RESEARCH PAPER

Green synthesis of titanium dioxide nanoparticles of leaf extract of Neolamarckia Cadamba for in vitro wound healing activity

Pankaj Kumar[1], Mehta Parulben D.[1], Somesh Saxena[1] and Rita Mourya[2]

1. SAM College of Pharmacy, Faculty of Medical and Paramedical Sciences, SAM Global University, Raisen, Madhya Pradesh, India-464551.
2. School of Pharmacy, Faculty of Medical and Paramedical Sciences, SAM Global University, Raisen, Madhya Pradesh, India-464551.

Abstract:

The present study was undertaken to develop and evaluate titanium dioxide nanoparticles (TiO_2NPs) using the leaf extract of *Neolamarckia cadamba* through a green synthesis approach and to investigate their potential for in vitro wound healing activity. The leaves of *Neolamarckia cadamba* were collected, authenticated, dried, powdered, and subjected to extraction using a suitable solvent. The plant extract, containing various phytoconstituents such as phenolic and flavonoid compounds, was used as a natural reducing and stabilizing agent during the synthesis of TiO_2 nanoparticles. The synthesized nanoparticles were characterized by ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, particle size and surface charge analysis, scanning electron microscopy, and energy-dispersive X-ray analysis to confirm their formation and physicochemical characteristics. The optimized TiO_2NPs were subsequently incorporated into a topical gel formulation and evaluated for physical properties, drug/nanoparticle content, spreadability, extrudability, pH, viscosity, and in vitro release characteristics. The wound healing potential was assessed using suitable in vitro methods, including antioxidant and protein denaturation/proteinase inhibition studies and a scratch wound assay using relevant cell cultures. The optimized formulation demonstrated satisfactory physicochemical properties and showed progressive wound closure in the in vitro scratch assay, indicating enhanced cellular migration and proliferation. The biological activity was further supported by the antioxidant and anti-inflammatory potential of the formulation. Overall, the findings suggest that green-synthesized TiO_2 nanoparticles from *Neolamarckia cadamba* leaf extract can be successfully incorporated into a topical gel and may provide a promising, eco-friendly nanotechnology-based approach for promoting wound healing. Further in vivo and clinical investigations are required to establish its therapeutic efficacy and safety.

Keywords: Neolamarckia cadamba, green synthesis, titanium dioxide nanoparticles, TiO_2 nanoparticles, leaf extract, topical gel, wound healing, antioxidant activity, scratch wound assay, nanotechnology

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1. Introduction

Wound healing is a complex and highly coordinated physiological process through which the body restores the structure and function of damaged tissue. It involves several overlapping phases, including haemostasis, inflammation, proliferation, and remodelling. During this process, inflammatory cells, fibroblasts, keratinocytes, endothelial cells, and extracellular matrix components work together to promote tissue repair. Any disturbance in these stages due to infection, oxidative stress, prolonged inflammation, or poor cellular migration can delay wound closure and may lead to chronic wounds. Therefore, the development of effective and safe therapeutic approaches for accelerating wound healing remains an important area of pharmaceutical and biomedical research.[1]

Oxidative stress and inflammation play important roles in delayed wound repair. Excessive production of reactive oxygen species can damage cellular components and interfere with fibroblast proliferation, collagen formation, and tissue regeneration. Similarly, prolonged inflammatory responses can prevent progression from the inflammatory phase to the proliferative phase of healing. Antioxidant and anti-inflammatory agents may therefore support the wound-healing process by reducing oxidative damage and controlling excessive inflammatory reactions. Evaluation of antioxidant activity and inhibition of protein denaturation and proteinase activity can provide useful preliminary information regarding the potential of a formulation to support tissue repair.[2]

Nanotechnology has gained considerable importance in modern drug delivery because nanoparticles possess unique physicochemical and biological properties. Their small particle size and large surface-area-to-volume ratio can enhance interaction with biological tissues and may improve the effectiveness

of therapeutic agents. Metal oxide nanoparticles, particularly titanium dioxide (TiO_2) nanoparticles, have attracted significant attention because of their physicochemical stability and reported antimicrobial, antioxidant, and other biological properties. These characteristics make TiO_2 nanoparticles interesting candidates for pharmaceutical and biomedical applications, including topical preparations for wound management.[3]

Titanium dioxide nanoparticles can be prepared using physical, chemical, and biological methods. Conventional physical and chemical approaches may require high energy input, elevated temperatures, and potentially hazardous reducing or stabilizing chemicals. These limitations have encouraged the development of green synthesis methods. Green synthesis utilizes biological materials such as plants, microorganisms, or their extracts to produce nanoparticles in a comparatively simple and environmentally friendly manner. Plant-mediated synthesis is particularly attractive because plant extracts contain a wide range of phytoconstituents that may participate in nanoparticle formation, stabilization, and surface capping.[4]

Medicinal plants are valuable sources of naturally occurring bioactive compounds, including phenolic compounds, flavonoids, alkaloids, terpenoids, tannins, and other secondary metabolites. These constituents may contribute to the reduction and stabilization of nanoparticles during green synthesis while also providing additional biological properties. Plant-mediated nanoparticle synthesis can therefore combine the advantages of nanotechnology with the pharmacological potential of medicinal plant constituents. This approach may offer an economical and sustainable alternative for developing novel therapeutic materials.[5]

Neolamarckia cadamba is a medicinal plant belonging to the family Rubiaceae and is traditionally associated with several therapeutic applications. Different parts of the plant contain various phytoconstituents that may contribute to its biological activities. The leaves are considered a useful source of phytochemicals, including phenolic and flavonoid compounds, which possess antioxidant and other pharmacological properties. These characteristics make *N. cadamba* leaf extract a suitable biological material for exploring plant-mediated synthesis of nanoparticles.[6]

Green synthesis of TiO_2 nanoparticles using *Neolamarckia cadamba* leaf extract may provide a promising combination of nanoparticle-based and plant-derived biological activities. During synthesis, phytoconstituents present in the leaf extract can interact with titanium precursors and assist in the formation and stabilization of TiO_2 nanoparticles. The resulting nanoparticles can subsequently be characterized using techniques such as ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, scanning electron microscopy, particle-size analysis, zeta-potential measurement, and energy-dispersive X-ray analysis to confirm their formation and determine their physicochemical characteristics.[7]

Topical gel formulations are widely used for localized delivery because they are easy to apply, provide good patient acceptability, and allow the active material to remain in contact with the skin for an appropriate period. Incorporation of green-synthesized TiO_2 nanoparticles into a topical gel may facilitate localized application at the wound site and potentially support the different biological processes involved in wound repair. A well-designed gel should possess suitable pH, viscosity, spreadability, extrudability, homogeneity, and stability to ensure satisfactory topical performance.[8]

In vitro wound-healing evaluation provides an initial method for investigating the biological potential of a formulation before further in vivo studies. The scratch wound assay is commonly used to assess cellular migration and proliferation by observing the closure of an artificially created gap in a cell monolayer. Greater wound closure over time indicates enhanced cellular migration and proliferation. Other investigations, such as antioxidant activity, protein denaturation inhibition, and proteinase inhibition, can provide additional evidence regarding the antioxidant and anti-inflammatory potential of the formulation.[9,10]

2. Methodology

Extraction of leaf extract of Neolamarckia Cadamba plant

In light of the plant's critically endangered nature, a minimal quantity (100 g) of plant material was selected for study. The materials were meticulously washed, sliced into fine segments, and air-dried in the shade under ambient conditions. Once dried, the material was finely ground using a mechanical grinder to obtain a uniform powder. A sequential extraction protocol was followed, utilizing solvents in ascending order of polarity—hexane, ethyl acetate, and methanol—over a 48-hour period. Each solvent extraction involved continuous agitation and gentle heating in a water bath, maintaining temperatures aligned with the respective boiling points of each solvent. Post-extraction, the mixtures were filtered through Whatman No. 1 filter paper. The filtrates were then concentrated by evaporating the solvents using a water bath. The resulting dried extracts were carefully labeled and stored at 4 °C for future use in phytochemical profiling and bioactivity assessments.[11,12]

Quantitative Analysis of extract

Determination of total phenolics by Folin-ciocalteau colorimetic assay

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols.They produce a blue colour upon reaction.This blue colour was measured spectrophotometrically.[13]

Determination of Total Flavonoid Content

Flavonoids are polyphenolic compounds ubiquitous in nature and are categorized according to their chemical structure as flavonols, flavones, flavanoes, isoflavones, catechins, anthocyanidins and chalcones. Favonoids react with $AlCl_3$ to produce a coloured product, which can be measured spectrophotometrically. Total flavonoids were measured by a colorimetric assay. An aliquot of diluted sample or standard solution of rutin was added to a 75 µl of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg Rutin/g dry weight (mg rutin/g), through the calibration curve of Rutin. All samples were analysed in three replications. Line of regression from rutin was used for estimation of unknown flavonoid content.[14,15]

In vitro antioxidant assay by Superoxide radical scavenging activity

The scavenging activity of superoxide anions by extract was assessed using the established protocol. This approach hinges on the ability to inhibit the formation of formazan from nitroblue tetrazolium (NBT) in the presence of superoxide ions. All solutions were formulated using a 100 mM phosphate buffer at a pH of 7.4. Specifically, a mixture was prepared comprising 1 ml of 156 mM NBT, 1 ml of 468 mM reduced Nicotinamide adenine dinucleotide (NADH), and 3 ml of extract at concentrations ranging from 20 to 100 µg/ml. The reaction initiation was triggered by the addition of 100 µl of 60 µM Phenazine methosulphate (PMS), followed by incubation at 25°C for a duration of 5 minutes. The absorbance was subsequently measured at 560 nm utilizing a UV-visible spectrophotometer, and the percentage inhibition of superoxide radicals was calculated.[16,17]

In vitro Anti-inflammatory Activity by Inhibition of protein denaturation method

The egg-albumin denaturation method is a simple in vitro model for preliminary screening of anti-inflammatory activity. Fresh hen's egg albumin is mixed with phosphate-buffered saline (PBS, pH approximately 6.4), and different concentrations of Neolamarckia cadamba leaf extract are added to obtain final concentrations such as 20, 40, 60, 80 and 100 µg/mL. A control containing the reaction mixture without extract is prepared, while diclofenac sodium is used as the standard/reference drug at corresponding concentrations. The reaction mixtures are incubated and then heated under controlled conditions to induce protein denaturation. After cooling, the absorbance is measured at approximately 660 nm. The percentage inhibition of albumin denaturation is calculated by comparing the absorbance of the treated samples with that of the control. A concentration-dependent increase in percentage inhibition indicates anti-inflammatory potential. A published N. cadamba study used 0.2 mL egg albumin, 2.8 mL PBS and 2 mL of test solution to obtain final extract concentrations of 20–100 µg/mL.[18,]

Synthesis of TiO_2 nanoparticles

For synthesis of TiO_2 nanoparticles, 500 mg of the prepared N. cadamba leaf extract was transferred into a 250 mL beaker and maintained at 60°C under continuous magnetic stirring. A 0.1 M titanium precursor solution was prepared by dissolving 2.84 g of titanium(IV) isopropoxide (TTIP) in 100 mL isopropanol. Alternatively, titanium(IV) oxysulfate may be used as the precursor. The titanium precursor solution was added dropwise (approximately 1 mL/min) to the 50 mL leaf extract with continuous stirring. The mixture was maintained at 60–70°C for 2 hours. The phytochemicals present in the leaf extract, particularly phenolic and flavonoid compounds, acted as reducing, complexing and stabilizing/capping agents during nanoparticle formation.

The pH was adjusted to approximately 8–9 using 0.1 M NaOH to promote hydrolysis and precipitation of titanium hydroxide intermediates. The resulting suspension was allowed to stand for 12 hours to complete nanoparticle formation. The obtained precipitate was separated by centrifugation at approximately 10,000 rpm for 15 minutes and washed three times with distilled water followed by one wash

with ethanol to remove unreacted materials and residual plant constituents. The washed precipitate was dried in a hot-air oven at 60°C for 12 hours to obtain crystalline TiO_2 nanoparticles. [19]

Characterization of biogenic TiO_2

UV–Visible spectroscopy

The phyto-reduction of sodium selenite by extract of is monitored by observing the colour change. After the formation of the deep brown colour, using a UV-Visible spectrophotometer, the absorbance of the nanoparticles is determined at various wavelengths running from 200 to 800 nm, with 1 nm wavelength intervals.[20]

Zeta-potential analysis of TiO_2

The hydrodynamic size of particles is calculated using this method by beaming a monochromatic light into the solution and observing the resulting Doppler shift. Once the laser light hits the moving particles, it changes the wavelength of the incoming light and scatters the light at an angle. This angle of scattered light is inversely propositional to the size of the particle. The size distribution of nanoparticles is monitored using Zetasizer (Nano ZS, Malvern instrument, United Kingdom). The Zeta-potential of the nanoparticles is measured to find the surface charge on it.

XRD Analysis

The almost close crystalline nature of NPs was analysed by XRD, and data were collected on the 2θ range from 5º–80º. Sample Characterization X-ray diffraction (XRD): The sample was measured by powder X-ray diffractometer instrument(XPERT-PRO) in the 2θ range of 10° -70°.The measurement was performed on a diffractometer operated at a voltage of 40 KV and a current of 30 mA using Cu Kα radiation at θ-2θ geometry. X′ pert high score software with search and match facility were used to identify different phases present in the synthesized samples, The synthesized nanoparticles were studied at voltages of 30 kV and 20 mA current with a scan rate of 0.030/s using Cu Kα radiation (λ = 1.5406 Å).

EDS Analysis

EDS was carried out to ascertain the elemental composition of NPs. Energy dispersive X — ray chemical analysis (EDS) Interaction of multiple-step droplet includes the logical strategy (EDS) that is customarily applied for optical investigation or synthetic depiction of a specimen. It relies on a relationship of some wellspring of X-beam stimulation.[21]

Particle size determination

Dynamic Light Scattering (DLS) principle was used to measure the particle size distribution of synthesized nanoparticles using a Malvern Zetasizer Nano ZS (Malvern Panalytical, UK). It was then diluted with deionized water to achieve the desired concentration of nanoparticles within the sample (to avoid multiple scattering), before MBS measurements were taken. The diluted sample was pipetted into a disposable polystyrene cuvette and measured at 25 °C where a laser beam illuminated the sample, scattering light changes due to Brownian motion of nanoparticles were observed. The measured diffusion coefficients were automatically converted into hydrodynamic particle size and polydispersity index (PDI) by the instrument software.

Formulation of topical gel

All the ingredients were collected according to the formula the given in table . Required amount of gelling agents HPC, Polaxomer and Sodium CMC were added in water with constant stirring at 500 rpm for about 2 hours. Drug was added to the above mixture. Glycerin, propylene glycol, methyl paraben and propyl paraben was added to it. Final weight was made with water. All the samples were allowed to equilibrate for 24 h at room temperature prior to performing evaluation test.[22]

Table 1: Formulation development for topical gel

No.	Ingredients %	Formulation Code								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	TiO_2 NPs	10	10	10	10	10	10	10	10	10
2	HPC	4	6	8	-----	-----	-----	-----	-----	-----
3	Polaxomer	-----	-----	-----	15	20	25	-----	-----	-----
4	Sodium CMC	-----	-----	-----	-----	-----	-----	4	6	8
5	Glycerin	1	1	1	1	1	1	1	1	1
6	Propylene glycol	1	1	1	1	1	1	1	1	1
7	Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

8	Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
9	Water	QS	QS	QS	QS	QS	QS	QS	QS	QS

Evaluation of gels

The formulated gels were examined for their physical properties, rheological properties and wound healing activity.[23]

Homogeneity

The gels were examined for their physical properties like color, clarity and phase separation by visual inspection. They are tested for the presence of any aggregates.

pH measurement

The pH of gel formulations were determined by using digital pH meter. 1gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation is done in triplicate and average values are calculated and reported.

Spredability

Concentric circles of different radius were drawn on graph paper and a glass plate was fixed onto it. 5gms of gel was placed on the centre of the lower plate. Another glass plate of 100±5 gm was placed gently on the gel and the spread diameter was recorded after 1 minute of each addition.

Extrudability

The gel formulations were filled in collapsible tubes. After being set in the containers, the extrudability of gel formulations was determined in terms of weight required in grams to extrude 0.5 cm. ribbon of gel in 10 sec.

Drug content

1 g gel was dissolved in 100 ml of phosphate buffer pH 5.8. Suitable dilutions were made using phosphate buffer pH5.8. Absorbance was measured using UV spectrophotometer.

In-vitro drug release study

In-vitro drug release studies were carried out using Franz diffusion cell. 0.5 g of gel was applied on cellophane membrane as donor compartment. Phosphate buffer pH 5.8 was placed in the receptor compartment as the dissolution medium. The whole assembly was place on magnetic stirrer with thermostat maintained at 37°c. samples were collected regular time interval and sink conditions were maintained by replacing with new buffer solution. Collected samples are analyzed using UV spectrophotometer.[24]

In vitro wound healing activity

Hydroxyproline assay for in vitro wound-healing activity

The hydroxyproline assay is used to estimate collagen production, which is an important indicator of tissue repair and wound healing. Hydroxyproline is a characteristic amino acid of collagen, and its estimation is therefore commonly used as an indirect measure of collagen synthesis. The colorimetric reaction involves oxidation of hydroxyproline by chloramine-T, followed by reaction with Ehrlich's reagent (p-dimethylaminobenzaldehyde) to produce a colored chromophore that can be measured spectrophotometrically around 550–560 nm.

For the in vitro study, HaCaT keratinocytes or human dermal fibroblasts are cultured in an appropriate complete culture medium and seeded into sterile culture plates. After the cells reach suitable confluence, they are divided into four groups: untreated control, optimized gel, standard drug, and marketed gel. The optimized gel, standard drug, and marketed gel are applied at predetermined non-cytotoxic concentrations. After an appropriate incubation period, cells from each treatment group are harvested and processed for hydroxyproline estimation. The cellular samples are hydrolyzed to release amino acids, including hydroxyproline. A validated hydrolysis procedure may use alkaline or acid hydrolysis; the hydroxyproline assay literature describes hydrolysis followed by chloramine-T oxidation and Ehrlich's reagent color development.

The hydrolyzed samples are appropriately neutralized and reacted with chloramine-T reagent and allowed to stand at room temperature for approximately 20 min. Ehrlich's reagent containing p-dimethylaminobenzaldehyde is then added, followed by incubation at approximately 60–65°C for 20 min to develop the characteristic chromophore. After cooling, absorbance is measured spectrophotometrically at approximately 550–560 nm against the reagent blank. Hydroxyproline standards are processed in the same manner to construct a standard calibration curve, and the hydroxyproline content of each sample is calculated from the corresponding absorbance. Samples should preferably be analyzed in triplicate.[25]

3. Result

Determination of Percent yield of extract

The maximum percent yield was obtained methanolic extract which was 16.2%

Quanlitative phytochemical screening

Three important secondary metabolites were extracted from the dried powdered material of extract and estimated. The content estimated was of total Phenols (14.74mg/ml) and of Flavonoids (19.91mg/100mg).

In vitro antioxidant activity by Superoxide radical scavenging activity (% inhibition)

Superoxide radical is known to be very harmful to cellular components and leads to tissue damage and various diseases. It plays an important role in the formation of other reactive oxygen species such as hydroxyl radical, hydrogen peroxide, or singlet oxygen in living systems.

Table 2: Superoxide radical

scavenging activity (% inhibition)

Concentrations (µg/ml)	VIT C	Plant extract
20	27.12±1.04	23.18±1.32
40	48.19±2.01	31.17±1.29
60	63.01±1.38	53.12±1.36
80	71.50±2.17	61.54±2.02
100	78.43±1.78	76.12±1.68

The antioxidant constituents present in the flower extract, particularly phenolic and flavonoid compounds, may scavenge free radicals and reduce oxidative damage at the wound site. This antioxidant effect can help protect fibroblasts, keratinocytes, and other cells involved in tissue regeneration, while also supporting collagen synthesis, cell proliferation, and re-epithelialization. Thus, the observed antioxidant activity of the flower extract provides supportive evidence for its wound-healing potential by helping to control oxidative stress and creating a favorable environment for tissue repair.

In vitro anti inflammatory activity

Table 3: Inhibition of protein/albumin denaturation assay(% inhibition)

Concentration (µg/mL)	% Inhibition – Leaf extract	% Inhibition – Diclofenac
20	23.72 ± 1.12	33.42 ± 1.05
40	34.35 ± 1.26	44.05 ± 1.18
60	45.54 ± 1.31	51.58 ± 1.24
80	60.26 ± 1.42	61.39 ± 1.37
100	71.75 ± 1.36	72.29 ± 1.31

The inhibition of protein/albumin denaturation assay and proteinase inhibition assay provide useful in-vitro evidence supporting the anti-inflammatory potential of the flower extract, which can be correlated with its wound-healing activity. During tissue injury, inflammation is an important early phase of wound healing; excessive inflammation can promote protein denaturation, proteinase-mediated tissue degradation, oxidative stress, and prolonged tissue damage, thereby delaying subsequent repair. The flower extract's ability to inhibit albumin/protein denaturation indicates a protective effect against inflammation-associated alteration of cellular proteins.

Green synthesis of flower extract loaded TiO_2 nanoparticles

The phytochemicals present in the plant extract, particularly phenolic and flavonoid compounds, facilitate the conversion of the titanium precursor into TiO_2 nanoparticles and help stabilize the newly formed particles. The formation of nanoparticles was indicated by the development of a milky-white colloidal suspension. The resulting suspension was centrifuged, washed several times with distilled water/ethanol, and dried to obtain the green-synthesized TiO_2 nanoparticle powder.

Characterization of Green synthesis of flower extract loaded TiO_2 nanoparticles

UV-Visible spectroscopy

The absorption maxima were found to be at 352 nm. Excitation of the surface plasmon resonance led to the development of a solution with a whitish colour, which indicated that titanium isopropoxide had been converted into elemental titenium. The phenolics, flavonoids, and alkaloid found in extract are responsible for the reduction of titanium isopropoxide .

XRD analysis

XRD studies were used to determine the structure of the synthesised NPs , which shows broader peak without any sharp Bragg's peaks and thus, it states that phytofabricated NPs are undoubtedly amorphous in character. The XRD pattern of the flower extract-loaded nanoparticles demonstrates a broad diffraction pattern rather than sharp, narrow peaks. The major diffraction feature centered approximately in the 27–30° 2θ region, together with weaker features at higher angles, is consistent with the formation of elemental nanoparticles.

Zeta potential of TiO_2

The current study found that the Zeta potential for nanoparticles has a distinct peak at -24.4 mV, indicating that the surface of nanoparticles is negatively charged. Reducing agents (phenolics, flavonoids, and alkaloid) in the flower extract of are responsible for providing nanoparticles with their negative charged potential value. As a result of their negative electrostatic forces, nanoparticles tend to remain in a dispersed state.

EDX spectrum of phytofabricated TiO_2 nanoparticles

The EDX spectrum of phytofabricated nanoparticles prepared using *Neolamarckia cadamba* flower extract confirms the successful formation of TiO_2

nanoparticles. Prominent peaks corresponding to titanium (Ti) and oxygen (O) were observed, along with a minor carbon (C) signal attributed to phytoconstituents acting as capping/stabilizing agents. The characteristic Ti peaks around 4.5–4.9 keV and O peak around 0.52 keV support the presence of TiO_2. The Ti:O atomic ratio was approximately 1:2.05, which is close to the theoretical 1:2 ratio of TiO_2, confirming successful nanoparticle synthesis.

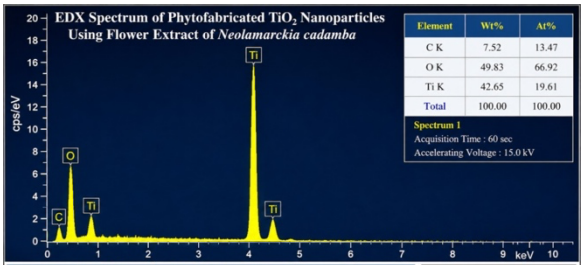

Figure 1: EDX spectrum of phytofabricated nanoparticles

Formulation and characterization of TiO_2 nanoparticles loaded topical gel

All the formulations showed good homogeneity with absence of lumps and grittiness and also Passed for other characterization parameters.

Table 4: Characterization of nanoparticles loaded topical gel

Code	Homogeneity	Appearance	pH	Spreadability (g·cm/s)	Extrudability (%)	Viscosity (cP)	Drug content (%)
F1	Good	smooth	6.21 ± 0.04	5.42 ± 0.12	78.4 ± 1.2	3850 ± 85	91.26 ± 0.84
F2	Good	smooth	6.28 ± 0.03	5.18 ± 0.10	80.1 ± 1.0	4120 ± 92	92.48 ± 0.76
F3	Good	smooth	6.34 ± 0.05	4.96 ± 0.14	81.6 ± 1.1	4380 ± 88	93.15 ± 0.69
F4	Good	smooth	6.18 ± 0.04	6.12 ± 0.11	84.2 ± 0.9	3650 ± 76	94.06 ± 0.72
F5	Very good	smooth	6.24 ± 0.03	5.84 ± 0.13	86.5 ± 1.2	3980 ± 81	95.18 ± 0.65
F6	Very good	smooth	6.31 ± 0.04	5.56 ± 0.12	87.8 ± 1.0	4260 ± 95	96.02 ± 0.58
F7	Very good	smooth	6.20 ± 0.03	6.48 ± 0.15	89.6 ± 0.8	4520 ± 87	96.74 ± 0.61
F8	Very good	smooth	6.27 ± 0.04	6.25 ± 0.13	91.2 ± 0.9	4780 ± 90	97.53 ± 0.54
F9	Excellent	smooth	6.35 ± 0.03	6.72 ± 0.12	93.8 ± 0.7	4950 ± 82	98.42 ± 0.46

In Vitro Drug Release study

Table 5: In vitro release of nanoparticle loaded topical gel

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
0	0	0	0	0	0	0	0	0	0
1	25.12	18.43	22.78	30.53	26.32	22.33	25.34	15.43	12.22
2	40.	30.	32	45	42	35	38	28	25

	32	23	.45	.89	.73	.54	.23	.73	.21
3	55.56	40.67	43.57	58.78	55.98	48.76	50.67	38.97	35.53
4	68.12	50.53	55.89	75.43	67.23	60.53	62.51	48.74	43.88
5	78.32	65.54	68.78	88.58	78.53	72.76	76.23	62.76	55.78
6	88.54	78.89	80.05	99.98	88.76	84.92	85.64	72.57	62.56
7	94.67	85.65	87.12	—	95.67	91.21	90.49	80.89	70.71
8	98.88	92.98	92.21	—	100.0	96.33	95.74	88.99	80.22
9	100.02	100.01	97.98	—	—	99.97	100.0	99.98	86.21
10	—	—	100.0	—	—	—	—	—	93.98
12	—	—	—	—	—	—	—	—	99.97

In vitro drug release kinetics

F9 considered the best formulation for sustained drug release based on its overall release-kinetic profile. Most importantly, F9 exhibited the highest Korsmeyer–Peppas R^2 value of 0.998, indicating the strongest fit to this model among all formulations. The corresponding release exponent (n = 0.988) indicates a release mechanism approaching Case-II transport, suggesting that polymer relaxation/swelling and drug diffusion contribute substantially to the release process.

Table 6: *In vitro* drug release kinetics of topical gels

Formulation codes	R^2 values of Kinetic Models					
	Zero order	First order	Higuchi	Hixson Crowell	korsmeyer peppas	Slope (n)
F1	0.942	0.913	0.987	0.606	0.975	0.764
F2	0.935	0.923	0.984	0.607	0.974	0.855
F3	0.955	0.913	0.984	0.65	0.984	0.979
F4	0.995	0.803	0.997	0.574	0.991	0.711
F5	0.994	0.85	0.995	0.729	0.998	0.714
F6	0.992	0.815	0.996	0.531	0.992	0.757
F7	0.976	0.878	0.993	0.569	0.994	0.853
F8	0.977	0.861	0.991	0.54	0.993	0.898
F9	0.988	0.994	0.995	0.502	0.998	0.988

Hydroxyproline assay for in vitro wound-healing activity

The optimized F9 gel showed the highest hydroxyproline content **(**35.92 ± 1.24 µg/mg tissue**)** compared with the control, Optimized gel (F9), standard drug, and marketed gel. The increased hydroxyproline level indicates enhanced collagen synthesis and deposition, suggesting improved extracellular-matrix formation and supporting the wound-healing potential of the optimized gel. The result was higher than that of the marketed formulation and standard drug, indicating that F9 may provide enhanced wound-healing activity.

Table 7: Hydroxyproline assay for in vitro wound-healing activity

Treatment group	Dose/concentration	Hydroxyproline content (µg/mg tissue)	% Increase vs. control
Normal control	—	18.42 ± 0.86	—
Standard drug (Dexpanthenol)	1% w/w	32.76 ± 1.12	77.85
Marketed gel (Dexpanthenol)	1% w/w	29.84 ± 1.05	62.00
Optimized gel (F9)	1% w/w	35.92 ± 1.24	95.01

Conclusion

The present study successfully demonstrated the potential of *Neolamarckia cadamba* leaf extract for the green synthesis of titanium dioxide nanoparticles (TiO_2NPs). The use of plant extract provides an environmentally friendly and comparatively simple alternative to conventional nanoparticle synthesis methods. The phytoconstituents present in the leaf extract, particularly phenolic and flavonoid compounds, may have contributed to the formation and stabilization of the synthesized nanoparticles. The successful synthesis was supported by physicochemical characterization of the prepared TiO_2NPs. In conclusion, F9 was selected as the best and optimized TiO_2 nanoparticle-loaded topical gel formulation because of its satisfactory physicochemical characteristics, favorable release behavior, and superior in vitro wound-healing activity. The study indicates that *Neolamarckia cadamba* leaf extract-mediated green synthesis of TiO_2 nanoparticles can serve as a promising and environmentally sustainable approach for developing topical wound-healing formulations. Further in vivo studies, toxicity evaluation, long-term stability studies, and clinical investigations are recommended to confirm the safety and therapeutic efficacy of the optimized F9 formulation.

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