Silk Waste Cocoon. In a 2L Erlenmeyer flask, 15 g of Bombay mori cocoons were weighed, along with 1L of ultra-pure water, and the flask was autoclaved for 60 minutes at 120°C. Sericin was extracted after autoclaving, with a few minor adjustments, in accordance with the methods outlined (Rocha *et al*., 2017). Following the removal of the surplus cocoon mass, the resultant solution was vacuum-filtered through a 4g filter using a Millipore filtration system. Before lyophilization, the filtered solution was divided among multiple glass flasks and properly frozen at 86°C. The crude extract was properly ground after lyophilization. For further examination, the resulting powdered crude sericin extract is employed.

Synthesis of SeNPs from essential oil of *W. tinctoria* and Sericin extract (Sr-Wt-SeNPs).

A standard protocol was followed for the synthesis of SeNPs from essential oil and sericin (Xia, 2007).

Biophysical Characterization of Sr-Wt-SeNPs. The Sr-Wt-SeNPs are further characterized by UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). The formation of Sr-Wt-SeNPs was confirmed by measuring the absorption spectra in UV–Visible spectroscopy (SHIMADZU 1800). The reaction mixture was scanned at the speed of 300 nm min^{-1} in 200–600 nm range. The Sr-Wt-SeNPs is scanned at the transmission mode ranging from 500 to 4000 cm−1 in a solid phase, at a resolution of 1 cm−1 using FTIR spectrophotometer (SHIMADZU, IR PRESTIGE 21). XRD pattern provides the details of the structure and composition of Sr-Wt-SeNPs. The sample was analyzed by Shimadzu XRD 6000diffractometer operated at 40 kV voltage, 30 mA current in a scanning mode range of θ–2θbetween 10° and 80° with sampling pitch of 0.1000° equipped with a Cu Kα radiation. The sample was placed on copper grids coated carbon films, dried at room temperature and analyzed at 200 kV.

***In-vitro* antioxidant activity.** The in-vitro antioxidant activity of Sericin extract, essential oil from W. tinctoria and Sr-Wt-SeNPs were analyzed for its ability to scavenge radicals at different concentrations (20, 40, 60, 80, 100 µg/ml). The DPPH radical scavenging activity and Reducing Power assay was performed according to Jain and Agrawal (2008) ; Yen and Duh (1993) respectively.

Antidiabetic Activity

α-amylase inhibitory activity. α-Amylase (0.05g in 100 mL of ice-cold distilled water) was pre-mixed with the Sr-Wt-SeNPs at various concentrations (100-12.5 g/mL) and sonicated at room temperature for 30 minutes to prevent agglomeration. To begin the reaction, a 0.5 % solution of starch was added as a substrate in each tube. Incubated for 20minutes,2 ml of DNS reagent (1% 3,5-dinitrosaliycilic acid and 12% sodium potassium tartrate in 0.4 M NaOH) was added to stop the reaction. Following 15 minutes of heating at 100 °C forthe reaction mixture, the activity of the α-amylase was determined by measuring the absorbance at 540 nm (Bernfeld 1955) . As percentage controls, inhibition rates were computed using the procedure below.

$\%I_{\alpha-Amylase} = (Control–Test/Control) \times 100$

α-Glucosidase Inhibitory Activity. To prevent agglomeration, α-glucosidase (0.05 g of α-glucosidase in 100 ml of ice-cold distilled water) was pre-mixed with Sr-Wt-SeNPs at different concentrations (100-12.5g/ mL). p-nitrophenyl- α-D-glucopyranoside (pNPG) (3 mM) was added to the mixture as a substrate in phosphate buffer to start the reaction. After 30 minutes of incubation at 37°C, the reaction was stopped by adding sodium carbonate (2ml, 0.1M). By detecting the release of pNPG at 420nm, α-glucosidase activity was identified (Daou et al., 2022). The following formula was used to calculate inhibition rates as percentage controls: $\%I_{\alpha-Glucosidase} = (A_{Control} – A_{Test} / A_{Control}) \times 100$

In which $A_{Control}$ is the absorbance of control, A_{Test} is the absorbance of Sr-Wt-SeNPs.

***In-vitro* Antithrombotic Activity**

The Prasad *et al.* (2007) approach was followed with a few minor adjustments to test the *in vitro* clot lysis activity of the Sr-Wt-SeNPs. From healthy volunteers, 7 ml of blood from venous was collected and transferred to sterilized pre-weighed micro-centrifuge tubes (1 ml/tube). Incubated at 37°C for 45 minutes, the blood samples are observed for clot formation. Without disturbing the clot, serum was completely discarded and each tube having clot was again weighed to determine the weight of the clot. Each micro-centrifuge tube containing clot was appropriately labeled and 100 µl of Sr-Wt-SeNPs with various concentrations (2, 4, 6, 8 and 10 mg/ml respectively) was added to the tubes accordingly. As a positive control, 100 µl of streptokinase and 100 µl of sterilized distilled water as a negative non-thrombolytic control were maintained. Incubated the tubes again at 37°C for 90 minutes, the clot lysis was observed. Discarded the fluids formed after incubation, the tubes are again weighed to observe the clot disruption. The percentage of clot lysis was then calculated from the weight differences using the following equation.

% of clot lysis = (wt. of released clot /clot wt.) × 100%.

Antimicrobial activity of Sr-Wt-SeNPs

Test microorganisms: Bacterial pathogens such as Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Micrococcus luteu*s, Gram-negative *Escherichia coli*, *Salemonella Typhi*, *Proteus Vulgauis* and *Klebsiella pneumonia* and 2 fungal strains such as *Aspergillus Niger* and *Candida albicans* were tested to analyze the antimicrobial properties of Sr-Wt-SeNPs for wound healing. The bacterial and mycelial cultures were acquired from the Microbial Type Culture Collection (MTCC), Chandigarh, India's Institute of Microbial Technology. The cultures are maintained in log phase until they are subjected to screening process.

***In vitro* antibacterial activity.** The antibacterial activity was screened using the agar well diffusion method (Shriniwas and Subhash 2017). The bacterial strains were inoculated in nutrient media and incubated at 37°C for 2-4 hours. At the exponential growth phase, the organisms are cultured in Muller Hinton agar plates in which six wells of about 5 mm diameter were bored. Different concentrations of Sr-Wt-SeNPs (25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml), 10 µg/ml of dimethyl sulfoxide (DMSO) (negative control) and chloramphenicol (positive control) were added to the wells.

***In vitro* antifungal activity.** The fungal strains were inoculated in Sabouraud Dextrose Agar in which the antifungal activity of Sr-Wt-SeNPs at different concentrations (25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml) are evaluated with fluconazole (10 µg/ml) as a positive control. The plates were incubated for 24 hours at 37 °C to observe the clear zones of inhibition (ZOI). The diameter of clear zones was measured and recorded that determines the antimicrobial property of NPs.

Anti-inflammatory activity

Inhibition of Albumin denaturation. Minor adjustments to the (Mizushima and Kobayashi 1968; Sakat *et al.*, 2010) methods were made to determine the protein denaturation. Sericin extract, *W. tinctoria* essential oil and Sr-Wt-SeNPs at different concentrations (20, 40, 60, 80, 100 µg/ml) are taken separately along with 1% aqueous solution of bovine albumin fraction. The samples are incubated for 20 minutes at 37°C, heated to 57°C for 20 minutes followed by cooling. The turbidity of the sample extracts is determined spectrometrically at 660 nm and Percentage inhibition of protein denaturation was calculated using the subsequent formula.

Percentage inhibition = ((A_C of control–A_C of test sample) × 100/A_C)

Where, A_C and A_S are the absorbance of the control and sample (at 600 nm) respectively.

Membrane Stabilization Test. Red blood cells (RBCs) suspension is made using the techniques described in (Sakat *et al.*, 2010; Sadique *et al*., 1989). 10 ml of fresh human blood samples was collected in centrifuge tubes containing heparin and centrifuged at 3000 rpm for 10 minutes and washed 3× with an equal volume of normal saline solution. The volume of the blood was measured and reconstituted as a 10% *v*/v suspension with normal saline. 1 ml of 10% red blood cell suspension was mixed with the 2 mlof Sericin extract, *W. tinctoria* essential oil and Sr-Wt-SeNPs at different concentrations (20-100 µg/ml) separately. For the control, saline was added instead of test samples. Aspirin was used as a standard drug (positive control). The samples were incubated at 56°C for 30 min, centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatant measured at 560 nm. The experiment was performed in triplicate. Percent membrane stabilization activity and percentage of protection was calculated using the following formula:

Percent of protection = 100 − A_S/A_C × 100

where A_C and A_S are the absorbance (at 560 nm) of the control and sample, respectively.

RESULTS

Extraction of essential oil and Sericin. Sericin and the essential oil of *W. tinctoria* were used to synthesis SeNP (Sr-Wt-SeNPs) and their *in vitro* antioxidant activity, anti-inflammatory effects, anti-diabetic activity,anti-thrombotic activity and wound healing capability have all beenstudied. From 150 g of *W. tinctoria* bark 3.5% of essential oil were extracted. 15 g of cocoons yielded approximately 4 g of sericin, which was confirmed by Biuret test.

Spectral Studies

UV-VIS Spectroscopic Analysis. The bioactive Sr-Wt-SeNPs were further characterized for their size, shape, and functional groups associated with them. The formation of Sr-Wt-SeNPs was initially detected by a change in color from pale yellow to a dark brick orange, caused by thereduction of sodium selenite to elemental selenium by the reducing agents present in the essential oil of *W. tinctoria* and sericin. A 2 ml of Sr-Wt-SeNPs suspension was subjected to UV–Vis absorbance spectra in the range of 200–400 nm. The maximum absorption peak is observed at 266nm (Fig. 1), indicating the synthesis of SeNPs.

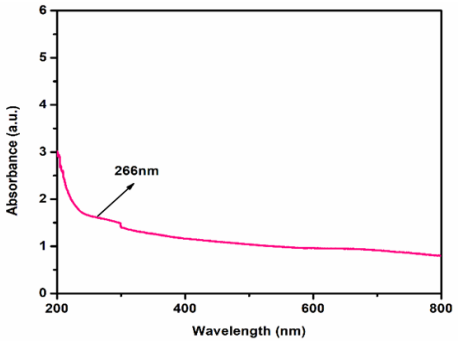


Fig. 1. UV-*Visible Absorption Spectrum* of Sr-Wt-SeNPs.

FTIR Spectroscopy for Sr-Wt-SeNPs. The FTIR spectrum of Sr-Wt-SeNPs revealed a major peak at 1633.83 cm^{-1}, which corresponded to the –NH bending (Fig. 2), indicating the presence of amide groups.

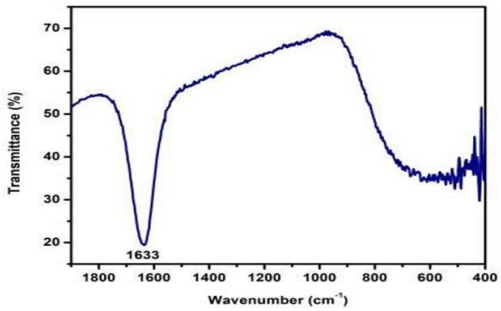


Fig. 2. FT-IR spectrum of Sr-Wt-SeNPs.

X-ray diffraction pattern for Sr-Wt-SeNPs. X-Ray Diffraction analysis was carried out to characterize the structures of Sr-Wt-SeNPs. Fig. 3 evidenced broad peak at 23.6°, 29.8°, 43.7°, 45.3°, and 51.6° corresponding to the (100), (101), (110), (102), and (111) hkl plane, confirming the formation of a very tiny Sr-Wt-SeNPs (Krysmann *et al.*, 2012). The diffraction peaks obtained also coincides with JCPDS file no. 06-0362.

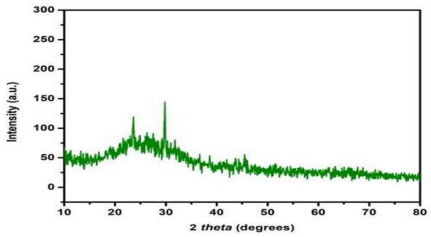


Fig. 3. X-rayDiffraction pattern of Sr-Wt-SeNPs**.**

***In-vitro* Antioxidant activity**

DPPH radical Scavenging Activity. The activity of DPPH radical scavenging of Sericin, essential oil and Sr-Wt-SeNPs are analyzed and compared with reference ascorbic acid as presented in Table 1, Fig. 4 at different concentration ranges (20, 40, 60, 80 and 100 µg/ml). At 100 g/ml, sericin, essential oil, and Sr-Wt-SeNPs each have substantial percentages of suppression of DPPH radicals (80.10%, 73.26%, and 94.1%, respectively). While ascorbic acid at 100 g/ml was found to have a 97.02% inhibitory rate. The IC_{50} values for Sericin, essential oil and Sr-Wt-SeNPs and ascorbic acid were 28.66 µg/ml, 58.05 µg/ml, 34.54 µg/ml and 7.34 µg/ml. The higher inhibition activity was recorded in Sr-Wt-SeNPs in dose dependent manner.

Table 1: DPPH free radical scavenging analysis of sericin, essential oil from *W. tinctoria* and Sr-Wt-SeNPs.

Sr. No.	Concentration (µg/ml)	% of Inhibition			
		Sericin	Essentialoil	Sr-Wt-SeNPs	Ascorbic Acid
1.	100	80.10	73.26	94.10	97.02
2.	80	75.50	62.31	85.11	89.17
3.	60	67.00	52.08	69.66	78.54
4.	40	54.49	42.15	57.22	68.09
5.	20	44.77	26.05	48.54	55.17
6.	IC_{50}Value	28.66	58.05	34.54	7.34

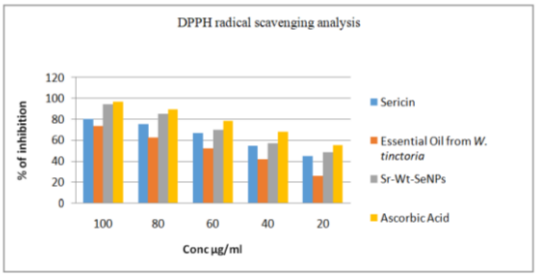


Fig. 4. Graphical representation of DPPH free radical scavenging analysis of sericin, essential oil from *W.tinctoria* and Sr-Wt-SeNPs.

Reducing Power Assay. The % of Inhibition that shows the reducing capacity of sericin, essential oil from *W. tinctoria* and Sr-Wt-SeNPs compared with the reference ascorbic acid are tabulated in Table 2, Fig 5. The reducing power of Sericin, Essential oil from *W. tinctora*, and SeNPs of Sr-Wt-SeNPs increased with dosage, similar to the antioxidant activity. The outcome reveals that all the samples consist of hydrophilic poly phenolic compounds that caused the greater reducing power. Comparatively, Sr-Wt-SeNPs exhibited higher reducing power activity.

Table 2: Reducing Potential of sericin, essential oil from *W. tinctoria* and Sr-Wt-SeNPs.

Sr. No.	Concentration (µg/ml)	% of Inhibition			
		Sericin	Essentialoil	Sr-Wt-SeNPs	Ascorbic Acid
1.	100	1.243	1.194	1.680	1.753
2.	80	1.089	0.936	1.477	1.507
3.	60	0.943	0.811	1.134	1.324
4.	40	0.807	0.750	0.963	1.008
5.	20	0.656	0.398	0.848	0.937
6.	IC_{50}Value	62.35	53.5	41.04	40.8

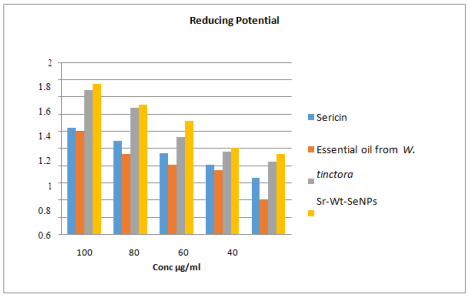


Fig. 5. Graphical representation of Reducing Potential of Sericin, Essential oil from *W. tinctoria* and Sr-Wt-SeNPs.

Antidiabetic activity. The *in vitro* α-Amylase inhibitory activity of Sr-Wt-SeNPs was illustrated in Table 3, Fig. 6. It was found that, there is increase in percentage inhibitory activitywith the increase in dosage against α- Amylase. As a standard drug, Acarabose was used with similar dosage to compare inhibitory capacity of the Sr-Wt-SeNPs. The analysis showed that, the % inhibitory activity of Sr-Wt-SeNPs had a minimum of 16.47±2.8 (at 12.5µg/ml) to a maximum of 71.07±1.0 (at 100 µg/ml) where as the standard drug Acarabose showed % inhibitory activity ranges from 27.51±3.0 (at 12.5 µg/ml) to a maximum of 79.05±1.2 (at 100µg/ml). The IC_{50} value of Sr-Wt-

SeNPs was 65.4 µg/ml and the standard drug Acarabose was found to be 42.5 µg/ml.

Table 3: α-amylase inhibitory activity of Sr-Wt-SeNPs.

Sr. No.	Test Conc (µg/ml)	% inhibition	
		Sr-Wt-SeNPs	Acarobose
1.	100	71.07±1.0	79.05±1.2
2.	75	56.23±1.6	62.38±2.4
3.	50	37.10±2.0	44.26±2.6
4.	25	20.30±1.5	31.94±1.5
5.	12.5	16.47±2.8	27.51±3.0
6.	IC_{50}	65.4	42.5

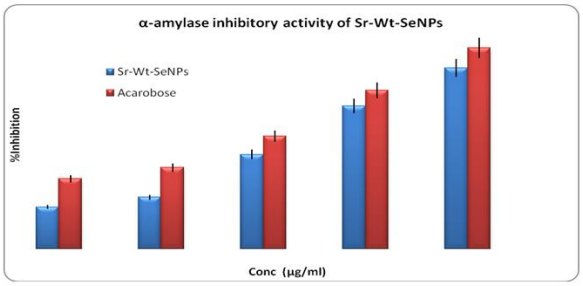


Fig. 6. Graphical representation of α-amylase inhibitory activity of Sr-Wt-SeNPs.

The inhibition rate of α-glucosidase was directly proportional to the concentration of the Sr-Wt-SeNPs. The maximum inhibition rate was 66.12±2.4 % at 100 µg/ml. There was a dose dependent increase in % inhibition with increase in concentration of Sr-Wt-SeNPs (Table 4 & Fig 7). The IC_{50} value was also comparable to the standard Acarobose.

Table 4: α-Glucosidase inhibitory activity of Sr-Wt-SeNPs.

Sr. No.	Test Conc (µg/ml)	% inhibition	
		Sr-Wt-SeNPs	Acarobose
1.	100	66.12±2.4	83.30±1.0
2.	75	53.19±2.5	71.26±3.4
3.	50	48.15±3.00	59.44±1.3
4.	25	35.06±2.2	42.60±2.4
5.	12.5	23.00±1.5	34.00±1.6
6.	IC_{50}	52	72.5

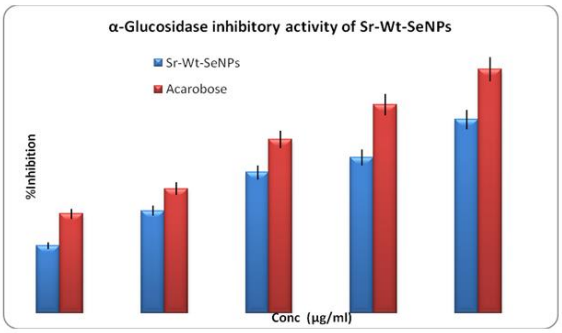


Fig 7. Graphical representation of α-glucosidase inhibitory activity of Sr-Wt-SeNPs

***In Vitro* Antithrombotic Activity.** The treatment and prevention of cardiovascular diseases requires primarily the inhibition of platelet aggregation (Antiplatelet Trialist Collaboration, 1994). The ability of Sr-Wt-SeNPs to lyse the blood clot is recorded as 50.76% (Table 5 & Fig. 8) and values are compared with standard streptokinase (67.19%) the negative control (4.76% lysis) and the positive control (67.19%).

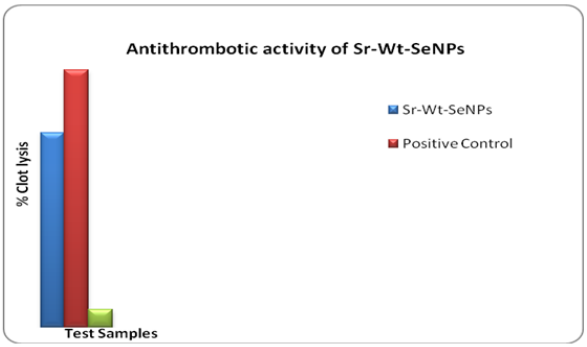


Fig. 8. Graphical Representation of Antithrombotic Activity of Sr-Wt-SeNPs.

Table 5: Antithrombotic activity of Sr-Wt-SeNPs.

Sr. No.	Weight of empty tube A (g)	Weight of tube with clot B (g)	Weight of clot C (B-A) (g)	Weight of tube with clot after lysis D (g)	Weight of lysis E (B-D) (g)	% of clot lysis (E/C)	Average % of clot lysis
1.	0.968	1.523	0.555	1.239	0.284	51.17	50.76%
2.	0.967	1.490	0.525	1.210	0.280	53.53	
3.	0.968	1.455	0.487	1.225	0.230	47.22	
4.	0.986	1.519	0.533	1.248	0.271	50.84	
5.	0.993	1.545	0.552	1.263	0.283	51.08	

Antimicrobial Activity of Sr-Wt-SeNPs. Sr-Wt-SeNPs showed increase in antimicrobial activity against the selected pathogens dose dependently. *S. aureus* formed a 23mm ZoI whilst *E. faecalis* shown a high level of inhibition of 24mm at 100 g/ml among the Gram-positive bacteria. While in Gram-negative bacteria; *P. vulgaricus* had a highest inhibition zone of 26mm, followed by *S. typhi* (24mm). Among the fungal pathogens, *C. albicans* showed highest inhibition zone of 22mm compared to *A. niger* (19mm). Overall, the Sr-Wt-SeNPs had potent activity against Gram-negative bacteria than Gram-positive and Fungal strains.

Table 6: Antimicrobial activity of Sr-Wt-SeNPs.

Sr. No	Microorganisms	Zone of inhibition(mm)				
		100	75	50	25	+ ve control
Gram-PositiveBacteria						
1.	*S. aureus*	23	21	20	18	27
2.	*B.subtilis*	22	20	18	16	25
3.	*E. faecalis*	24	21	19	17	25
4.	*M. leuteus*	22	21	19	16	27
Gram-NegativeBacteria						
5.	*E.coli*	23	22	21	20	28
6.	*S.tyohi*	24	21	18	15	26
7.	*P. vulgauis*	26	23	21	18	26
8.	*K.pneumonia*	23	21	19	18	27
Fungal						
9.	*A. niger*	19	17	15	13	24
10.	*C.albicans*	22	19	17	14	23

Anti-inflammatory properties

Inhibition of albumin denaturation. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of different solvent extracts of plants are studied for protein denaturation. It was effective in inhibiting heat induced albumin denaturation (Table 7 & Fig. 9). Maximum inhibition 74%, 71% and 82% was observed from sericin, Essential oil and Sr-Wt-SeNPs. Aspirin, a standard anti inflammation drug showed the maximum inhibition 87% at the concentration of100µg/ml.

Membrane Stabilization Analysis. Test samples (20-100µg/ml) inhibited the heat induced hemolysis of RBCs to varying degree as shown in Table 8 & Fig. 10. It showed the maximum nhibition of 77%, 66% and 82% at 100µg/ml. Aspirin, standard drug showed the maximum inhibition, 89% at100µg/ml.

Table 7: Anti-inflammatory activity (Albumin denaturation).

Sr. No.	Concentration (µg/ml)	% of Inhibition			
		Sericin	Essential oil	Sr-Wt-SeNPs	Aspirin
1.	100	74.39	71.60	85.92	87.43
2.	80	66.40	59.73	75.81	75.56
3.	60	57.24	43.11	61.45	67.03
4.	40	44.62	30.14	43.09	52.36
5.	20	31.25	18.21	37.76	52.36
6.	IC_{50}	51.18	68.06	43.27	31.21

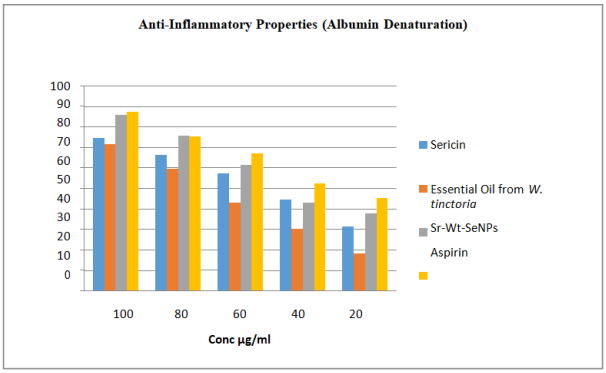


Fig. 9. Graphical representation of anti-inflammatory activity (albumin denaturation).

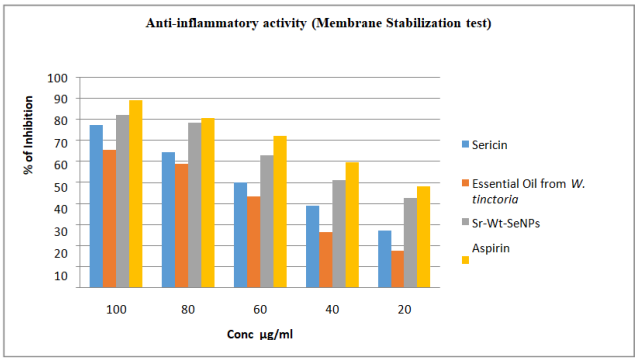


Fig. 10. Graphical representation of anti-inflammatory activity (membrane stabilization).

Table 8: Anti-inflammatory activity(Membrane Stabilization test).

Sr. No.	Concentration (µg/ml)	% of Inhibition			
		Sericin	Essential oil	Sr-Wt-SeNPs	Aspirin
1.	100	77.42	65.57	82.05	89.16
2.	80	64.36	58.91	78.44	80.68
3.	60	50.11	43.24	62.82	72.33
4.	40	39.00	26.31	51.08	59.49
5.	20	27.13	17.40	42.55	48.10
6.	IC_{50}	57.51	72.04	34.87	21.41

DISCUSSION

Essential oils extracted from various medicinal plants reported to possess various pharmacological properties such as antibacterial activity, antioxidant activity, chemo preventive, anti-inflammatory activity, cytotoxic activity, allelopathic activity and repellent and insecticidal activity (Dhifi *et al*., 2016) as well as used in folkloric medical practices. Sericin, a protein based nanoparticle have prospective use in therapeutic applications. Essential oil loaded Nanodelivery systems and sericin nanoparticles possess antimicrobial activity and various biopharmaceutical applications (Dupuis *et al.,* 2022; Sana *et al.,* 2021; Das *et al.,* 2021).

In our study, Sr-Wt-SeNPs showed the colour change of the reaction mixture to a dark brick orange which may be due to the reduction reaction that may lead to the excitation of surface plasmon resonance property. By UV-VIS Spectroscopic analysis, the absorption peaks for Nanohybrid and many green synthesized SeNPs were reported (Vahdati and Tohidi 2020; Rajeshkumar *et al.,* 2018) that was very similar with the result obtained in our study. The FTIR spectrum of Sr-Wt-SeNPs revealed the presence of the functional groups from the essential oil of *W. tinctoria* and Sericin that render the SeNPs' stability and also serve as reducing agents in the conversion of sodium selenite to elemental selenium (Coates 2000). Similar interactions of selenium with sericin were observed (Dutta *et al*., 2020). The bioactive compounds from the essential oil also constitutes for the functionalization of SeNPs. X-Ray diffraction analysis showed diffraction peaks evidently representing the crystalline nature of Sr-Wt-SeNPs which is in agreement with the works of Alagesan and Venugopal (2019), who showed that the SeNPs obtained from *W. somnifera* leaf aqueous extract were crystalline in nature.

Several researchers proved that natural sources like plants and seaweeds have strong antioxidant activity (Arive *et al.,* 2017; Sarvjeet Singh *et al.,* 2019). The antioxidant activity of sericin was in concurrence with earlier reports (Pachiappan *et al.,* 2020; Takechi *et al.,* 2014). The DPPH radical scavenging activity of *W. tinctoria* leaf oil was tested and reported by Jesy and Jose (2017) ; Jesy and Jose (2017) who showed that the compounds in oil is responsible for the scavenging activity. The ethanolic extract of *W. tinctoria* bark was also reported Khyade and Vaikos (2014). But the current analysis is the first report on the antioxidant properties of *W. tinctoria* bark oil and Sr-Wt-SeNPs that showed a remarkable quenching property of DPPH radical. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the determination of reducing power activity, the Fe^{3+} to Fe^{2+} transformation was investigated in the presence of the samples chosen for the study. Antioxidants' ability to inhibit chain initiation, attach to transition metal ion catalysts, degrade peroxides, stop further hydrogen absorption, have reductive capacity, and scavenge free radicals are only a few of the mechanisms associated with their activity (Yildirim, 2000). Free radicals' production in body induces many acute and chronic disease complications such as cancer, diabetes, matrix deterioration, cell-cell interaction, etc. Radical scavengers may shield cell tissues from these free radicals and protects from the oxidative stress that involves preventive and repair mechanisms, physical and antioxidant defences. Based on many reported literatures, the plant *W. tinctoria* was more effective at scavenging DPPH and other free radicals than common cruciferous vegetables (Borowski and Siegbahn 2006). Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these DPPH radical quenching assay and reducing power assays are commonly used (Djeridane *et al.,* 2006). In both the assays Sr-Wt-SeNPs synthesized from the sericin and *W. tinctoria* essential oil combination showed effective antioxidant properties when compared with standard ascorbic acid, as well as the other samples. The functional groups of bioreductant molecules capping the surface of SeNPs may implicate the free radical scavenging abilities of these NPs (Kokila *et al.,* 2016). The presence of compounds exerts their action by breaking the free radical chain through donating a hydrogen atom (Rice-Evans *et al.,* 1997).

Diabetes mellitus is a metabolic disease caused by the body's incapacity to produce insulin or by the ineffective use of the insulin produced. Green synthesized NPs are well known to keep the structural integrity of insulin and have an active role in the secretion of insulin from pancreatic cells. In addition to oral agents and insulin therapy, phytonanotherapy is an alternative that offers a wide range of natural resources in nano form with hypoglycemic effects. The α-amylase inhibitors either prevent or slows the absorption of starch into the body by blocking the hydrolysis of 1,4-glycosidic linkages of polysaccharides into monosaccharides. The Sr-Wt-SeNPs had showed significant α amylase inhibitory activity which is most likely to be due to polar compounds and is worth

investigating further and isolating pure active compounds (Wickramaratne *et al.,* 2016). In 2015, Nazarizadeh and Asri-Rezaie (Nazarizadeh and Asri-Rezaie 2016) carried out a study to compare the antidiabetic activity and oxidative stress of AgNPs and sericin in diabetic rats. They found that AgNPs with small dimensionsat higher doses (3 and 10 mg/kg) had a much greater antidiabetic effect compared to ZnSO4 (30 mg/kg). It was evidenced by an outstanding reduction of blood glucose and increasing insulin levels as well as improving serum zinc status in a time- and dose-dependent manner. Raj *et al*. (2019) investigated the hypoglycemic and hypolipidemic activity of petroleum ether extract of *W. tinctoria* in alloxan induced diabetes in albino Wistar rats. In addition, our results showed that the Sr-Wt-SeNPs had potent antidiabetic effects that can be developed as a novel drug candidate.

Antithrombotic drugs are generally used to treat an acute thrombus conditions. These drugs include thrombolytic, anticoagulant, and antiplatelet aggregation medications that target various stages of thrombus formation. Thrombin converts fibrinogen to fibrin during the formation of thrombus, causing the formation of insoluble fibrin clots. Studies shown that the biosynthesized metallic NPs are selectively active against the clots and have more anticoagulant properties (Shanthi *et al.,* 2021; Lateef *et al*., 2018; Ajarem *et al.,* 2021) by inhibiting the conversion of prothrombin to thrombin, a critical step in the formation of insoluble fibrin strands and the catalysis of other clotting factors (Jin and Gopinath 2016). From the result obtained, it was evidenced that Sr-Wt-SeNPs had potent antithrombotic activity as the positive control, which may be due to the sericin and essential oil content that caps the nanoparticles and its nano size that increase the bioavailability of the drug molecules.

The scientific community is directed to hunt for antibiotic alternatives by literature reporting the rising appearance of drug resistant bacteria in the clinical setting to reduce the danger from bacterial infections. The complex blend of volatile compounds found in Dodson rigorous essential oils may have bactericidal properties when used against different human pathogenic microorganisms. Many reported (Pungle *et al.,* 2022; Ferro *et al.,* 2021; Dhifi *et al.,* 2016; Dupuis *et al.,* 2022) on the efficacy of essential oils against human pathogenic microorganisms. The hydrophobicity of essential oils, which causes an increase in cell permeability and consequent leakage of cell components, is linked to their capacity to kill bacteria. Reactive oxygen species (ROS) are also produced as a result of SeNPs' capacity to degrade cell membranes and their permeability, which increases their effectiveness against drug-resistant bacterial strains (Abinaya *et al.,* 2018). They asserted that SeNPs' effects on Gram-positive bacterial strains were stronger than those on Gram-negative ones. From our studies, it was also found that Gram Positive *E. faecalis* and Gram-positive *M. luteus* had more powerful bactericidal effect than Gram Negative *K. pneumoniae* and *E. coli*.

According to Rajalakshmi *et al.* (2012) the HRBC method was used to test the ethyl alcohol and aqueous extract of *W. tinctoria* for anti-inflammatory efficacy. Anti-inflammatory activity was measured by the prevention of hypotonicity-induced HRBC membrane lysis, and the extracts exhibit biphasic effects. Their effects are contrasted with those of the common medication diclofenac sodium. Stabilization of the RBCs membrane was studied to further establish the mechanism of anti- inflammatory action of sericin, Essential oil and Sr-Wt-SeNPs. The test samples were effective in inhibiting the heat induced hemolysis at different concentrations. They provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This sample may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage (Korkmaz *et al*., 2010). The study was in consistence with the reports of Uddin et *al*. (2016) ; Uddin *et al.* (2021). The satisfactory amount of flavonoid content in *W. tincoria* bark is responsible for a significant and positive effect with membrane stabilization activities. The extracts exhibited membrane stabilization effects by inhibiting hypotonicity induced lyses of erythrocyte membrane (Rajalakshmi and Harindran 2012). According to Soares *et al.* (2019) the hydro-alcoholic extract from *W. tinctoria* bark has the ability to stop protein denaturation. Secondary metabolites like flavonoids could be the cause of the extract's anti-inflammatory effects. The putative anti-inflammatory properties of flavonoids are particularly remarkable. Thus Sr-Wt-SeNPs exhibited potent anti-inflammatory properties.

CONCLUSIONS

Silk protein sericin is an emerging substance with vast pharmacological properties. In the present study, the Sr-Wr- SeNPs were synthesized by using sericin and essential oil from *W. tinctoria* and was characterized The synthesized Sr-Wr- SeNPs showed potent antioxidant, antimicrobial activity, anti-inflammatory, antidiabetic and antithrombotic activities when compared to sericin & essential oil the Sr-Wr- SeNPs. Further work is needed to develop wound healing gauze –robustness and efficiency to make it suitable for commercial production.

FUTURE SCOPE

The formation of smaller sized selenium nannoparitcle was confirmed by UV-Vis spectroscopy, by FT-IR the role of characteristic functional groups was analyzed and XRD confirmed the crystalline selenium nanoparticles. This biogenic synthesis of highly stable selenium nanaoparticles is a simple, low-cost and ecofriendly method. Waste to wealth concept efficiently implemented with environmental consciousness in reducing the waste sericin.

Acknowledgement. The authors are grateful to Tamil Nadu State Council for Science and Technology, Department of Science and Technology, Government of India, New Delhi for their financial support under students' project scheme (Project code: MS-413). Authors also thank the VMKVEC, Salem and GRD Bioresearch Clinical Centre, Nammakal for providing the research facilities.
Conflict of Interest. None.

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How to cite this article: M. Sridevi, R. Siva Renjith, A. Surya and C. Nirmala (2023). Wound Healing Gauze Formulation through Synthesis of Selenium Nanoparticles from Sericin and *Wrightia tinctoria* – A Green Approach. *Biological Forum – An International Journal, 15*(5): 1103-1114.