

**Original Research Article**  
**Augmented hybrid sunscreen for Albinistic skin types from *Terminalia sericea* biosynthesised Silver doped ZnO NPs, efficacy and safety investigation**

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**Abstract**

Albinism is a congenital hypopigmentation disorder characterized by errors in melanisation leading to the partial or complete absence of melanin, the skin's primary defense against actinic damage. Actinic damage in albinism summarizes all solar induced consequences from freckles to fatal skin cancers. Of the 16 sunscreens approved by regulators, 14 chemical sunscreens were stripped of GRASE (Generally Recognizable as Safe and Effective) status in 2020 by the FDA through Over-the-Counter Monograph M020, the remaining 2 physical sunscreens (ZnO and TiO<sub>2</sub>) are unaesthetic, leaving unpleasant persistent white casts. The hunt for new effective and safe sunscreen agents is on and photoprotective polyphenolics from tropical plants including *Terminalia sericea*, maybe the solution. After plant phyto screening for secondary metabolites, our objectives were to develop and evaluate the effectivity and safety of a pioneering albinistic skin biased sunscreen-based on *T Sericea* mediated, biosynthesised Ag doped ZnONPs, so as to leverage the novel sun protection efficacy of aesthetic ZnONPs with the antibacterial effects of AgNPs as well as the photoprotective effects from *T sericea* polyphenolics. Our observations from the study confirm that *T sericea* possesses abundant polyphenolic compounds capable of mediating bio reducing and capping of metallic nanoparticles. UV-Vis identified the nanostructures as Ag-ZnONPs, TEM confirmed their cubic and oblong agglomerated morphology. DLS estimated the nanostructures to be between 30 and 40nm. The resultant sunscreen had a high SPF of 30 and the safety tests using OECD guideline 406 determined the nano alloys to be negligible skin sensitizers. It was concluded that the biosynthesis of multifunctional Silver doped ZnONPs mediated by *T sericea*, was feasible and that the resultant safe multifunctional emulsion was efficacious in retarding biomarkers of actinic damage and is a potential pioneering albinistic skin type actinic damage retarding sunscreen.

**Key words:** *Albinism, Polyphenols, Photoprotection, sunscreen, terminalia sericea*

## 1 Introduction

### 1.1 Albinism

Albinism is a congenital pigmentation disorder that affects all known vertebrates. The term refers to the various resultant phenotypes from failures in differentiation or consequent migration of melanocytes to the skin, hair or eyes of afflicted individuals. The anomaly exhibits a wide spectrum of forms from partial to complete absence of melanin<sup>1</sup>. Organisms with complete absence are known as 'albino' and those with a partial absence are known as 'albinoid'. Hypo-pigmentation errors manifest in the hair, skin and eyes are medically referred to as oculocutaneous albinism (OCA). While those errors primarily localized to the eyes are described as ocular albinism (OA)<sup>2</sup>. Due to the absence of natural skin protectant mechanisms from sun damage, people living with albinism are prone to all forms of detrimental solar attacks causing all conceivable symptoms of actinic damage from simple sunburn to fatal cancers including basal and squamous cell carcinoma. There is no cure that can replace the lack of melanin or repair the impairment in melanogenesis<sup>3</sup>. No specific treatment is commercially available to retard actinic damage in people living with albinism (PLWA). To avoid excessive sunburn, PLWA within tropical areas use sunscreens but due to the high cost, they mostly rely on physical methods and barriers including abstinence from outdoor activities and appropriate clothing.

### 1.2 General sunscreen products and albinism

The geographical bias of albinism prevalence towards tropical Africa has been observed and reported implying that the higher populations of people living with albinism are found in Sub-Saharan Africa which coincidentally is a region with some of the highest global UVR onslaughts<sup>4</sup>. Most of these people belong to the "bottom billion" group, which is the billion people living in the lowest economic tier. The sunscreens available for albinistic persons in these areas are mostly from donors and well-wishers and are therefore developed for the western Caucasian users<sup>5</sup>. Despite the perceived protective effect from sunburn, the 16 approved sunscreens by the FDA do not protect albinistic persons from most forms of actinic damage. Sunscreens are photo-protective cosmeceuticals that primarily protect exposed skin from ultraviolet radiation (UVR) onslaughts and subsequent sunburn which often leads to solar keratosis, the main precursor of skin cancers in PLWA<sup>6</sup>. Most commercial sunscreens contain UVR filters that reflect or absorb UVR rays. Two main types of UVR filtration modes exist which include physical and chemical mechanisms. Chemical sunscreen materials are often organic filters that absorb UVR, converting and dissipating the energy within skin layers. Examples include cinnamates, and benzophenones<sup>7</sup>. Physical sunscreens are typically inorganic filters that reflect and scatter UVR radiation on applied skin surfaces. Examples include titanium dioxide and zinc oxide<sup>6</sup>. The ability of the treatment to protect the skin against UVR induced actinic damage is defined as the sunburn protecting factor (SPF). This dimensionless factor, which is a ratio of how long you can stay in the sun after applying the sunscreen without developing barely perceptible sunburn compared to how long you can stay in the sun before developing sunburn without any sunscreen is mostly dependent on the formulation. It is influenced by the choice of sunscreen, emulsifiers used in the cream, emollients, the choice of other functional ingredients and the patient adherence to application recommendations<sup>5,7</sup>. The FDA approved a standardized *in-vivo* SPF testing method in its 1993 tentative final monograph (TFM) currently in use. Before that, researchers used the FDA 1978 proposed monograph test method<sup>7</sup>. The prescribed procedure is to determine the minimum erythral dose (MED) of a sunscreen first. The MED is defined as the amount of radiation required to produce barely perceptible erythema at between 22 to 24 hours after exposure to the irradiation. The MED is determined by exposing the unprotected skin to a series of five incidences of geometrically increasing UVR. The radiation must be from a solar simulator xenon lamp, which emits radiation between 290 nm and 400 nm. The lamp must have a spectrum similar to the 10° solar zenith angle<sup>5</sup>. The simulator should emit radiation simulating both UVA and UVB at sea level. The exposures exponentially increase at a rate of 25% of the previous exposure. After 22-24 hours, a trained practitioner appraises the exposed site for erythema<sup>8,9</sup>. The MED obtained for the unprotected skin here is referred to as the MED<sub>US</sub>. The second stage is now to repeat the exposures, but this time with sunscreen-protected skin. The sunscreen is applied to the patient's back and given 15 minutes to dry. The site is then exposed to seven, (not five as in MED<sub>US</sub>) geometrically increasing UV irradiation<sup>9</sup>. The geometric progressions are based on the expected SPF from the product. The MED<sub>PS</sub> determined is the lowest dose that produces barely perceptible sunburn between 22-24 hours after the incident. The product SPF value is therefore the ratio between the MED<sub>US</sub>/MED<sub>PS</sub>. However, to minimize the use of human subjects *in-vitro* methods have been

developed which use specially designed spectrophotometers with comparable results to the *in-vivo* evaluations<sup>9</sup>.

### 1.3 *Terminalia Sericea*

*Terminalia sericea* (*T Sericea*), is a deciduous tree prevalent and native to most parts of Southern Africa which belongs to the *Combretaceae* family. The tree, which is also known as the silver cluster leaf due to the leaf morphology, has widespread use in traditional medicine and it is reported to be among the 50 most popular medicinal plants in Africa<sup>10</sup>. It has been reported that Infusions and decoctions from the plant's stem and root barks are highly antibacterial and have been extensively used to treat STDs and other opportunistic infections associated with HIV in Southern African traditional medicine<sup>11</sup>. Other studies have also described antimicrobial potency with minimum inhibitory concentrations (MICs) as low as 1 mg/mL for bacterial and fungal infections. The anti-HIV properties of the plant have also been reported, indicating that the hydro-ethanolic plant extracts of *T. sericea* can inhibit 100% HIV-1 RT at 100 µg/mL<sup>12</sup>. The tree is widely known as the 'elixir of youth' after the scientific validation of the anti-aging properties of the stem and root bark. These 'elixir of youth' claims are believed to stem from the compound sericoside (a major constituent of the bark extract), which has proven anti-inflammatory, anti-wrinkle or anti-aging claims<sup>13</sup>. The medicinal benefits of *T. sericea* have transcended African borders and the stem bark is now available on the European market. Metabolomic studies confirm that *T Sericea* is rich in both primary and secondary metabolites. It is believed that the medicinal attributes of the plant are due to the interaction of the inherent, intricate and concatenated complex of secondary metabolites and bioactive compounds present. Almost all plants from the species regardless of geographical location and habitat are composed of complex phytochemical pools of polyphenolic compounds in unique combinations with bioactive potential for use in biomedical applications<sup>12,13</sup>. The use of natural plant phenols in biomedical applications has been hampered by lack of standardization of activity and toxicological profiles, this standardization is inherently complex and duly extenuated by apparent variations in phytochemical constituents and their concentrations due to genetic traits, environmental factors and inducing factors that influence the production of secondary metabolites, since these secondary metabolites are produced to deal with existential threats in a plant's habitat<sup>14</sup>. When working with widely thriving plants in different habitats like *T Sericea*, it is imperative to acknowledge the secondary metabolite variation within the species, and to comprehend how these aberrations are linked to the resultant discrepancies in the bioactivity of extracts prepared. With a view to enhance the exploitation of the *T sericea*, this study sought to harness the different anti-bacterial, anti-aging, anti-inflammatory and photoprotective polyphenols of the plant as well as their capability in mediating the biosynthesis of photoprotective and anti-inflammatory ZnONPs alloyed to antibacterial AgNPs in ambitious pioneering actinic damage retarding albinistic treatments.

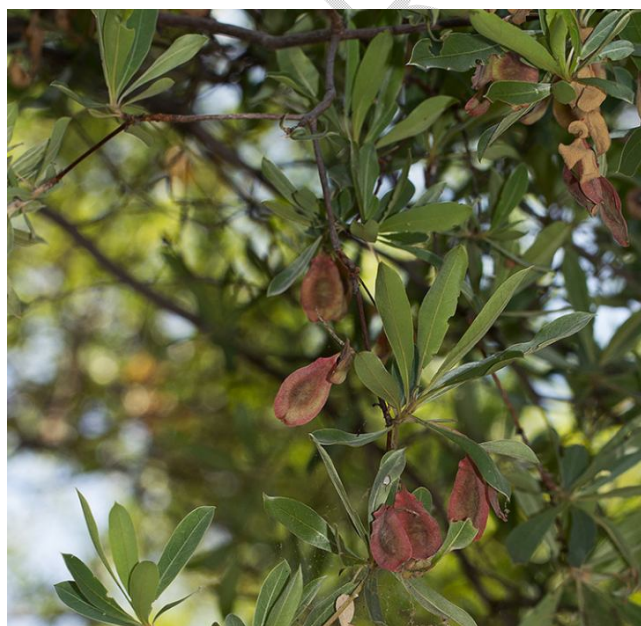


Figure 1 ; Leaves and fruits of *Terminalia sericea*.

#### 1.4 Polyphenols and photoprotection

Polyphenols are secondary metabolites naturally existing in living, plant and animal species which have cardinal survival and restorative functions such as anti-inflammatory, immunomodulatory, anti-oxidative and anti-ultraviolet properties and anti-bacterial effects<sup>15</sup>. Among other attributes, it is their impeccable radiation absorption and reactive oxygen species scavenging capabilities that have put them in the spotlight as potential photoprotective sunscreens or sunscreen boosters<sup>16</sup>. It is also, their unique structural morphology and functionality from various moieties, which render them capable of bio reducing and capping photoprotective nano metallic oxides coupled with their easy integration into multifunctional biomedical platforms that make them exciting precursors in the fabrication of hybrid photo protective active platforms<sup>15,17</sup>. This has ushered in new opportunities and expanded the scope of polyphenolic sunscreens. Various studies have confirmed the correlation between the total phenolic content, the antioxidant activities, and the Sun Protection factor (SPF) of sunscreen formulations in which they are incorporated<sup>18</sup>. These factors confirm the potential for using polyphenols in unique sunscreen formulations for genetically compromised skin types like albinism and vitiligo.

#### 1.5 Biosynthesis of nanometric bimetallic alloys of Silver and Zinc Oxide

Fabrication of novel metallic alloys through bio-reduction and capping of metallic salts by the bioactive polyphenols of known clinical value from medicinal plants is attracting increasing interest due to the intrinsic potential to harness the medicinal properties of the natural polyphenolic extracts and conjugate them to the inherent amplified photoprotective properties of the metallic oxides<sup>19</sup>. Biosynthesized metallic nanocomposites promise enhanced therapeutic competencies in comparison with the simple natural polyphenols. In experimental models' metallic nanocomposites demonstrate remarkable augmented treatment outcomes due to the additive or synergistic dual activities of the constituents<sup>20</sup>. The ability to control and fine-tune resultant desired properties through varying fabrication conditions including pH, temperature, concentration and rate of reaction adds a valuable dimension to their practicality in therapeutics. The same fabrication conditions can also be optimized to control particle size and shape of the metallic nanoparticles which has a gigantic effect on phase aggregation as well as therapeutic, bio and chemical equivalence<sup>19,21</sup>. In the present investigation we present the novel formulation of a pioneering anti-actinic damage cream specially formulated for albinistic skin types, incorporating biosynthesized metallic alloys of conjugated known antimicrobial agent, ionic silver and anti-inflammatory and photoprotective ZnO nanoparticles. This nano composite bimetallic alloy fabrication being mediated by the various bioactive polyphenolic constituents from *T sericea*. We further investigate the characteristics of both the bimetallic nano alloys as well as the sunscreen albinistic cream.

## 2 Materials and methods

The investigation protocols and the animal ethics handling procedure were approved by the institutional review board of the Joint University of Zimbabwe and Parirenyatwa research ethics committee (JREC) and the study was conducted according to the Declaration of Helsinki and the International Conference on Harmonization of Technical requirements for Registration of Pharmaceuticals for Human Use Guidance for good clinical practice.

### 2.1 Plant Material

The *T sericea* root bark samples were obtained from 5 different mature individual trees from the area surrounding Harben Park in Gweru Zimbabwe (19° 27' 0" South and 29° 49' 0" East) in December 2023. The trees were taxonomically authenticated as *T sericea* by the Zimbabwe national Herbarium.

### 2.2 Extract preparation.

The roots were debarked using a scalpel and subsequently washed to remove the debris. The bark samples were air dried in the laboratory for 4 weeks. After confirming that the moisture content was below 5 %, the roots bark samples were ground into a fine powder using a coffee grinder. A 60g portion of powder was soaked in 900 mL 70:30 ethanol/water (analytical grade, Merck, Germany) for 72 hours with intermittent shaking 3 times a day. After the extraction, the mixture was shaken one more time and filtered (Whatman No 4 filter paper). The filtrate was evaporated to dryness using a rotary evaporator

(Buchi, Switzerland) at 60 °C, cooling water temperature at 20° C and the vacuum set at 175 mbar. The crude extracts were ultra-low temperature lyophilized at -80 °C with the vacuum pump set at 0.01 mbar (ThermoScientific™KF Apex 96DW). The obtained lyophilized extracts were stored at 4 °C

### 2.3 Secondary metabolites analysis of *T Sericea*

In a 200ml round bottomed flask, 10g of the lyophilized hydro-ethanolic extracts of *T sericea*, were dissolved in 100g of distilled water and subjected to various phyto-screening techniques to confirm the presence or absence of relevant phytoconstituents of pharmacological interest to this study. The following qualitative tests were conducted:

#### 2.3.1 Tests for alkaloids

The presence of alkaloids was determined through the Mayer's test. To 5 ml of the lyophilized extract liquor in a test tube, two drops of Mayer's reagent were added. The presence of alkaloids was determined by the development of a white creamy precipitate at the bottom<sup>22</sup>.

#### 2.3.2 Tests for tannins and phenolics

The presence of tannins in the extract was determined by the ferric chloride test. To the test tube, 2-3 drops of ferric chloride was added to 5 ml of the prepared extract liquor. The test sample was observed for the presence of catechic tannins signaled by the development of a green blue color or a blue-black colour development which indicates the presence of Gallic tannins<sup>23</sup>.

#### 2.3.3 Test for flavonoids

The presence of flavonoids was determined by means of the alkaline reagent test. To 5ml of the lyophilized liquor in a test tube, 2 to 3 drops of a 50 % NaOH lye were added. The development of a deep yellow colour which gradually pales to a colorless hue after the addition of 3 to 4 drops of dilute HCL, confirms the presence of flavonoids<sup>24</sup>.

#### 2.3.4 Test for terpenoids

To confirm the presence of terpenoids. To a test tube with 5ml of the extract liquor, 2 or 3 granules of tin metal in 2 ml thionyl chloride solution were added. The formation of a pink colour indicates the presence of terpenoids<sup>25</sup>.

#### 2.3.5 Tests for steroids

The presence of steroids in the hydro-ethanolic extract of *T Sericea* was confirmed by adding 5 ml of chloroform to 5 ml of the extract liquor in a test tube, followed by the addition of 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. The development of a reddish brown colour indicates the presence of sterols in extract<sup>26</sup>.

#### 2.3.6 Test for saponins

To determine the presence of saponins in the test sample, a simplified foam test was used. In a 100ml measuring cylinder, 5ml of the extracted liquor was added to 30ml distilled water, the mixture was shaken for 2 minutes and the development of at least 1 cm head of foam in the test tube confirms the presence of saponins<sup>27</sup>.

### 2.4 Preparation of silver doped Zinc oxide nanoparticles of *T Sericea*

Ag doped ZnO nanoparticles were synthesized by dissolving 4g silver acetate (C<sub>2</sub>H<sub>3</sub>AgO<sub>2</sub>) and 8g zinc acetate dihydrate [CH<sub>3</sub>COO)<sub>2</sub>Zn.2H<sub>2</sub>O] in 100 mL of distilled water. After vigorous shaking, the mixture was titrated with 100ml of a 5% lyophilized *T sericea* extract in distilled water solution. The reaction was conducted at 50 °C under continuous stirring with a magnetic stirrer for 30minutes. The reaction mixtures were left overnight to complete the reduction process. The mixtures were then centrifuged at 10000 rpm. The samples obtained were washed thrice with distilled water and then calcined in a furnace at 500°C for 2 hours and stored for further studies. The obtained nanocomposites synthesized were subjected to a series of confirmatory tests for their characteristics. Various techniques including UV-vis Spectrophotometer (PerkinElmer, Lambda 30) to confirm the synthesis and to determine the optical density and energy gaps. Morphological features of the biosynthesized nanoparticles were analysed using Transmission electron microscopy (TEM) (Hitachi HT7800 RuliTEM).

## 2.5 Albinistic sunscreen prototype formulation

The materials used in preparing the cream were cosmetic Ingredient Review (CIR) approved ingredients as follows:

Stearic acid, Ceto stearyl alcohol, GMS and acetyl alcohol was obtained from Savanna Chemicals (South Africa), Liquid paraffin, petrolatum were supplied by Engen South Africa, Triethanolamine was obtained from Merck Chemicals South Africa, EDTA, Carbopol 940®, Glycerin, MPG, methyl paraben, propyl paraben, *Trichilia emetica* butter were supplied by KAZA natural oils, Zimbabwe, Silver doped Zinc oxide nanoparticles were fabricated in the lab.

### 2.5.1 Method for preparing the emulsion sunscreen

**Step 1:** A water phase was homogenized in a stainless steel thermal jacketed heating vessel, it contained 75% of the required amount of deionized water, EDTA, glycerin, Carbopol 940® MPG and methyl paraben. The materials were homogenized by a Silverson mixer and heated to 85°C for 5 minutes. The silver doped Zinc oxide nanoparticles were dispersed into this water phase.

**Step 2:** In a separate vessel, the oil phase was prepared simultaneously by adding all the required amounts of the following materials and heating them to 90°C for 5 minutes. The materials included propyl paraben, *Trichilia emetica*, GMS, cetyl alcohol, castor oil and stearic acid.

**Step 3:** The clear hot oil phase from step 2, was added into the water phase vessel from step 1 while consistently agitating with an emulsifying mixer at 3000rpm. Triethanolamine was then added to the newly formed emulsion.

**Step 4:** The buffered emulsion was then cooled down naturally by removing sources of heat down to 50°C. The *T sericea* lyophilized extract was then incorporated at this point. The remaining 25% of the deionized water was also added in to make up the required volume.

## 2.6 In-Vitro SPF determination of the sunscreen

### 1.1.1 Determination of SPF, UVAPF and the critical wavelength

Directives on Sunscreen testing and labelling of products prescribed by the FDA and COLIPA were used as guides in this determination of the SPF<sup>28</sup>. The *in vitro* SPF determinations (from 290-400nm) were carried out using a Spectrophotometer equipped with two photodiode array spectrographs and coupled to an integrating sphere, Ultraviolet Transmittance Analyzer (UV-2000S, Labsphere, USA). The spectrophotometer had a xenon flash lamp, which permitted emission of the required continuous peak less spectrum of radiation<sup>28</sup>. The lamp supplied energy in the spectral range between 290–450 nm. The incremental step was 1nm and the irradiance was conveniently kept low so as not to introduce potential photo stability to the sample. The sunscreen formulated above was applied at a rate of 2mg/cm<sup>2</sup> to square Polymethylmethacrylate (PMMA) plates which were roughed on one side (Helioplate™ HD6, HelioScreen, France) and was spread evenly over the plates with a fingertip covered in a vinyl glove. The sunscreen, 50mg in total per plate, was directly weighed and applied in droplets onto the plates. Care was taken to prevent any material losses. Three plates were prepared for each sample and the filmed plates were kept in the dark to equilibrate for 15 minutes at 28 degrees Celsius. The equilibrated plates were then subsequently mounted onto the light-path of the Ultraviolet Transmittance Analyzer (UV - 2000S). The UV radiation transmittance patterns through the mounted samples were measured using the equipment settings above at 6 different sites of the plates. The blank was prepared by mounting onto the PMMA plates, the base emulsion without the 50% Aloe gel as per recommended guidelines. The built in equipment software used the recorded transmission patterns to calculate and determine In-vitro UVA/B photo protection efficacy accordingly: The UVR photo protection efficacy of the sunscreen was determined through the calculation of the UVB protection efficacy (SPF) and the UVA protection efficacy (UVAPF), the UVA/UVB ratio and the critical wavelength  $\lambda_c$ .

The *in vitro* SPF was evaluated as per the following equation 1.

$$\text{SPF}_{\text{in vitro}} = \frac{\int_{\lambda=290\text{nm}}^{\lambda=400\text{nm}} E_{\lambda} \times I(\lambda) \times d(\lambda)}{\int_{\lambda=290\text{nm}}^{\lambda=400\text{nm}} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda)} \times d\lambda}$$

Equation 1

Where?

$E(\lambda)$ : erythema action spectrum,

$I(\lambda)$ : spectral irradiance,

$A_0(\lambda)$ : mean monochromatic absorbance before UV exposure,

$\Delta\lambda$ : wavelength step (1 nm).

The whole experiment procedure was repeated with a COLIPA SPF 30 standard, as an assaying guide.

## 2.7 Skin sensitivity tests

The skin sensitivity tests conducted on the final formulation were guided by OECD technical guideline 406 with minor amendments using 3 adult male New England breed white laboratory rabbits, weighing between 1.3-1.8 kgs<sup>29</sup>. The rabbits were checked for their suitability for the study over a 7-day acclimatization period. The rabbits were kept in a rodent facility in a limited access facility. They fed on a typical commercial rabbit diet and had unlimited access to drinking water. Prior to the test, the backs of the rabbits were shaved by depilatories and the shaved area was divided into two marked parts measuring 25cm<sup>2</sup> each. The first marked area was used for the application of the test cream and the second demarcated area was used as the control for testing the irritation according to OECD guidelines<sup>29</sup>.

### 2.7.1 Cream application

To the test area, 5ml of the test cream was applied by a syringe and spread evenly over the 25cm<sup>2</sup> demarcated shaved area of each animal. The application site was covered by gauze and the area was lightly covered by non-sticky bandages. The treated rabbits were then returned to their respective cages and observations were made to the sites at 24, 48 and 72 hours. Any sensitivity or reactions to the treatment was evaluated by the following criteria (Table 1) as per the documented method<sup>30</sup>.

### 2.7.2 Score of primary irritation index (SPI)

Table 1: Observed irritation classification<sup>30</sup>

Reaction	Observation	Score
Erythema	No erythema	0
	Very slight erythema	1
	Well-defined erythema	2
	Moderate to severe erythema	3
	Severe erythema to eschar formation	4
Oedema	No oedema	0
	Very slight oedema	1
	Well defined oedema	2
	Moderate oedema (raising 1mm)	3
	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
<b>Total score for primary irritation</b>		<b>8</b>

The marked control site was treated in the same way with the base cream without the nanomaterials. Observations were made in the same manner as the treated sites. The Score of Primary Irritation (SPI) was calculated by the following equation 2 for both the treated and the control sites<sup>30</sup>.

$$SPI = \sum \frac{\text{erythema and oedema grade at 24,48,72hrs}}{\text{number of observations}}$$

Equation 2

### 2.7.3 Primary irritation index (PII)

The Primary Irritation Index (PII) was derived from the differences between the summed SPI scores for the treated site and the control sites. The PII was calculated by the following equation 3:

$$PII = \frac{\sum SPI(test) - \sum SPI(base)}{\text{number of animals}}$$

Equation 3

The degree of irritation was then categorized according to the Draize irritation response categories in Table 2<sup>31</sup>.

Table 2: Irritation response categories<sup>31</sup>

CATEGORY	PRIMARY IRRITATION INDEX (PII)
Negligible irritation	0-0.4
Slight irritation	0.5-1.9
Moderate irritation	2-4.9
Severe Irritation	5-8

## 3 Results and discussion

### 3.1 Plant preparation

Plant collections were done from 5 individual trees because a single tree could not provide enough root samples for the complete analysis since according to Zimbabwean traditional medicinal practices, only roots from the eastern side of plants can be harvested for treatment purposes. Our observation is that this is done simply for conservation purposes to protect highly medicinal plants from over harvesting of roots from individual plants leading to plant demise. The trees were flowering during the time of root sampling. The preparation was done using the root bark even though there have been reports of decoctions from fruits, leaves and stems of *T sericea* used for medicinal purposes, decoctions from the root bark are the most frequently reported in traditional medicine. The root bark was a greyish-brown colour and easily peeled away in strips. In Southern Africa, *T. sericea* root decoctions are administered for the treatment of a range of conditions, such as stomach ailments, diabetes, asthma, and constipation, but the main applications are associated with bacterial infections and as an anti-inflammatory, anti-aging and burns treatment<sup>10,11,12</sup>.

### 3.2 *T Sericea* phytochemical screening

Table 3 : Phytochemicals present in *T Sericea* hydro-ethanolic and distilled extracts.

Evaluate	Presence in hydro-ethanolic extract	Presence in distilled water extract
Alkaloids	+++	+
Phytosterols	+	-
Flavonoids	+++	++
Saponins	-	-
Phenolic compounds	+++	++
Tannins	+++	++
Glycosides	+++	+
Terpenoids	+++	+

(-) Indicates absence, (+) Indicates presence, (+++) indicates strong presence.

The preliminary metabolomics analysis of the *T Sericea* root extract revealed the presence of various secondary metabolites including phenolics, glycosides, tannins, terpenoids, flavonoids and alkaloids (Table 3). This correlates well with various other phyto screening studies which revealed that the major constituents of *T Sericea* include the pentacyclic triterpenoid sericic acid, its glycoside sericoside and the flavonoid dilactone and its derivatives which are believed to be the major sources of the medicinal effects of *T Sericea* extracts<sup>13</sup>. This correlates with our results which confirmed the presence of terpenoids and glycosides. In Japan, sericoside derived from *T. sericea* is patented for skin lightening preparations<sup>11</sup>. Flavonoids are widely known in the medical world for their medicinal benefits, including antibacterial, antiviral, antifungal, anticancer, antioxidant and anti-inflammatory attributes<sup>12</sup>. The wound healing effects of this plant have been substantiated by scientific studies which showed that the crude extract and the abundant Terminalic acid helped in wound healing by decreased the exudate formation and erythema of the wounds<sup>14</sup>. Laboratory tests confirmed the wound healing effects of *T sericea* when topically applied on induced wounds on rat models. Various other observations on biological activity support the use of the plant extracts in the treatment of wounds. The photoprotective effects of polyphenols in alleviating actinic damage symptoms including UV-induced acute, subacute and chronic skin inflammation, keratinocytes proliferation, actinic DNA and protein damage as well as dysregulation of important pathways that lead to solar keratosis and carcinomas have been scientifically confirmed by many studies<sup>13</sup>. Since these symptoms are characteristic of actinically damaged albinistic skin, it is realistic to assume that incorporation of polyphenolic rich natural medications in their sunscreens would be beneficial to people living with Albinism.

In phyto analysis, emphasis is not only given to the proven biomedically active components<sup>32</sup>, for it has been scientifically observed that in phytomedicine the activity of crude extracts is resultant from a delicate balance between the active and non-active constituents. The presence of these metabolites substantiates the traditional use of *T Sericea* as an antibacterial agent in traditional medicine and suggests its potential use in biosynthesis of nano-platforms from metallic salts. Reported studies on biosynthesised nanometric ZnO from *T sericea* observed that the nanoparticles successfully inhibited potent human pathogens and had excellent free radical scavenging activity comparable to the reference ascorbic acid and the *T. sericea* extract also revealed good antioxidant properties against more stable radicals (DPPH and ABTS)<sup>13</sup>.

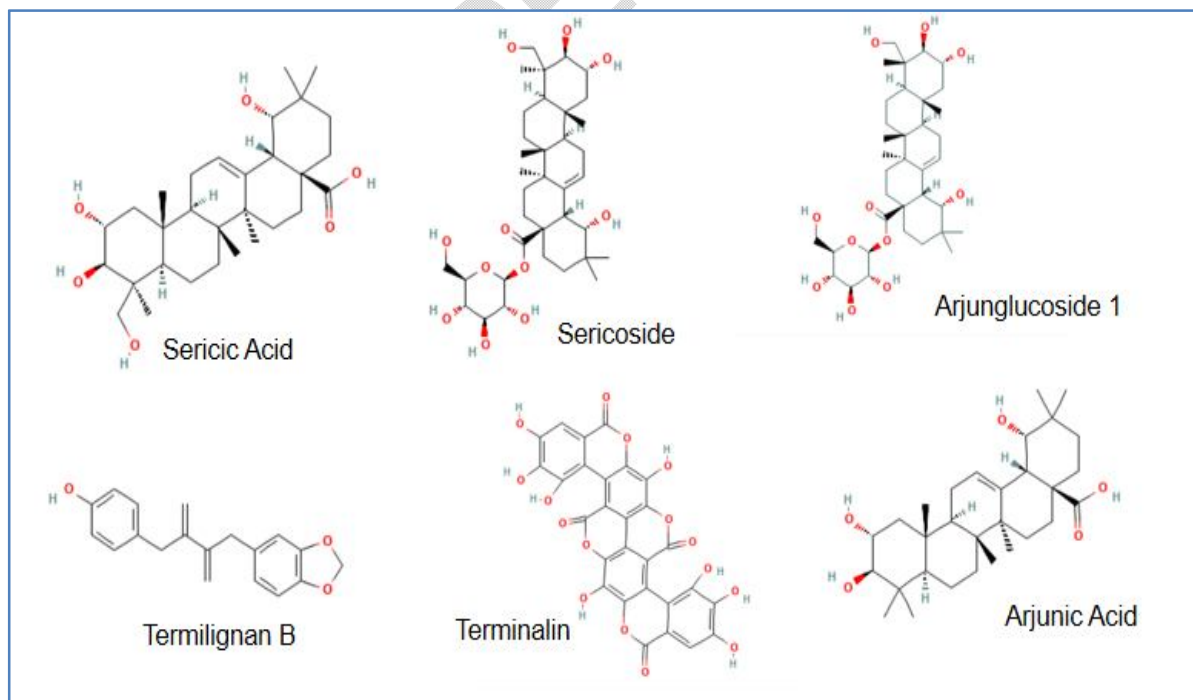


Figure 2 , Polyphenols, glucosides and terpenoids from *T sericea*.

### 3.3 Characterization of silver doped Zinc oxide nanocomposites

#### 3.3.1 TEM (Transmission electron microscopy) and UV-Visible spectroscopy

The main objective behind the characterisation of the biosynthesised nanoparticles was to confirm that Silver doped Zinc oxide nanoparticles had been formed, to measure the particle size distribution as well as to determine the morphology of the nanocomposite structures. Extensive evaluation assays and reports on the nanocomposites was therefore not dwelt upon since this was not the main objective of the study. Our objectives were to successfully fabricate Silver-doped Zinc oxide nanoparticles and incorporate them into a specially formulated albinistic sunscreen and evaluate its safety and efficacy.

UV-Visible absorption spectroscopic analysis was performed to confirm correspondents of the electronic transitions in the biosynthesised structures to characteristics associated with nanometric Zinc oxide and Silver to confirm successful doping. Absorbance spectra for ZnO nanoparticles exhibited expected absorption peaks between 300 and 380nm. Doping with silver resulted in the shift of the absorption peaks to an absorption band at 440 nm which is characteristic of the surface plasmon resonance of Ag.

The TEM images of the ZnO: Ag nanoparticles synthesised from *T Sericea* roots extract as shown in Figure 3 were between 50 and 80nm. As shown in close frames (a) and frame (b), the nanoparticles exhibited a mixture of roughly quartzite and irregular shapes and were agglomerated to a large extent. The agglomeration is very common in the case of metal oxide nanoparticles.

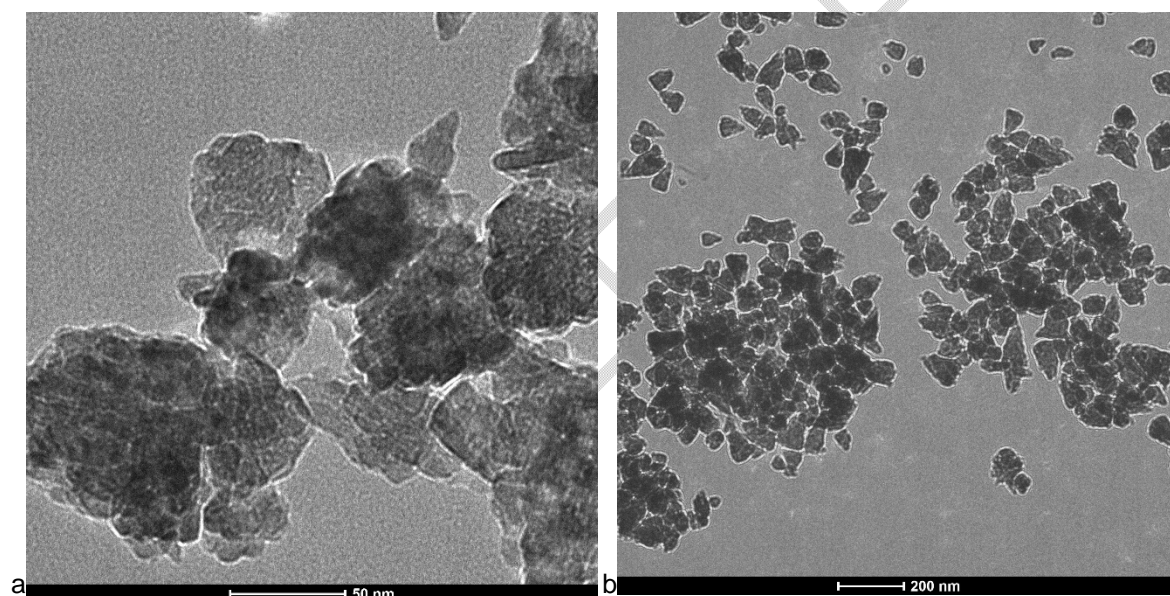


Figure 3: TEM images of the fabricated nano composites.

#### 3.4 Sunscreen formulation

Albinism, also called achromia is a severe form of amelanosis. A congenital inability of the epidermal melanin unit to synthesize, distribute or store the biopolymer, melanin, in response to UVR assault or as part of the wound healing process. To protect the underlying structures from actinic damage the body employs two mechanisms: melanisation and keratinisation<sup>33</sup>. Melanin which is synthesised from tyrosine under direction from the enzyme tyrosinase through the raper-mason pathway has the capacity to absorb harmful UVR through a process referred to as 'ultrafast internal conversion'<sup>34</sup>. The second mechanism of protection against actinic damage is through keratinisation, whereby the skin basal cell membrane hyperproliferates and thickens to protect the dermal layer and retard cell damage. Keratinisation is what is commonly observed as severe skin roughness on exposed albinistic skin especially the outer forearms, face and neck area<sup>33</sup>. This impairment in the epidermal melanin unit functions in albinistic persons renders them vulnerable to biological endpoints of actinic damage including sunburn, skin roughness, solar urticaria, solar keratosis, skin wrinkles, inflammation and malignancy. Ordinary commercial

sunscreen formulations do not address the above issues since they are designed for the Caucasian leisure market and normal healthy skin<sup>6</sup>. It was therefore prudent to steer clear from typical sunscreen formulation protocols and develop something exemplary and unique to deal with problems pertinent to people living with albinism especially in the tropics and sub tropics. To alleviate the excessive roughness and dryness observed in People Living with Albinism (PLWA), the formulation under review here incorporated a high ratio of protective emollient oils including *Trichilia emetica* (Table 4). These fixed oils are also believed to be excellent photo protectant agents<sup>35,36</sup>. Albinistic skin is often associated with syndromes that make the skin bruise easily like the Hermansky-Pudluk syndrome and impaired wound healing mechanisms which make the afflicted persons prone to skin infections<sup>37</sup>. The formulation therefore included anti-bacterial and anti-fungal agents to thwart microbial threats, in this case we used the lyophilised active extract from *T sericea* which is very rich in polyphenolic, flavonoid, alkaloid and other secondary metabolites. The sunscreen effect in the formulation is provided by the biosynthesised multifunctional nanoplatform conjugating alloyed ZnO and Ag nanoparticles to *T sericea* polyphenolic compounds. To give a unique high SPF sunscreen at low active ingredient levels. The sunscreen was optimised to have a pH of 5.8 consequent to the fact that our previous research reported that the average skin pH for Albinistic skin was 5.8 (Table 5), which is slightly higher than normal skin pH at 5.5.<sup>6</sup>

**Table 4: protype albinistic sunscreen formulation**

Material	% Incorporation
Stearic acid	5
Cetyl alcohol	2
Ceto stearyl alcohol	2
Glycerol monostearate	2
Cocoa butter	1
Castor oil	1
Lyophilised T Sericea	Q. S
Mafura butter (Trichilia emetica)	2
Glycerin	4
Monopropylene glycol	1
Carbopol 940 <sup>®</sup>	0.07
Methyl hydroxybenzoate	0.2
Propyl hydroxybenzoate	0.2
Triethanolamine	0.6
Colloidal Ag-ZnONPS	Q. S
EDTA	0.3
Distilled Water	Q. S

**Table 5: Treatment cream analytical report**

Parameter	Result
Description	Off white smooth viscous cream, esthetically pleasing slippery feel when rubbed between two fingers.
pH	5.80
Odor	Characteristic of un-fragranced base cream

### 3.5 SPF determinations

#### 3.5.1 In-Vitro SPF determination and photo stability of lyophilized *Aloe excelsa* extract

The *in-vitro* photo protection testing for SPF and UVAPF was done following the “Sunscreen Testing According to COLIPA 2011/FDA Final Rule 2011 Using UV/Vis LAMBDA Spectrophotometers” guidelines. After testing materials for photoprotective activity using the protocols, sunscreen SPF’s are labelled as either low, moderate, high or very high as shown in table 6. According to these guidelines, marketed consumer products with SPF’s below 6 cannot be classified as sunscreens because the protective effect is low, and the objectives of sun protection cannot be achieved. According to the mandates above commercial sunscreen SPF’s can either be low, moderate, high or very high<sup>38</sup>.

**Table 6: The four protection classes for SPF labelling by COLIPA 2011/FDA Final Rule 2011<sup>38</sup>**

Label SPF	Protection class
6	low
10	low
15	moderate
20	moderate
30	high
50	high
50+	very high

For ease of commercial application and to ensure that products compliance can be achieved from 1 testing guideline, the FDA Final Rule 2011 test protocol parameters are aligned to COLIPA 2011 sunscreen guidelines. Apart from the SPF, both directives require proof of UVA protection factor (UVAPF) from products which should be equal to or more than 1/3 of the SPF, The directives also specify an *in-vitro* Critical Wavelength value greater than 370 nm, in order for a product to comply with requirements for broad spectrum sunscreen protection<sup>38,39</sup>.

**Table 7: *In vitro* photo protection and photo stability of the Albinistic Sunscreen**

Parameter	Time, minutes				
	0	30	60	90	120
SPF	31.60±0.38	31.20±0.44	30.45±0.50	30.20±0.60	30.12±0.65
UVAPF	14	14	14	14	14
$\lambda_c$	385	385	384	383	383
%SPF <sub>eff</sub>	-	98.7	96.3	95.6	95.3
%UVAPF <sub>eff</sub>	-	100	100	100	100

According to the reported results, above (Table 7) the albinistic cream has a high SPF above 30 and a UVAPF of 14 and a critical wavelength of 385. We can therefore confirm that the *in-vitro* photoprotective efficacy of the albinistic cream as depicted by SPF and the UVAPF and the photostability were successfully evaluated. In these studies, we did not only analyze the SPF which only measures perceptible sunburn, but we evaluated other parameters that contribute to other forms of actinic damage. To conform to COLIPA and FDA guidelines and to relate our formulation to the concept of broad-spectrum photo protection, we also measured and reported the UVAPF, the UVB/UVA ratio as well as the critical wavelength  $\lambda_c$  as shown in Table 7. In the same experiments we also report the results from the extended photostability potential of the cream evaluated over a 2-hour period. These findings register that the cream retains the obtained broad spectrum photo protection efficacy 2 hours post irradiation without any significant deviations from expected parameters as depicted by the calculated *in-vitro* efficacy percentages: %SPF<sub>eff</sub> and %UVAPF<sub>eff</sub> which are all above 95%.

As discussed above, the methods for evaluating the efficacy of sunscreens through SPFs are standard. They are mandated by the FDA, OECD, COLIPA and the EU guidelines method M389/EN through council directive 76/768/EEC of 27 July 1976<sup>40</sup>. Only 2 techniques exist and are recognized by the bodies

as suitable methods to approximate the SPF of a sunscreen product. One is the *in-vivo* methods based on visually appraising the effects of increased erythral doses of UVR on human subjects and the second is the *in-vitro* spectrophotometric methods. In this study, the *in-vitro* testing protocols using Optometric 290S were employed. The *in-vitro* method was preferred to the *in-vivo* methods due to numerous concerns and reservations about envisaged inadequacies and unsuitability of the testing protocols and irrelevance of using *in-vivo* SPF measurements as the basis for actinic damage protection in albinistic skin for the following reasons.

- i. SPF is a measurement of sunburn, oedema and urticaria induced by UVB on skin. It does not adequately measure the chronic effects of UVA. While UVA might not cause much acute damage in normal skin, it is the precursor of chronic damage in albinistic skin and all melanin deficient skin types, including various cancers, skin fragility and premature aging. The declaration of product SPFs without giving similar emphasis on the measurement of UVA protection is inadequate, for use as a protection factor for albinistic treatments.
- ii. The second major concern in choosing a protocol for determining actinic damage protection, in relation to albinistic persons is that the related mandated protocol is the persistent pigmentation darkening method (PPD). This method observes potential actinic damage protection by the ability of sunscreen to protect the skin from persistent darkening on exposure to UVR. Pigment darkening is a function of melanisation and is a self-protective mechanism for the body during UVR onslaughts. This mechanism is absent in albinistic persons, the lack of melanin makes it impossible to react to sunlight in the way the mandated testing methods evaluate skin damage. Such a method cannot be relevant to genetically compromised skin, which does not tan, like albinistic skin.
- iii. Melanin is part of the wound healing system. The mandated SPF testing method is based on observing responses to photo onslaughts on normal skin with well-known UVR damage pathways. Albinistic skin is not normal skin. The pathways are different, the wound healing system lacks melanin and even benign UVR damage is not reversible. It is therefore not possible to approximate the sunburn protection factor of compromised skin using the mandated *in-vivo* methods.
- iv. The mandated *in-vivo* SPF measurement method is based on Fitzpatrick skin categories<sup>41</sup>. A set of skin types that were put into compartments with regards to color and potential response to UVR damage. Albinistic skin is not included in the Fitzpatrick classification system on which the mandated test methods are based on.
- v. The mandated *in-vivo* test methods seem to ignore the influence of altitude and latitude on UVR incidences and photo damage. The test methods were developed in temperate areas. In the FDA TFM (1993)<sup>38</sup> mandated method of SPF testing, the radiation must be from a xenon solar simulator lamp, which emits radiation between 290nm and 400nm. The lamp must have a spectrum like the 10° solar zenith angle at sea level. The simulator should emit radiation simulating both UVA and UVB at sea level. Albinism is mostly biased towards people living in tropical Africa with geographic and radiation patterns unrelated to the above mandated protocols, It was reported in our previous studies<sup>5</sup> that, the in-effectiveness of most commercial sunscreens on albinistic humans in Zimbabwe can be attributed to the various discrepancies in climatic and geographic conditions that are not found in temperate regions where the products are developed and intended for use.

The SPF for the albinistic cream was determined to be  $31.60 \pm 0.38$  by Optometrics<sup>®</sup> spectrophotometric 290s instrumentation which is regarded as a high SPF.

### 3.6 Skin sensitivity tests

The skin irritation and sensitivity test on rabbits for the treatment cream and the control base cream exhibited the following:

#### 3.6.1 Erythema

After 24 hours, the skin irritation score for erythema and oedema in all 3 rabbits ranged from 0-1 for both the treatment cream and the control base cream. The results were identical for both the control and the test creams. No erythema or oedema values above 1 were observed on any rabbit in all the studies

carried out (table 8). The limited sites which recorded very minimal erythema scores of 1 after 24 hours, recorded scores of 0 after 72 hours, showing disappearance of the slight erythema in limited time.

### 3.6.2 Oedema

For the oedema, the results for the treatment and the control base cream were also identical. No animal exhibited any signs of oedema formation after application of both creams. After the 72 hours, time frame, no rabbit was exhibiting any form of erythema or oedema, and the experiments were concluded as per the guidelines.

**Table 8: Irritation, erythema and oedema scores for rabbit skin sensitivity**

Rabbit	Sensitivity reactions	Treatment cream			Control base cream		
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
1	Erythema	0	0	0	0	0	0
	oedema	0	0	0	0	0	0
2	Erythema	1	0	0	1	0	0
	oedema	0	0	0	0	0	0
3	Erythema	1	1	0	1	0	0
	oedema	0	0	0	0	0	0

### 3.6.3 Primary irritation index

For both creams, the Primary irritation index, for all animals, was found to be between 0.04 and 0.09. According to the scoring criteria, this classifies the cream as a 'negligible irritant'.<sup>31</sup>

Adverse effects of formulations on skin which are referred to as skin irritations are due to numerous factors. These include the concentration of the irritants in the product, the duration of the contact time, the body site that has been exposed to the potential irritant, the skin permeability and toxicity profile of the potential irritant. The determination of the potential to cause irritancy by any skin care product is therefore necessary and mandated. The OECD guideline 406<sup>29</sup>, COLIPA as well as the FDA recommend the rabbit skin irritancy evaluation based on the methods documented by Draize (Draize H: 1944)<sup>31</sup> employed in this study.

For ethical reasons, OECD guideline 406 recommends a decision-making protocol prior to any *in-vivo* animal test for skin irritation and corrosivity based on the product analytical report evidence and product pH which was adhered to in this study. Examples of the decision-making considerations done include Substances with pH below 2 or with pH above 11.5 should never be tested on animals under the Draize tests due to the obvious adverse effects on animals and that any substances previously found to be corrosive in any other sensitivity tests documented under Annex V of Dir. 67/548/EEC<sup>42</sup> should never be included in the Draize tests. In this study, the product analytical report and pH justified the use of the Draize tests.

## 4 Conclusion

We presented a pioneering approach to the development of specialised hybrid UV filtering sunscreens for people living with Albinism incorporating advanced bimetallic nanocomposites and natural photoprotective plant metabolites possessing antioxidant and UV ray absorption abilities in an aesthetically pleasing multifunctional cosmeceutical formulation. The photoprotection as measured by mandated protocols is in the category of high protection, however unlike typical physical sunscreens our formulation doesn't leave the 'whitish' cast on users. The safety of the formulation has also been confirmed by the skin sensitivity evaluations and the albinistic sunscreen is not only effective, but it doesn't also have any detrimental effects on human health. With all the available new information on the photoprotective mechanisms action of natural polyphenols and their ability to bio reduce, cap and stabilise metallic oxides, it is prudent for

formulators to employ as well as support their potential use in skin photoprotection and prevention of actinic damage in humans especially those with genetically compromised skin types.

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