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Research

Development And Evaluation Of Nanosponge Loaded Topical Herbal Gel Of Wrighia Tinctoria

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Abstract

Psoriasis is the prevailing chronic autoimmune disorder. Nevertheless, the topical application of this substance is hindered by frequent adverse effects such as skin shrinkage, steroidal acne, hypopigmentation, and allergic contact dermatitis. The primary aim of this work was to create topical gels of Wrightia tinctoria extract utilizing a cross-linker and polymer through the solvent emulsion diffusion method. It is employed in the treatment of Psoriasis. The nanosponges were produced using the solvent emulsion diffusion technique and assessed for Fourier transform infrared spectroscopy (FTIR) analysis, particle size determination, polydispersity index measurement, zeta potential measurement, drug content analysis, scanning electron microscopy examination, in vitro dissolution analysis, and stability assessment. The drug release and entrapment efficiency of all nanoparticles were assessed using in vitro methods. The tailored nanospheres were included in the gel to create a nano topical gel. All gel formulations underwent evaluation experiments to assess homogeneity, viscosity, spreadability, pH, and in vitro properties. The gels that were synthesized exhibited transparency, favorable viscosity, and spreadability. SEM images verified that the produced formulation exhibited a mostly spherical shape and possessed porosity. The drug release in the in vitro diffusion experiments was found to be 92.15% within a 24-hour period. The study indicates that the extract contains the active components for treating psoriasis, and the topical gel made from this extract has a notable impact in delivering the medication over a prolonged period of time.

Keywords: Hypopigmentation, Nanosponges, skin atrophy, Psoriasis, Topical gel, Wrightia tinctoria.

INTRODUCTION

Psoriasis is the prevailing chronic autoimmune disorder. Nevertheless, the topical application of this substance is hindered by frequent adverse effects such as skin shrinkage, steroidal acne, hypopigmentation, and allergic contact dermatitis. The primary aim of this work was to create topical gels of Wrightia tinctoria extract

utilizing a cross-linker and polymer through the solvent emulsion diffusion method. It is employed in the treatment of Psoriasis.

Nanotechnology

In last century, Drug delivery research has established to be innovative in the foundation of nano-drug delivery systems. Improvements in a range of medication delivery systems, both in the educational and scientific fields as well as in industrial and commercial administrations, have been a vital role in facilitating the new establishment. Data was largely created as a consequence of a revolutionary development that incorporated numerous scholarly articles and multiple patented investigations from around the world [1].

Nanoparticle drug delivery systems are engineered technologies that employ nanoparticles to deliver therapeutic medications with pinpoint accuracy and precision. A modern pharmaceutical delivery method should minimise side effects while reducing dosage and frequency. Nanoparticles have a lot of value because of their medication delivery potential. Nanoparticle medication delivery aims to improve pharmacological efficacy while lowering cytotoxicity. While fine-tuning nanoparticle properties for effective pharmaceutical delivery, the following challenges must be addressed. The surface-area-to-volume ratio of nanoparticles can be altered to facilitate enhanced ligand binding to the surface. Increased ligand binding efficiency can lower dosage and lessen nanoparticle toxicity. The mass of nanoparticles per mass of drug is reduced when the dosage or frequency is reduced, resulting in greater efficiency. Traditional pharmacological therapy has a number of drawbacks, including a lack of selectivity, limited efficacy, poor biodistribution, and a short duration of action [2].

Nanotechnology is an imperative part of engineering revolution since the industrial age. Nanotechnology resulted in multifarious formulations like nanoparticles, nanocapsules, nanospheres, nanosuspensions, nanocrystals, nano-erythrosomes etc. Nanoparticles are a newer development which is accessible in several forms like polymeric nanoparticles, solid-lipid nanoparticles, nanoemulsions, nanosponges, carbon nanotubes, micellar systems, dendrimers etc. [3]

Nanosponges

Nanosponges are tiny mesh like nonporous particular structure in which a large variety of substances can be encapsulated or suspended and then be incorporated into a dosage form. They have a proven spherical colloidal nature, reported to have a very high solubilization capacity of poorly soluble drugs by their inclusion and non-inclusion behavior. Recently nanosponges have been developed and proposed for delivery of poorly soluble drug to increase their bioavailability and prolonged release. Nanosponges are able to encapsulate both hydrophilic and lipophilic drug molecules because of their inner hydrophobic cavities and external hydrophilic branching, thereby offering unparalleled flexibility. Nanosponges are more like a 3 dimensional network or scaffold. The backbone is a long length of polyester which is mixed in solution with small molecules called crosslinkers that act like tiny grappling hooks to fasten different parts of the polymer together. The Nanosponge technology put frontward the elegant of excipients and is believed to situate in towards lower side effects, enhanced stability, improved elegance and enhanced formulation flexibility. Sponges are non-mutagenic, non- allergenic, non-irritating, and non-toxic. Nanosponges are miniature mesh-like structures. As compare to supplementary NPs, sponges are insoluble in water and organic solvents, porous, harmless and stable at elevated temperatures up to 300ºC. In the commencement, nanosponge drug delivery system emerged only as a topical system, but in the 21st century, nanosponges can be taken by oral as well as IV route.

Nanosponge drug delivery system colloidal transporters have been newly forwarded for drug delivery, since their application can dissolved poorly water-soluble drugs and give prolonged discharge, as well as enhance the actives bioavailability by changing the pharmacokinetic traits of actives.[4]

Nanosponges are poised of hypercrosslinked cyclodextrin linked in a 3-D network. Nanosponges form spongy NPs with sizes less than 500 nm, so they effortlessly travel in the systemic circulation. As ‘sponges’, they can soak up toxins, fragments and secretions obtained by cancerous cells themselves. Their globular shape and negative surface charge give them a good capacity for embedding small molecules, ions, gases and macromolecules within their structure. Therefore, NSGs have been intended to enhance chemotherapeutic effectiveness by targeting drug-resistant cells. The erythrocyte membrane can be employed as a cloak consisting of >3,000 nanosponges. Once they are entirely embedded with toxins, nanosponges are safely inclined of by the liver with lesser toxicity. Consequently, which are designed to work with any category of cancer or poisoning that demonstrates dysregulation of, or abnormalities in, cellular membranes which is depicted in Fig 1.

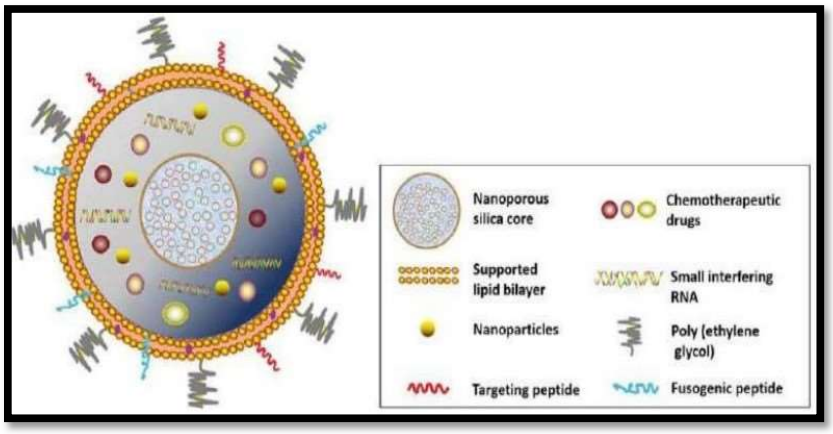

Fig 1: Lipid bilayer-wrapped nanoporous drug delivery system in protocells.

Mechanism of drug release from nanosponges

The sponge particles have an open structure, and the active moiety is free to move in and out from the particles and into the vehicle until equilibrium is reached. In case of topical delivery, once the finished product is applied to the skin, the active that is already in the vehicle will be absorbed into the skin, depleting the vehicle, which will become unsaturated, therefore disturbing the equilibrium. This will start a flow of the active from the sponge particle into the vehicle and from it to the skin until the vehicle is either dried or absorbed. Even after that the sponge particles retained on the surface of stratum corneum will continue to gradually release the active to the skin, providing prolonged release overtime. [5]

Epidermis

Epidermis is the most superficial layer of the skin and is composed of Stratified epithelium which varies in thickness in different parts of the body. It is thickest on the palms of the hands and soles of the feet. Epidermis is approximately 0.4-1.5 mm in thickness compared with the 1.5-4mm full thickness of skin. Epidermis is the cellular external layer of the skincomposed keratinocytes but it also contains other cells. The cells in the epidermis include. Epidermis is generally main target of most skin problems. It is made up of several layers. It protects the skin from bacterial and fungal attacks as well as from mechanical injury. The epidermis is composed of 4 or 5 layers, depending on the region of skin being considered.

Aim and objectives

The aim of present work was Formulation, Development and Evaluation of Nanosponges for Topical Application. To formulate and characterize drug loaded nanosponges by emulsion solvent diffusion method. To evaluate prepared nanopsonges by Percentage yield, % Entrapment Efficiency, % Drug release and particle size. To prepare and optimize drug loaded nanosponges based hydrogel. To evaluate prepared nanopsonges based hydrogel with respect to compatibility study by FTIR, DSC analysis, particle size distribution analysis, zeta potential, entrapment efficiency, SEM analysis & P-XRD etc. To study in vitro release studies. To study stability of optimized nanosponges based hydrogel as per ICH guidelines.

MATERIALS & METHODS

Pharmacognostic Investigations

Plant Material

Based on the ethanomedical information and literature survey the plants selected for the dissertation work were

- Wrightia tinctoria R.Br. (Family: Apocynaceae)

Collection of Plant Material

The plant *Wrightia tinctoria* leaves was collected from the dense forest areas of Tirupathi, Tirupathi district, Andhra Pradesh. The fresh leaves were collected in February 2023, shade dried and coarsely powdered. The *Wrightia tinctoria* was identified and authenticated by Dr. K. Madhava Shetty, Department of Botany, Sri Venkateshwara University, Tirupati, 517 502, Andhra Pradesh.

Fig 2: Image of *Wrightia tinctoria*

Preparation of the *Wrightia tinctoria* Extract

The *Wrightia tinctoria* leaves powder of 0.20 kg was subjected to Soxhlet extraction using 0.65 liters of ethanol. The leaves extract was collected, concentrated and stored. The yield obtained for *Wrightia tinctoria* leaves methanol extract was 0.030 Kg.

Physical characteristics

By visual examination the *Wrightia tinctoria* ethanol extract was tested for its physical characters like colour, odour and texture.

Solubility test

Wrightia tinctoria Ethanol extract (about 1mg) was taken in a test tube and solubility in ethanol, water, dichloromethane and chloroform was tested.

Preparation of Buffer Solutions

Phosphate buffer pH 6.8: An accurately weighed quantity of 28.80gm of disodium hydrogen phosphate and 11.45gm of potassium dihydrogen phosphate was dissolved in sufficient water to produce 1000ml.

Preparation of stock solution

The standard stock solution of ethanol extract of *Wrightia tinctoria* was prepared by transferring accurately weighed quantity (10 mg) of *Wrightia tinctoria extract* in 100 ml of volumetric flask. The extract was dissolved in few ml of ethanol and the volume was made up to 100 ml with ethanol to get a stock solution of 100 µg/mL.

Selection of Wavelength

The standard stock solution was scanned in the range of 400 to 800 nm in UV spectrophotometer using phosphate buffer pH 6.8 as blank. The absorption maximum was found at 288 nm.

Construction of calibration curve of *Wrightia tinctoria*

From the standard stock solution of *Wrightia tinctoria* 2, 4, 6, 8 and 10 ml were withdrawn to 10 ml volumetric flask and then made up volume with phosphate buffer pH 6.8 to get a concentration range of 2-10 µg/mL. The absorbance of these solutions was measured at 418nm using JASCO V-530 UV 1600 UV- visible spectrophotometer. Phosphate buffer pH 6.8 was used as blank. The calibration curve was plotted between concentration and absorbance [6].

Drug Excipient Compatibility Studies

FT-IR spectrum of extraction was recorded using FT-IR Spectro photometer (Shimadzu JASCO 4100). The diffuse reflectance technique was utilised in the mid IR 4000-400 cm-1 spectral region. The procedure consist of dispersing the sample in KBr(100mg) using a mortar, triturating the materials into a extraction bed into the holder using compression gauge. The pressure was around 5 tons for 5 minutes. The pellet was placed in the light path and the spectrum was recorded. The characteristic peaks of the functional groups were interpreted. The FTIR spectrum of *Wrightia tinctoria ethanol extract*, polymers ethyl cellulose and Dimethyl carbonate were recorded

and to check for their compatibility.

Formulation of Silymarin Nanosponges by Emulsion Solvent Diffuion Method

Emulsion solvent diffusion method was used to formulate *Wrightia tinctoria ethanol extract* loaded nanosponges by using a suitable polymer. Dispersed phase consists of specified amount of drug and polymer which was dissolved in 20 ml of an organic solvent, dichloromethane. Aqueous phase consists of specified amount of Dimethyl carbonate dissolved in 100 ml distilled water. Disperse phase was added drop by drop into aqueous phase by stirring on magnetic stirrer at 1000 rpm for about 2 hours. The nanosponges formed were collected by filtration and dried in oven at 40°C for about 24 hours. They were then kept in the vacuum desiccators to remove the residual solvent. The *Wrightia tinctoria* nanosponges were formulated using polymers ethyl cellulose [7].

Characterization of Nanosponges

FTIR Spectroscopy of Nanosponges

Before formulating a drug substance into dosage form, it is essential that it should be chemically and physically compatible. Compatibility studies give information needed to define the nature of the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the fabrication of a dosage form. This study was carried out by using infrared spectrophotometer to find if there is any possible chemical interaction between the *Wrightia tinctoria* and polymers.

A few mg of sample (*Wrightia tinctoria* nanosponges) was weighed and mixed with 100 mg of potassium bromide (dried at 40-50°C). The mixture was taken and compressed under 10- ton pressure in hydraulic press to form a pellet. The pellet was scanned from 4000-400 cm-1 in IR spectrophotometer.

Entrapment efficiency (EE)

Determining the drug amount embedded in NS is of major significance, because it determines the release characteristics and consequently the therapeutic potency. To calculate the EE, accurately weighed amount of NS (150 mg) was dissolved in ethanol, sonicated for 15 min to break the NS complex and for complete solvation of extract followed by centrifugation. The obtained supernatant was then filtered, diluted suitably using 6.8 pH phosphate buffer solution (PBS), and analyzed using the UV-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 418 nm. All measurements were performed in triplicate under ambient environmental conditions. EE was calculated by using the below mentioned formula:

$$\text{Entrapment efficiency (\%)} = \frac{Initial\ amount\ of\ drug\ added - Drug\ amount\ in\ supernatant}{Initial\ amount\ of\ drug\ added} X100$$

Preparation of NS based gel

Precisely weighed amount of Carbopol-934 was soaked in water (around 5 mL) for 2 h and neutralized with triethanolamine (TEA) and stirred continuously. Guar gum and drug loaded NS (equivalent to topical doses of drugs) were dissolved in propylene glycol. This mixture was then transferred to the carbopol mixture and mixing was done for further 20 min. The dispersion was kept aside for 60 min, for complete hydration and swelling of gel components. Before performing viscosity studies, all the prepared gel samples were allowed to equilibrate for at least 24 h at room temperature [24]. Table Table22 represents formulation chart of prepared NS based gel formulations.

Table 1: Effect of *Wrightia tinctoria*-Gel formulation.

S.No.	Formulation	Carbopol 934 (%)	PG (mL)	TEA (mL)	Water
1	G1	0.5	0.5	1	Q.S
2	G2	1.0	0.5	1	Q.S
3	G3	1.5	0.5	1	Q.S
4	G4	-	0.5	1	Q.S
5	G5	-	0.5	1	Q.S
6	G6	-	0.5	1	Q.S
7	G7	1.0	0.5	1	Q.S

Characterisation of *Wrightia tinctoria*-NSG

Visual Observation

The prepared carbopol gel was inspected visually for its colour, homogeneity, grittiness, and syneresis [8].

Viscosity Determination

The prepared gel viscosity was determined using a programmable Viscometer. The carbopol gel prepared was taken in a beaker wherein the T-bar spindle (spindle-C, S-96) was immersed at 90° such that the spindle and the base of the beaker are not in contact. The speed of rotation of the spindle was maintained at 50 rpm. The evaluation is made after a duration of 30 s post which the *Wrightia tinctoria*-NSG system prepared is stabilized [9].

pH Determination

The pH meter was used to determine the formulation pH. In a known quantity of distilled water (100 mL), the prepared *Wrightia tinctoria*-NSG was dissolved and kept for 2 h. The electrode was then immersed in the mixture produced and observed at room temperature in triplicate [10].

Spreadability

Spreadability (g·cm/s) is referred to the time in sec required by two slides to slip over the *Wrightia tinctoria*-NSG placed between them upon the influence of external stimuli. The glass slides of 7.5 cm in length were employed along with the load on the upper plate being 20 g [11]. Spreadability was evaluated using the formula below mentioned:

$$\textbf{Spreadability} = \textbf{Weight (g)} \times \textbf{Lenght (cm) Time (s)}$$

Texture Analysis

The mechanical property of *Wrightia tinctoria*-NSG was evaluated with the help of software-texture analyzer TA (XT Plus Stable Micro System, UK equipped with 5 kg load cell). The sample weighing 100 mg was placed in the beaker cautiously to avoid air bubbles in it. The speed of the probe for analysis was fixed at 1.0 mm/s for pre-test, 2.0 mm/s for the test, and 10.0 mm/s for post-test analysis. The probe was immersed with a load cell capacity of 5.0 g and a distance of 10.0 mm. Data interpretation was undertaken using the Texture Exponent software installed within the equipment. The resultant force-time plot gave the values of different mechanical parameters [12].

The prepared *Wrightia tinctoria*-NSG (50 mg) was dissolved in 100 mL phosphate buffer pH 6.8 and shaken for 2 h. This move is intended to ensure optimum drug solubility during mechanical shaking. The solution was purified and spectrophotometrically quantified at 413 nm.

Scanning Electron Microscopy (SEM)

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared silymarin nanosponges. Nanosponges were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy (Hitachi X650, Tokyo, Japan) in different magnifications .Samples were placed on glass slide kept under vacuum and then by using sputter coater unit, samples were coated with a thin gold layer, operated at 15kv acceleration voltage [13].

Ex-Vivo permeation studies

A freshly excised hairless abdominal rat skin was selected and placed between the compartments of donor and receptor of Franz-type diffusion cells with an effective permeation area of 2 cm2 and with the stratum corneum facing the donor compartment. The receptor solution consisted of 200 mL of phosphate buffer pH 6.8 maintained at 37 ± 0.5 °C and continuously stirred with a magnetic bar at 60 rpm. Then, 1 mL of the cubosomal dispersions and *Wrightia tinctoria* aqueous solution was added to the donor compartment. Samples from the receptor compartment (1 mL) were withdrawn periodically over 24 h and analyzed for drug content spectrophotometrically at 413 nm. The 1 mL aliquots were substituted by an equal volume of phosphate buffer pH 6.8 maintained at 37 ± 0.5 °C. At the end of the experiment and in order to determine the amount of *Wrightia tinctoria* deposited in the skin, the rat skin was cleaned 5 times with a cotton cloth soaked in ethanol. The skin was then finely divided and immersed for 6 h in 6 mL of ethanol under constant stirring at room temperature. Extraction dispersions were centrifuged at 4000 rpm for 15 min and filtered through a 0.22 μm filter. Diffusion cells free of formula were also established. Samples collected from permeation of drug-free systems were used as a blank, and filtrates were studied at 413 nm using a spectrophotometer. The slope of the curve plotted for the cumulative amount of *Wrightia tinctoria* infused per unit area as a function of time was used to determine the drug steady-state flux (Jss) [14]. The permeability coefficient (kp) of *Wrightia tinctoria* through the skin from the investigated cubosomes was calculated as follows:

$$Kp = Jss/C$$

Where Jss: steady-state drug flux;
C: drug concentration in the donor compartment.

Stability study

A stability study was carried out for the optimized formulation as per International Conference on Harmonisation guidelines. The NS were placed in glass vials and stored at 25±2°/60±5 % RH and 40±2°/75± 5 % RH atmospheric conditions in a stability chamber (Macro Scientific Work Pvt. Ltd., Delhi, India) for a period of 1 mo. Samples were analysed for particle size, % EE and in vitro drug release after the stability period.

Plant authentication

Wrightia tinctoria leaves were collected from dense forest areas of Tirupathi, Tirupathi district, Andhra Pradesh. The authentication of the plant was done by Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, 517 502, Andhra Pradesh. The Voucher specimens (SVU/2023/3324) were deposited in the institutional museum, College of Pharmaceutical Sciences, SV University, Tirupati.

RESULTS & DISCUSSION

Wrightia tinctoria leaves have been investigated in a systematic way covering phytochemicals characterization and pharmacological studies. Literature survey revealed that no work was done on this plant. Therefore, it was thought worthwhile to carry out the phytochemical characterization and pharmacological studies on this plant.

Preliminary phytochemical screening of selected plant results shown in Table 1. The phytochemical screening of *Wrightia tinctoria* water, Hexane, ethyl acetate, pet. Ether, methanol and ethanol extract showed the presence of alkaloids, tannins, steroids, purines, carbohydrates, proteins (Table 1). The extracts of plant of *Wrightia tinctoria* was subjected to qualitative phyto-chemical screening to identify the phyto constituents present [15].

Present study deals with qualitative analysis of leaves extract of *Wrightia tinctoria* Table no. 1 shows the results of phytochemical analysis of leaves of *Wrightia tinctoria* water extract of leaves of *Wrightia tinctoria* shows the presence of Steroid, Saponin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Flavonoids whereas Tannin, Anthocyanin, Emodins, Proteins, Phytosterol, Phlobatannin, Leuco-anthocyanin and Cardial Glycosides were absent. Ethanol extract of leaves of *Wrightia tinctoria* shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Diterpenes, Phenol and Flavonoids whereas Emodins, Proteins, Amino acids, Phytosterol, Phlobatannin, Leuco-anthocyanin and Cardial Glycosides were absent. Methanol extract of leaves of *Wrightia tinctoria* shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Phlobatannin whereas Emodin's, Proteins, Phytosterol, Leuco-anthocyanin, Cardial Glycosides and Flavonoids were absent.

Different phytochemicals present in all the extracts were identified. Results obtained were also given in table. From the results, it can be concluded amino acids, alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, triterpenoids are present in the extract.

Table 2: Phytochemical test for *Wrightia tinctoria*

Phytochemical test	Extractions					
	Water	Hexane	Ethyl acetate	Pet. ether	Methanol	Ethanol
Carbohydrates						
Molish's test	-	+	+	-	+	+
Fehling's test	-	-	-	+	+	+
Barfoed's test	-	-	-	-	+	-
Benedicts's test	-	+	+	-	+	-
Proteins &Amino acids						
Millions test	+	-	+	+	+	+
Biurette test	+	-	+	-	-	+
Ninhydrin test	+	-	-	+	+	+
Fats &fixed oils						
Saponification test	-	-	-	-	-	-
DETECTION OF SECONDARY METABOLITIES						
Alkaloids						
Mayers test	+	-	-	+	+	-
Wagners test	+	-	-	-	+	+
Dragondroffs test	+	-	-	-	+	+
Hagers test	+	-	+	+	+	-
Steroids & terpenoid's						

Libermann-Burchard's Test	+	+	+	+	+	+
Salkowsky's Test	+	-	-	-	+	+
Phenolic compounds & Tannins						
Ferric Chloride Test	-	-	-	+	-	-
Lead Acetate Test	+	+	+	+	+	+
Bromine water test	+	-	+	-	-	+
Flavonoids						
Shinoda test	+	-	+	-	+	+
Alkaline reagent test	+	-	+	-	+	+
Saponin Glycosides						
Foam test	-	+	-	-	-	-
Glycosides						
Borntrager's Test	-	-	-	-	-	-
Keller-Killiani Test	+	-	-	+	+	+

(+) denotes presence and (-) denotes absence

Phytochemical investigation is one of the excellent tools for the quality assessment of medicinal plants. Our study demonstrated presence of different groups of phytochemicals viz. alkaloids, terpenoids, glycosides, tannins, saponins, anthocyanins, steroids flavonoids, polyphenolics and lignans are present in water extracts of *Wrightia tinctoria.* The detected phytochemical compounds support that selected plant has medicinal importance. Many alkaloids and terpenoids isolated from medicinal plants demonstrate biological activities like, anti-inflammatory, antimalarial, anticancer, antimicrobial etc. Likewise, steroids isolated from plants are reported as cardio tonic effect and also have antibacterial, insecticidal properties. According to research, Tannins are known to possess antibacterial, antitumor and antiviral activities. Other phytochemicals such as glycosides flavonoids and polyphenolics have been used to treat immunomodulatory, antioxidant, congestive heart failure, anticancer and other biological activities [16]. We scrutinized that all the studied plant parts showed diverse groups of compounds which we extracted in different solvent extracts later and assessed their wound healing activity. The presence of different class of phytochemicals may be correlated with different biological properties of *Wrightia tinctoria.* (leaves).

Preformulation Studies

Ultraviolet (UV) spectrum

Development of an analytical method for estimation of *Wrightia tinctoria.* during development is a key task of preformulation studies. *Wrightia tinctoria.* has been in use in India for anti-psoriasis and as an anticoagulant, antifungal, and insect repellent. *Wrightia tinctoria.* containing multiple unsaturated bonds which make it absorb UV radiations and exhibit a spectrum in the range of 400-800 nm. The UV absorption spectrum of *Wrightia tinctoria.* was observed in water and buffers containing one percent sodium lauryl sulphate (SLS) as shown in figure 1. *Wrightia tinctoria.* was found to exhibit an absorption maximum (λmax) at 413 nm in methanol and buffer containing 1% SLS respectively [17].

Characterization of *Wrightia tinctoria*

In order to determine the genuineness and purity profile, the extraction was characterized by using various techniques, which includes:

UV-Visible Spectroscopy

Ultraviolet spectroscopy is the phosphate buffer used to determine the purity of the sample as it helps in determination of absorption maxima i.e., peak maxima of the drug at a particular wavelength. Thus, a scan of the drug dissolved in pH 6.8 buffer solution (1% v/v) was obtained and from the peak λmax (413 nm) was calculated which was found to be comparable with the reported λmax (415 nm) of the extract in the standard literature. Thus confirms the purity of the drug.

Calibration curve of Wrightia tinctoria (in methanol, phosphate buffer pH 6.8)

The UV absorption spectrum of *Wrightia tinctoria* is characterized by absorption maxima at 413 nm which is reported. The wavelength of 413 nm showing maximum absorption was selected as analytical wavelength for further study. The calibration curve of *Wrightia tinctoria* in methanol. The absorbance values with corresponding concentrations. It was found to be linear in the concentration range of 0-50 µg/ml, at 413 nm with coefficient of regression (R^2) value of 0.982.

Formulation of Nanosponges

The nanosponges possess a large surface area, which decorates them with desirable features for the application of *Wrightia tinctoria* methanolic extract and encouraging their uniform release. In addition, their small size promotes their encapsulation in dermatological products and facilitate comfortable topical application. Nanosponges present some benefits for topical delivery owing to their delayed and controlled drug release as well as enhancement of drug residence time in the skin. Other benefits of this system include protection of labile molecules from chemical degradation by UV light and a reduction in cutaneous irritation. Further, this nanoformulation can be easily entrapped in hydrophilic vehicles like hydrogels. Therefore, nanosponges loaded hydrogel has been proposed in the present investigation for topical delivery of *Wrightia tinctoria*. *Wrightia tinctoria* has poor water solubility (2.00 mg/L). These properties and structure of this molecule make it suitable for encapsulation in ethyl cellulose and PVA nanosponges. Taking all these considerations together, *Wrightia tinctoria* nanosponge loaded hydrogels have been fabricated to prevent UV photodegradation of this drug and to control its release, resulting in reduction of associated side effects. Additionally, solubilisation efficiency of *Wrightia tinctoria* was also expected to enhance by this delivery system.

Table 3: Composition of *Wrightia tinctoria* nanosponge batches

F.code	Drug (g)	PVA	Ethyl cellulose	Tween 80	Results
NSF1	1	100	100	2	Less yield
NSF2	1	100	200	2	Less yield
NSF3	1	100	300	2	Product obtained
NSF4	1	100	100	2.5	Product obtained
NSF5	1	200	100	2.5	Less yield
NSF6	1	300	100	2.5	Less yield

Characterization of Nanosponge

Entrapment efficiency (%), Drug content

Drug content was determined by centrifugation method. The redispersed nanosponge suspension was centrifuged at 15,000 rpm for 40 min at 25 ^{0}C to separate the free drug in the supernatant. Concentration of *Wrightia tinctoria* in the supernatant was determined by UV-Vis spectrophotometrically at 413 nm after suitable dilution.

Drug content = weight of drug in nanosponge / weight of nanosponge x 100

The Entrapement efficiency of Nanosponge dispersion was determined by centrifugation method. Nanosponge dispersion (containing an equivalent to 1 gm of *Wrightia tinctoria*) was centrifuged at 15000 rpm for 30min in a refrigerated centrifuge to collect the supernatant liquid. The collected liquid was filtered to measure the free drug concentration after suitable dilution with a fresh phosphate buffer saline pH 6.8.The absorbance was measured at 413 nm in a UV spectrophotometer to calculate the entrapement efficiency using the following formula:

% Entrapment efficiency = Wt.of drug incorporated/Wt.of drug initially taken x 100

The results were shown in Table 12 and Figure 19. It revealed that the entrapment efficiency and drug content were obtained in all the formulations. Among all the formulations that F6 showed 92.8% and F7 87.6% respectively. Drug content of F6 showed 90.6 and F7 showed 89.6 respectively.

F.Code	Drug Content	EE	PDI	Size	Zeta potential
NSF1	69.35±0.6	59.34±0.25	0.425±0.01	385.42±5.32	-24.51±0.25
NSF2	78.54±0.25	69.58±0.41	0.361±0.05	296.84±6.24	-23.61±1.24
NSF3	91.53±0.42	82.41±0.32	0.298±0.04	183.57±3.12	-28.94±1.36
NSF4	86.43±0.13	76.54±0.41	0.315±0.03	213.54±5.82	-31.24±2.01
NSF5	75.26±0.25	68.43±0.25	0.415±0.12	296.84±3.49	-26.57±1.15
NSF6	68.34±0.31	59.24±0.11	0.468±0.06	325.42±4.75	-22.64±2.14
NSF7	73.51±0.16	65.23±0.29	0.513±0.08	395.46±6.32	-19.84±2.03

Preparation and Evaluation of Wrightia tinctoria-NSG

Preliminary tests were conducted to determine the best formulation to prepare the optimum gel. Table 4 indicates a different carbopol composition. The Carbopol 940 composition ranged from 0.2% to 1.5% w/w. However, the compositions of other constituents remained constant at a fixed concentration. The effects of the compositions on the following parameters were tested. These properties are instrumental in establishing the physical stability of the prepared hydrogel [8]. The purpose of following such objectives was to optimize the

formulation on the basis of experimental demonstrations. Table 5 demonstrates the results obtained for different *Wrightia tinctoria* -NSG preparations.

Evaluation of Topical Nanogel

Results of pH, drug content, viscosity, and spreadability assessments are summarized in Table 5.

Table 5: Physicochemical characteristics for evaluation of Carbopol hydrogel

F code	Visual Characterization			Viscosity (Centipoises) (n = 20) ± SD	pH (n = 3) ± SD	Spreadability (g·cm/s)	Texture				Drug content (%) ± SD
	Colour	HO	G				H	A	E	C	
G1	Creamy	----	-- --	0.403±0.005	5.86±0.02	2.45±0.04	185.37	-186	0.295	0.516	86.43 ± 1.38
G2	Creamy	----	-- --	0.506±0.002	5.69±0.01	1.62±0.03	196.46	-226	0.638	0.781	93.58 ± 0.18
G3	Creamy	----	-- --	0.834±0.001	6.58±0.04	1.45±0.05	204.57	-234	0.792	0.803	99.34 ± 0.43
G4	Creamy	----	-- --	1.269±0.008	6.48±0.26	0.86±0.01	246.83	-268	8.08	0.843	99.82 ± 0.22
G5	Creamy	Slightly clumpy	-- --	1.443±0.005	5.93±0.03	0.62±0.03	298.34	-284	0.835	0.869	92.15 ± 0.35
G6	Creamy	Slightly clumpy	-- --	1.836±0.002	5.36 ±0.01	0.37±0.04	265.37	-293	0.759	0.927	90.58 ± 0.12

** Homogeneity= (HO), Grittiness (G), Hardness (H), Adhesiveness (A), Elasticity (E) and Cohesiveness (C)*

pH

The pH of *Wrightia tinctoria* -NSG was found to be 5.43±0.04 - 6.35±0.26 which specifies that the pH of the formulation was similar to the pH of the skin indicating no irritation to the skin by the formulation. The pH value of the prepared 6.35±0.26 was found to be 5–6, i.e., within the appropriate limits, and does not cause skin irritation upon application. Additionally, the pH values of different formulations did not change significantly in time.

Drug content

The drug content of the gel formulation was measured employing a single-point standardization method which was found to be 99.34 ± 0.43% and the drug was uniformly distributed in the nanogel formulation. The percentage of drug content for the prepared *Wrightia tinctoria*-NSG were ranging from 86.43 ± 1.38 to 99.34 ± 0.43%. Thus, indicating the drug was uniformly distributed throughout the gel. According to previous studies, the hardness and compressibility of prepared gel should be strong should be low. Furthermore, high cohesiveness is a desirous attribute. Therefore, based on the results obtained from the above-performed studies it could be concluded that G4 with 1% Carbopol is the optimized formulation.

Viscosity

Wrightia tinctoria -NSG possessed a viscosity of 3684 cps signifying the preparation of excellent nanosponge formulation as optimal viscosity is fundamentally responsible for defining the diffusion of the drug from the nanogel system. Viscosity was testified as an important rheological physical parameter for topical formulations, whichever affects the rate of drug release into the skin. The prepared hydrogels had a viscosity of 0.413±0.005 centipoise. The lowest viscosity was observed in a lower polymer containing hydrogel. The shear rate increases with the decrease in viscosity suggesting the shear-thinning pseudoplastic nature of the formulation. The finding was corroborated with previous literature.

Spreadability

The spreadability of *Wrightia tinctoria* -NSG was found to be 0.96±0.01 indicating the prospect of devising a good topical nanogel formulation. Spreadability is the degree to which a gel spreads upon application. It was founded that therapeutic effectiveness depends largely on any hydrogel's spreading value. The result showed spreadability within 0.25±0.04 –2.13±0.04 g·cm/sec. Good spreadability is a requirement for ideal gel formulation. The result verified that increased polymer concentration reduces HG's spreadability.

Solid state Characterizations

FTIR

The obtained spectrum of the drug was similar to spectra reported in the literature [2]. The drug showed peaks due C-H stretching band at 2940.75 cm-1, O-H stretching band at 2698.66 cm -1, C=C stretching band at 1614.44 cm-1, presence of C=C stretching bands at 1498.98 cm-1 of the benzene ring and C-F band at 967.9 cm-1. Presence of C-H aliphatic peak at 2892.80 cm -1 and 1055.16 cm -1 with hydroxyl group peak at 3450.87 cm -1 confirmed the spectra of HPMC E5 (Figure 11). Figure shows all the peaks related to the drug (C-H stretching band at 2941.22 cm-1, O-H stretching band at 2723.66 cm-1, C=C stretching band at 1614.12 cm-1, presence of C=C stretching bands at 1498.58 cm-1 of the benzene ring and C-F band at 967.91 cm-1), hydrophobic polymer (sharp ester peak at 1724.80 cm -1, peaks at 1418.58 cm -1 and 1135.76 cm -1 due to indicating the presence of aliphatic amines and CO of ester group respectively) and hydrophilic polymer (Presence of C-H aliphatic peak at 2896.54 cm -1 and 1106.4 cm -1with hydroxyl group peak at 3223.41 cm -1), thus indicating that there is no interaction between the polymers and drug.

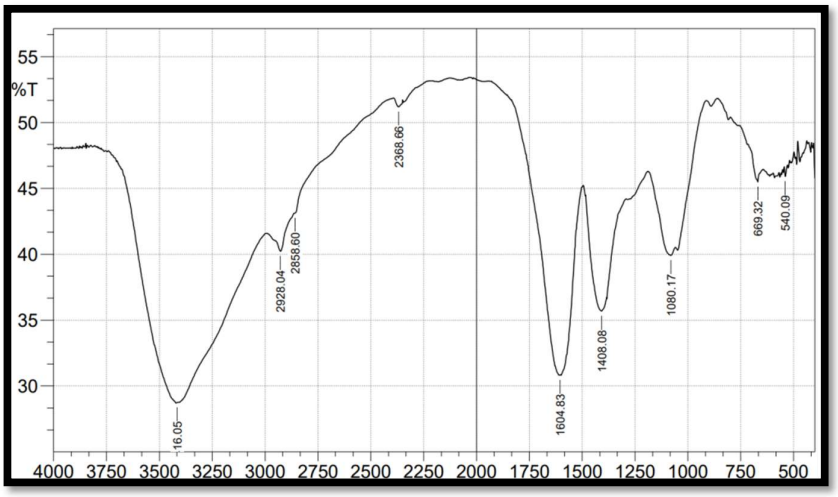

Fig 3: FTIR spectrum of pure leaves extract of *Wrightia tinctoria*

In-vitro drug rlease profile

In-vitro drug released profiles of Rosuvastatin Nanosponge were performed in each formulation diluted by phosphate buffer saline (pH 7.4). It was represented in table 13 and showed in figure 36.

Table 13-Cumulative % amount of drug release of *Wrightia tinctoria* Nanosponge and *Wrightia tinctoria* nanosponge gel

Time (h)	% Cumulative drug release (NSF3)	% Cumulative drug release (NSG)
0	0	0
1	9.53	5.34
2	16.57	8.97
4	24.75	12.45
6	36.95	18.36
8	43.15	25.49
10	56.28	31.27
12	64.97	35.28
14	72.45	43.01
18	83.26	49.86
20	89.47	52.76
24	98.64	63.25

Among all the 7 formulations only two formulations such as NSF3 and G3 selected for the In-vitro diffusion study based on the particle size, zeta potential and polydispersibility index. The formulations containing hibiscus rosa sinensis and tamarind seed powder showed better release but urad dal containing formulation showed controlled release. Urad dal showed the greater particle size, less zeta potential and more PI.

Stability study

A stability study was carried out for the optimized formulation as per International Conference on Harmonisation guidelines. The optimized nanoponsge (NSF3) were placed in glass vials and stored at 25±2°/60±5 % RH and 40±2°/75± 5 % RH atmospheric conditions in a stability chamber for a period of 1 month. Samples were analysed for particle size, zeta potential, % EE and polyderpersity index the stability period. The stability study of optimized formulation was carried out for the period of 1 month with the specified storage conditions. The EE (%), particle size, zeta potential and polydispersity index were evaluated at the end of study as shown in Table 15. It was found that there were no significant changes in the formulation with respect to performed evaluation parameters and thus, it could be concluded that formulation was stable after 1 month stability study. The result of optimized formulation found at accelerated conditions (40±2°/75±5 % RH) may produce long term reliability of formulation besides the studied period.

Table 6: Effect of storage on % EE, Vesicle size, %CDR, PDI, and zeta potential of optimized *Wrightia tinctoria* -NSG.

Temperature	Parameters	Initial	1 Month	2 Month	3 Month
25±2◦C/60±5%RH	%EE	82.41±0.32	80.37±0.01	79.68±0.21	78.32±0.31
	Vesicle Size	183.57±3.12	194.67±2.18	200.57±3.51	205.37±5.13
	PDI	0.298±0.04	0.296±0.02	0.291±0.02	0.293±0.02
	Zeta	-28.94±1.36	-27.64±0.35	-25.37±0.51	24.31±1.36
40±2◦C/75±5%RH	% EE	82.41±0.32	79.61±0.01	75.03±0.21	72.14±0.31
	Vesicle Size	183.57±3.12	198.51±1.82	205.27±5.11	210.52±8.82
	PDI	0.298±0.04	0.294±0.01	0.290±0.22	0.288±0.12
	Zeta	-28.94±1.36	-27.13±2.03	-24.05±1.52	-22.19±2.07

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SUMMARY AND CONCLUSION

The *Wrightia tinctoria* was widely used in the antipsoriatic drug. This research work mainly focussed on therapeutic effects of the drug to increased bioavailability. Nanosponge is a novel drug delivery system and are proposed for improving therapeutic efficacy of the drugs. One of the major advantages of this carrier system is predictable drug release as compared to other nano particulate systems where burst release is of major concern. On reaching target site, nanosponges can release the drug in predictable and controlled manner which helps in determining effective dosage levels. Nanosponges are microscopic particles with few nanometers' wide cavities, within which an outsized form of substances will be encapsulated. The aim of present study is to formulate, develop and Evaluate *Wrightia tinctoria* loaded Nanosponges hydrogel for Topical Application utilizing a variety of polymers for anti-psoriasis research and to ensure a consistent and predictable release of the medication. The negative effects of this formulation were decreased, as was the frequency of dosage and the dose. Preformulation studies of pure *Wrightia tinctoria* and excipients was carried out such as solubility, melting point, UV spectroscopy, compatibility studies by FTIR etc.

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