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FORMULATION AND EVALUATION OF NIOSOME LOADED GEL OF *WALTHERIA INDICA* FOR ANTI-MICROBIAL ACTIVITY

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ABSTRACT

This study aims to formulate and evaluate a niosome loaded gel containing *Waltheria Indica* extract for its antimicrobial activity. Niosomes, as vesicular carriers, offer advantages such as enhanced drug delivery and stability. The gel formulation provides a suitable topical delivery system for effective application. The *Waltheria Indica* extract, known for its antimicrobial properties, is incorporated into the niosome gel to harness its therapeutic potential. Various formulations of niosome gels were prepared using different ratios of surfactants and cholesterol to optimize the formulation. The prepared niosome gels were characterized for particle size, pH, Spreadability and entrapment efficiency. The niosomes showed high entrapment efficiency, with a viscosity of 5981±0.51cps, a ph of 6.2, and spreadability of 11.01. The antimicrobial activity of the optimized formulation was evaluated against selected microbial strain. As the concentration of the niosomal gel increases from 0.5 mg/ml to 2 mg/ml, there is a noticeable increase in the zone of inhibition, indicating enhanced antimicrobial activity against *E. Coli*. The results demonstrated promising antimicrobial efficacy, indicating the potential of the developed niosome gel as a topical antimicrobial agent.

Keywords: Niosomes, Gel, *Waltheria Indica*, Antimicrobial Activity, Formulation, Evaluation.

1. INTRODUCTION

Topical medication administration is a localized drug delivery strategy that can be administered anywhere in the body via ophthalmic, rectal, vaginal, or cutaneous channels. The stratum corneum, or top layer of the epidermis, is the skin's main barrier. Drugs with low molecular weight (≤500 Da), lipophilicity, and low dose are excellent for transdermal administration.[1] Thus; the therapeutic efficacy of existing medications is increased by formulating them in an advantageous manner. A transdermal drug delivery system has recently been created, with the goal of achieving systemic treatment via topical application to the intact skin surface. The skin serves as both a main target and a primary barrier for transdermal medication administration.

In recent decades, there has been a lot of interest in developing a new medicine delivery mechanism. Chemical and physical treatments have been tested to reduce stratum corneum barrier characteristics and improve permeability. These methods include tape stripping, iontophoresis, electroporation, and vascular systems like

liposomes and niosomes. Liposomes and niosomes are two of the most commonly employed techniques in the cosmetic and dermatological areas to improve drug absorption through the skin.[2] Vesicles can help model biological membranes as well as convey and target active substances. There are various kinds of pharmaceutical carriers, such as cellular, polymeric, macromolecular, and particulate carriers. Lipid particles, microspheres, nanoparticles, polymeric micelles, and vesicular systems are examples of particulate type carriers, commonly referred to as the colloidal carrier system.[3] Niosomes have also been extensively researched for their potential as focused and regulated medication delivery vehicles. Similar to liposomes, niosomes exhibit in vivo behavior by extending the drug's circulation to modify its distribution throughout the body and stability during metabolism, or by extending the drug's duration of contact with the tissues in consideration.

Niosomes, also known as non-ionic surfactant vesicles, are microscopic lamellar structures that are produced by mixing cholesterol (CHO) with non-ionic surfactants of the alkyl or dialkyl polyglycerol ether class, then hydrating the mixture in aqueous medium.[4] Because of its many benefits, including chemical stability, high purity, content homogeneity, affordability, easy storage of non-ionic surfactants, and a multitude of surfactants accessible for niosome design, niosomes are gaining a lot of attention.[5] A promising method of drug delivery is via niosomes. The use of niosomes to encapsulate pharmaceuticals can reduce drug inactivation and degradation after administration, as well as boost drug bioavailability and targeting to the diseased area and avoid unwanted side effects.[6] Due to their ability to dissolve functional compounds and remove the mucous layer; surfactants also serve as penetration enhancers. Because of their amphiphilic nature, the non-ionic surfactants in aqueous environments form a closed bilayer vesicle with the help of energy, such as heat and physical agitation. Their physiological characteristics, including composition, size, charge, lamellarity, and application circumstances, all have a significant impact on how effective they are. Studies have also examined the use of niosomes as delivery systems for oral vaccines, anticancer, antitubercular, anti-leishmanial, anti-inflammatory, and hormonal medications.[7]

Waltheria Indica L. is a plant growing in many regions of the world. It has been used in traditional medicine for the treatment of several pathologies in Hawaii, [8,9] West Africa;[10] East Africa,[11]South Africa,[12]South America[13,14,15]and India[16]. Considered as indigenous plant in the Hawaiin Islands, *Waltheria Indica* has been used to treat asthma, neuralgia and pain [17,14]. In Burkina Faso, this plant is used in traditional medicine for management of respiratory affections, such as cough and asthma[18]. Hence, in the present investigation, an attempt is made to develop and characterize niosomal gel formulation of *Waltheria Indica* plant extract. *Waltheria Indica* is selected as a drug candidate for the present study to treat microbial infections more efficiently.

2. MATERIAL AND METHOD

2.1 Plant collection

The medicinal plant *Waltheria Indica* (300 gm) was collected. After cleaning, plant parts (whole) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - Medicinal plant *Waltheria Indica* was authenticated by a plant taxonomist in order to confirm its identity and purity.

2.2 Extraction

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Waltheria Indica* was place in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dry and afterward re-extracted with methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporate using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use[19].

2.3 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Standard procedures were used[20].

2.4 Formulation of Niosomes

Niosomes were formulated using the thin film hydration method. An accurate amount of surfactant that is span 60 and cholesterol was dissolved in 10ml chloroform. Then the following mixture was subjected to thin film hydration at a temperature of 50°C until the thin film was found. The film was hydrated with 10ml phosphate buffer of pH 5.5 containing extract.[21]

Table 1: Composition of niosomes formulation

Ingredients	NS 1	NS 2	NS 3	NS 4	NS 5
Extract (mg)	200	200	200	200	200
Span 60 (mg)	100	150	200	250	300
Cholesterol (mg)	50	100	150	200	250
Chloroform (ml)	10	10	10	10	10
Phosphate buffer (ml)	10	10	10	10	10
Stirring time (min.)	60	60	60	60	60

2.5 Characterization of Niosomes

2.5.1 Particle size and Zeta potential

The particle size is one of the most important parameter for the characterization of niosome. The size of niosomes was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette. The size data is documented in Table 5[22]. The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the niosome

was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements. The zeta potential data is documented in Table 5[23].

2.5.2 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the extract loaded niosomes were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography[24].

2.6 Formulation of niosome loaded Gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then triethanolamine (Drop wise) was added to neutralize the pH and noisome of optimized formulation was incorporated into the dispersion to obtained Gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps[25-26].

Table *2*: Composition of gel formulation

S. No	Excipients	Quantity (gm)
1.	Carbopol 934	1.00 gm
2.	Carboxymethyl cellulose	1.00 gm
3.	Propylene glycol	0.5 ml
4.	Methyl paraben	0.2 ml
5.	Niosomes	10 ml
6.	Triethanolamine	q.s
7.	Water	100 ml

2.7 Characterization of Niosomal Gel

2.7.1 Physical appearance, pH and Viscosity

The prepared Gel formulation was evaluated for appearance, Color, Odor, and homogeneity by visual observation[27]. pH of the formulation was determined by using Digital pH meter (EI). The meter was allowed to stabilize as necessary and properly calibrated, begin by rinsing the probe with deionized or distilled water and blotting the probe dry with lint-free tissue paper[28]. The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 61 at 100 rpm at the temperature of 25^0C[29].

2.7.2 Spreadability

An ideal topical gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1 g of formulation on a glass slide. Another glass slide of the same length

was placed above that, and a mass of 50 mg was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the gel to travel the distance from the place of its position was noted down. Spreadability was determined by the following formula

$$S = M*L/T$$

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T-Time for spreading gel in sec[30].

2.7.3 Skin irritation test

The intact skin of Wistar rats of either sex with average weight 150– 200 g was used. The hairs were removed from the rat 2-3 days before the experiment. The gel was applied on the properly shaven skin of rat. The animals were treated daily for 2-3 days, and undesirable skin changes, i.e., change in color, change in skin morphology was checked for a period of 24 h and erythema and edema on the treated skin were examined[31-32].

2.8 Anti-microbial activity

2.8.1 Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

2.8.2 Well Diffusion Assay

The bacterial suspension of *E. Coli* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate[33]. The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the noisome loaded Gel (0.5, 1 and 2 mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well.

3. RESULTS AND DISCUSSION

3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Waltheria Indica* is shown in Table: 3

Table 3: Percentage Yield of crude extracts of *Waltheria Indica* extract

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Waltheria Indica*	Pet ether	298	1.59	0.53%

2		Methanol	289.27	6.58	2.27%

3.2 Preliminary Phytochemical study

Table 4: Phytochemical testing of extract

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Methanolic extract
Alkaloids		
Dragendroff's test	Absent	Present
Mayer's reagent test	Absent	Present
Wagner's reagent test	Absent	Present
Hager's reagent test	Absent	Present
Glycoside		
Borntrager test	Absent	Present
Legal's test	Absent	Present
Killer-Killiani test	Absent	Present
Carbohydrates		
Molish's test	Absent	Present
Fehling's test	Absent	Present
Benedict's test	Absent	Present
Barfoed's test	Absent	Present
Proteins and Amino Acids		
Biuret test	Absent	Present
Flavonoids		
Alkaline reagent test	Present	Absent
Lead Acetate test	Present	Absent
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Saponin		
Foam test	Present	Present
Test for Triterpenoids and Steroids		
Salkowski's test	Absent	Absent
Libbermann-Burchard's test	Absent	Absent

3.3 Characterization of Niosome

3.3.1 Particle Size and Zeta potential

Table 5: Particle size and Zeta potential

Formulation code	Particle size (nm)	PI Value	Zeta potential
Niosomes F1	175.3 nm	0.012	-40.5 mV
Niosomes F2	187.4 nm	0.212	-35.9 mV
Niosomes F3	198.4 nm	0.212	-59.3 mV
Niosomes F4	216.3 nm	0.081	-22.5 mV
Niosomes F5	**165.2 nm**	**0.083**	**-19.7 mV**

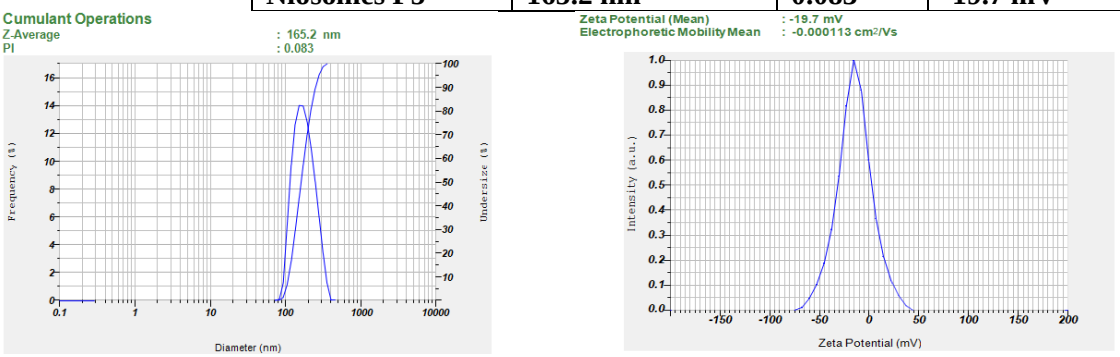

Figure 1: Particle size *and* Zeta potential (F5)

The particle size is one of the most important parameter for the characterization of niosomes. The average particle size of the prepared drug loaded niosomes

was measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of extract loaded niosomes was found to be range 165.2 nm to 216.3 nm. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. If the particles in niosome have a large positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating for niosome. Zeta potential of all formulations was found to be range -19.7 mV to -59.3 mV with peak area of 100% intensity. These values indicate that the formulated niosome are stable.

3.3.2 Scanning electron microscope (SEM) of F5 Formulation (Optimized)

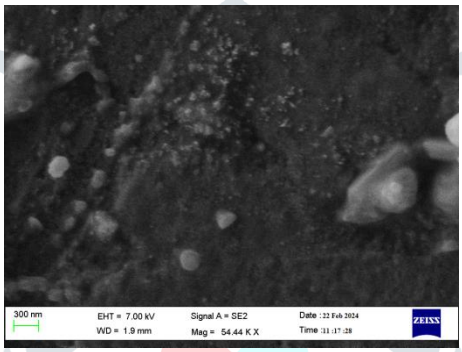

Figure 2: Scanning electron microscope

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared niosomes. Niosomes were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared niosomes at 54.44 kx magnification showed that the niosomes were smooth surface morphology and spherical shape. The spongy and porous nature of niosomes was clearly observed in the SEM images.

3.4 Characterization of Niosome loaded gel

3.4.1 Physical appearance

Table 6: Physical appearance

Parameter	Result
Colour	Green colour
Odour	Odourless
Appearance	Semisolid
Homogeneity	Homogeneous

An evaluation of the gel, including color, odor, appearance and homogeneity, was conducted. Gel was discovered to have a green color to it when tested. Gel does not have a distinctive odor and has a semisolid appearance, according to research conducted on it. Gel exhibited the same color, odor, and Appearance as the I.P. requirements for these characteristics.

3.4.2 Viscosity, PH determination and Spreadability of Gel

Table 7: Viscosity, pH and Spreadability

Formulation	Viscosity (cps)	pH	Spreadability (g.cm/s)
Gel	5981±0.51	6.2	11.01

The viscosity was measured by the Brookfield viscometer spindle no. 7 at 100rpm. The viscosity of Gel was found to be 5981 ±0.51 centipoise respectively. The pH of the gel formulation was found to be 6.2, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time. The physicochemical properties of prepared gel formulation were in good agreement. Good spreadability is one of the most important requirements for a gel. Spreadability is influenced by the formulation's viscosity as well as the physical properties of the polymers utilized. Poor spreadability would result from a more viscous composition. The word "spreadability" refers to the amount of skin that the gel easily spreads over after being applied. A formulation's spreading value affects how effective it is as a medicine as well. The spreadability of Gel formulation is found to be 11.01g.cm/s.

3.5 Anti-microbial activity

3.5.1 Results of antimicrobial activity of Niosomal gel formulation

Table 8: Antimicrobial activity of Niosomal against *E.coli*

Sample name	Zone of Inhibition (mm)
Niosomal gel (0.5 mg/ml)	7 mm
Niosomal gel (1 mg/ml)	12mm
Niosomal gel (2 mg/ml)	17 mm

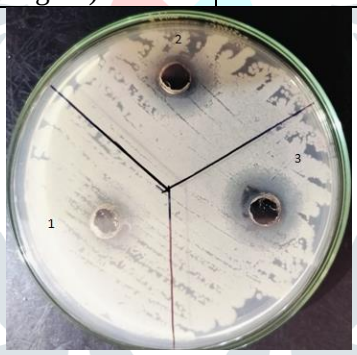

Figure 3: Antimicrobial activity against *E. Coli*

The results of antimicrobial activity testing against *E. Coli* for different concentrations of niosomal gel formulation are presented in Table 8 and illustrated in Figure 3. As the concentration of the niosomal gel increases from 0.5 mg/ml to 2 mg/ml, there is a noticeable increase in the zone of inhibition, indicating enhanced antimicrobial activity against *E. Coli*. At a concentration of 0.5 mg/ml, the niosomal gel exhibited a modest zone of inhibition of 7 mm. However, at higher concentrations of 1 mg/ml and 2 mg/ml, the zones of inhibition increased significantly to 12 mm and 17 mm respectively. This concentration-dependent increase in antimicrobial activity suggests that the niosomal gel formulation effectively delivers the antimicrobial agent, likely *Waltheria Indica* extract, to the target microorganism. The observed antimicrobial activity of the niosomal gel against *E. Coli* is promising and suggests its potential application as a topical antimicrobial agent. The increasing zone of inhibition with higher concentrations indicates a dose-response relationship, reinforcing the effectiveness of the niosomal gel formulation.

4. CONCLUSION

The study focuses on the phytochemical screening of *Waltheria Indica*, revealing the presence of active metabolites such as alkaloids, carbohydrates, glycosides, saponin, tannin, phenols, protein, and amino acid. The

niosomal gel formulation was prepared using the thin film hydration method, with polymers such as Span 60 and Cholesterol selected for further study. The niosomes were observed in electron micrographs and SEM images, with particle size and zeta potential determined using the Malvern Zeta sizer. The niosomes showed high entrapment efficiency, with a viscosity of 5981±0.51cps, a ph of 6.2, and spreadability of 11.01. This study highlights the potential of niosomal gel formulations in enhancing bioavailability and the potential of the noisome-based drug delivery approach to improve the therapeutic efficacy of phytochemicals by improving their absorption and bioavailability. The study highlights the potential of niosomal gel formulations in enhancing phytochemicals' therapeutic efficacy.

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