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Formulation Development of Vitex Negundo Nanoemulgel for Topical Delivery

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ABSTRACT: The present study was aimed to formulate and evaluate antimicrobial activity of vitex negundo nanoemulsion by Cup plate method and developing its nanoemulgel formulation. The MIC value of Vitex negundo oil and nanoemulsion against E. coli was found to be 0.625 mg/ml and 0.3125 mg/ml. Whereas MIC value of Vitex negundo oil and nanoemulsion against Candida albicans was found to be 2.5 mg/ml and 0.625 mg/ml respectively. Nanoemulsion of Vitex negundo oil was prepared by high speed homogenizer. 2^2factorial design with five batches were formulated and evaluated. The optimized batch NE3 shows particle size of 210 nm, zeta potential -27.5 mV and polydispersity index 0.3 and promising antimicrobial activity. The nanoemulsion after formulation was transfer into nanoemulgel by using different concentration of Carbopol 934 ranging from 0.5 to 2% w/v (F1 to F4). The nanoemulgel formulations were evaluated for pH, viscosity, spreadability, extrudability and physical appearance. The nanoemulgel formulation prepared from 1% w/v of Carbopol 934(F2) showed excellent extrudability with semisolid consistency and viscosity of 4260±0.80 CPs at 10 RPM. The developed formulation gives desired physicochemical properties and stability studies.

KEYWORDS: Vitex negundo oil, MIC, nanoemulsion, nanoemulgel, Carbopol 934

I. INTRODUCTION

Now a day's microbial infections are more common. Although antibiotics are used to treat microbial infections, there is need to search for herbal drugs with lesser side effects. Today most of pharmaceutical companies are focusing their attention to folk medicines for developing better drugs against microbial infection. [1] vitex negundo oil is medicated oil extracted from leaves and roots of plant vitex negundo belonging to family Verbenaceae.[2] vitex negundo oil used in the treatment of rheumatism, insecticidal, antimicrobial, pruritus, helminthiasis, etc. The antimicrobial activity of vitex negundo oil and nanoemulsion was evaluated by cup plate method. The optimized nanoemulsion was converted into nanoemulgel by using Carbopol 934 hydrogel. Nanoemulgels are three dimensional hydrophilic networks that have the tendency to imbibe water without changing the internal network structure. Nanoemulsion prepared by either low energy or high energy emulsification methods. High-energy emulsification method includes high pressure homogenization, ultra-sonication, micro-fluidization whereas low-energy emulsification method includes phase inversion temperature method, phase inversion composition method, spontaneous emulsification method. [3] In this method, the nanoemulsion is formed by high pressure homogenization method. Nanoemulsions obtained when the size of an emulsion globule reaches approximately 20-500 nm. The small droplet size can resist the physical destabilization caused by gravitational separation, flocculation and coalescence. It also avoids the creaming process because the droplet's Brownian motion is enough to overcome the gravitational separation force. The size and Polydispersity index of Nanoemulsion can affect properties such as particle stability, rheology, appearance, color, and texture. [4] The present study was designed to develop the anti-microbial herbal nanoemulgel formulation containing vitex negundo oil.

II. MATERIALS AND METHOD

Vitex negundo oil was obtained as a gift sample from Pollen Pharmaceutical Pvt Ltd. Sangli, India. Tween 80, propylene glycol andtriethanolamine are purchasedfrom Research Lab Fine Chemicals Pvt Ltd.Mumbai. Carbopol

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934 and propyl parabenare purchased from Ajinkya Enterprises Pune. All the strains of microorganismswere obtained from National ChemicalLaboratory; Pune.

Nanoemulsion formulation:

Nanoemulsion developed by high-energy method using90 % (w/w) of sterile water, 5 % (w/w) of vitex negundo oil and 5 % (w/w) of varying proportion of Tween 80 and Isopropyl alcohol. Stirred the nanoemulsion at 10,000 RPM using high speed homogenizer for 60 minutes. Water was added dropwise at a flow rate of 3.5 ml/min. Nanoemulsion was evaluated and stored at room temperature.[5]

Figure 1: Oil in water nanoemulsion of vitex negundo oil

Table 1: Nanoemulsion batches obtained by 2^2 full factorial design by using State Ease Design Expert Software Version 12.

Batch Code	Vitex Negundo oil (g)	Tween 80 (g)	Isopropyl Alcohol (g)	Sterile Water (50 g)
NE 1	2.5	1	1	Q. S
NE 2	2.5	1	2	Q. S
NE 3	2.5	2	1	Q. S
NE 4	2.5	3	1	Q. S
NE 5	2.5	4	1	Q. S

Evaluation of Nanoemulsion Formulation:

Droplet Size analysis:

Droplet size and polydispersity of the formulated nanoemulsion was measured by particle size analyzer (NANOPHOX-NX0088). 1ml of liquid formulation was further diluted to 2 ml by double distilled water to avoid the interactions of particles. The average droplet size was measured as the mean diameter. [6]

Zeta Potential analysis:

For zeta potential measurements, the optimized nanoemulsion was 10-fold diluted with filtered distilled water and then analyzed. Each measurement was derived from a total of 30 scans using zeta sizer (Malvern, USA).[8]

pH measurement:

The pH of nanoemulsion formulation was determined by using calibrated digital pH meter. pH measurement was taken in triplicate and average of it was calculated.

Physical Stability:

The physical stability of a nanoemulsion is also important to its performance, which can be adversely affected by precipitation of the drug in the excipient matrix. Physical stability was determined by phase inversion temperature and phase inversion duration.

In Vitro Testing of Vitex negundo oil and nanoemulsion for Antimicrobial activity:

Minimum Inhibitory Concentration (MIC)

This testing was done in the seeded broth by two-fold serial dilution technique. The solutions of vitex negundo oil and nanoemulsion batches were prepared in DMSO. For both bacterial and fungal microorganism a series of 7 assay tubes of concentration ranging from 0.3125,0.625,1.25,2.5,

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5,10,20 mg/ml were prepared. One positive control for each microorganism was prepared by adding nutrient broth with respective microorganism inoculum was added and kept for 24 hours incubation at 37°C. After incubation period the growth of microorganism was measured by comparing the turbidity of test sample with that of positive control using turbidimeter.[9]

Table 2: Minimum inhibitory concentration of vitex negundo oil and nanoemulsion against microbial strains.

Microorganism tested	MIC Value of Vitex negundo oil (mg/ml)	MIC value of nanoemulsion (mg/ml)
E. coli	0.625	0.312
Candida albicans	2.5	0.625

Antimicrobial activity

Antibacterial assay:

Method used: Cup plate method

Bacterial strain: E. coli

In this method 0.2 ml of the seeded broth containing 10-7 test microorganism (E. coli) was inoculated on the plates of solidified agar and spread uniformly. Then five plates were used to checked the antibacterial activity of formulated nanoemulsion batches of concentration dissolved in DMSO. Each plate was cut into a hole by using a sterile borer of 4 mm diameter. Zone of inhibition in mm obtained around the well was measured which has been shown in Table 4.[11],[12]

Antifungal assay:

Method used: Cup plate method

Fungal strain: Candida albicans

Antifungal study was carried out through the same procedure as that of antibacterial study only different media was used i.e. sabouraud dextrose agar media and different test microorganism was used i.e. Candida albicans.

Preparation of Nanoemulgel:

The optimized nanoemulsion was mixed with carbopol 934 solution and stir for 30 minutes at 800 RPM. Propylene glycol solution was added into it with stirring for 20 minutes. Carbopol dispersion was neutralized by using Triethanolamine. [14]

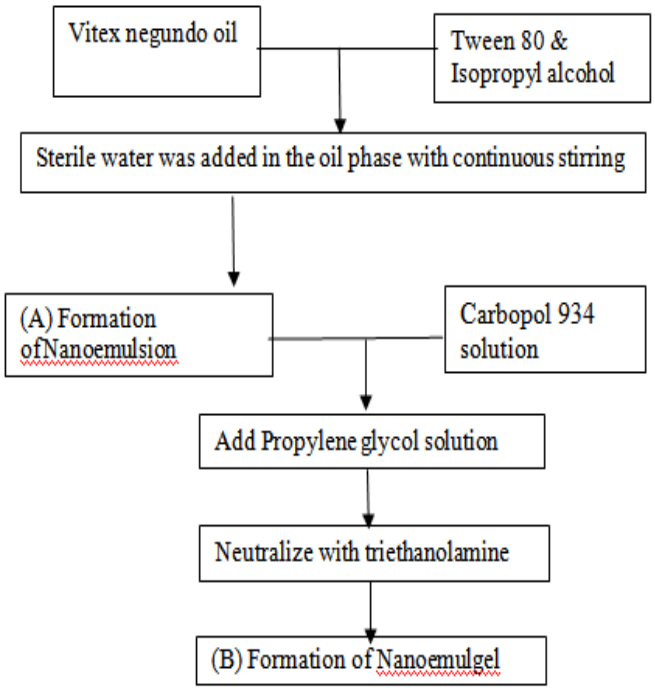

Figure 2: Procedure for the preparation of(A)Nanoemulsion and (B) Nanoemulgel

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Table 3: Nanoemulgel formulation with Carbopol 934

Batch Code	Carbopol 934 % (w/v)	Propylene glycol (g)	Triethanolamine (g)	Sterile water (50 ml)
F1	0.5	2.4	Q. S	Q. S
F2	1	2.4	Q. S	Q. S
F3	1.5	2.4	Q. S	Q. S
F4	2	2.4	Q. S	Q. S

Evaluation of Nanoemulgel formulation:

Measurement of pH:

The pH of nanoemulgel formulation was determined by using calibrated digital pH meter. Dip the glass electride and the reference electrode completely into the nanoemulgel and measure pH in triplicate and average value was calculated.[15]

Homogeneity:

Developed nanoemulgel formulation was tested for Homogeneity by visual inspection after the gel has been set in the container. The gel was tested for their appearance and presence of any aggregates.

Grittiness:

The nanoemulgel formulation was evaluated for the presence of particular matter. If no appreciable particulate matter is seen under light microscope, the gel preparation fulfills the requirement of freedom from particular matter and from grittiness as desired for any topical preparation.[16]

Rheological Studies:

The viscosity and rheological behavior of nanoemulgel formulations were determined using a cone and plate viscometer (Brookfield Engineering Laboratories). All measurements were carried out at a temperature of 25°C±1, using Spindle CP52 at 10 rpm.[19]

Spreadability:

The therapeutic efficacy of the formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. The spread ability was determined by parallel plates method which is widely used for determining and quantifying the spread ability of semisolid preparations. Spread ability is inversely proportional to viscosity.A weight of 1 g of gel was allowed to rest on the upper glass plate for 60 sec. The increase in the diameter due to spreading of the test formulation was noted. [21]

$$S = \frac{m \times l}{t}$$

Where S =Spreadability, m= weight placed on upper slide, l=length of upper slide and t= time taken.

III. RESULTS AND DISCUSSION

Herbal Nanoemulsion were prepared using 5% concentration of vitex negundo oil and 5 to 15 % varying concentration of Tween 80 and Isopropyl alcohol (1:1, 1:2, 2:1, 3:1, 4:1).All the five nanoemulsion batches were in therange of 200-500 nm. The batch NE 3 gives globule size of 210 nm with polydispersity index (PI) 0.3 indicating mid-range disperse system.

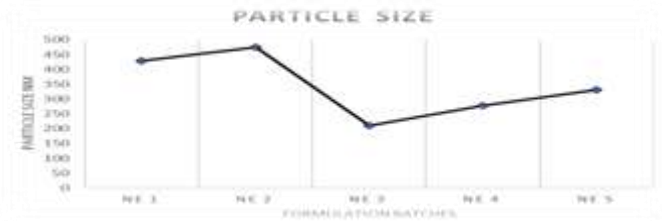

Figure 3: Particle size of nanoemulsion batches

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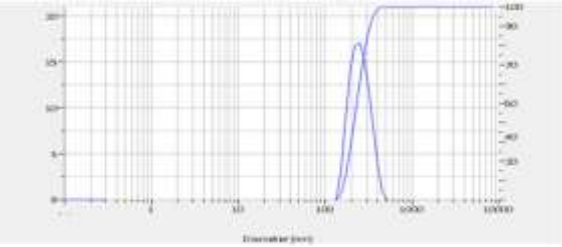

Figure 4: Particle size analysis of NE 3

The Zeta potential for all the five batches were in the range of -12 to -40mV. pH of all the five formulation batches found in the range of 6.6 to 7.1 which is close to the neutral pH and hence it is safe for topical application to the skin .

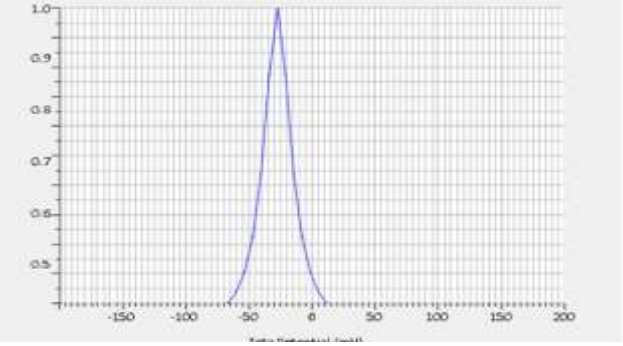

Figure 5: Zeta potential analysis of NE 3

Table 4: Evaluation of nanoemulsion formulation

Batch code	pH	Particle size (nm)	Zeta potential (mV)	PI	Antibacterial activity (mm)	Antifungal activity (mm)
NE 1	7.1	428	-40.7	0.7	7.6±0.8	10.7±0.1
NE 2	6.7	475	-40.1	0.5	6.2±0.3	9.0±0.2
NE 3	6.6	210	-27.5	0.3	9.1±0.4	13±0.1
NE 4	6.9	278	-12.9	0.4	7.0±0.8	11±0.5
NE 5	6.8	331	-24.3	0.6	8.3±1.2	12±0.8

Inhibition zone including 4 mm bore diameter

Antimicrobial activity of all the five nanoemulsion batches was determined by Cup plate Method. Zone of inhibition was measured in mm (Including bore size of 4mm). All the nanoemulsion formulations have considerable antibacterial activity against bacterial strain E. coli and antifungal activity against fungal strain Candida albicans. Among all the five nanoemulsions NE 3 nanoemulsion has maximum antimicrobial activity**.**

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(A) (B)

Figure 6: Antimicrobial activity produced by vitex negundo oil

(A): Antibacterial activity of vitex negundo oil against E. coli
(B): Antifungal activity of vitex negundo oil against candida albicans

The optimized nanoemulsion (NE 3) is coverted into a nanoemulgel formulation by using varying concentration of Carbopol 934 (0.5, 1, 1.5 and 2% w/v). Four nanoemulgel formulations F1 to F4 prepared using Carbopol 934 polymer were evaluated for physical appearance, pH, viscosity, spreadability and extrudability. Results of the test study were in acceptable limits and are discuss in the Table 5.

Batch code	Carbopol conc (%w/v)	pH	Viscosity at 10 RPM(CPs)	Spreadability (g.cm/sec)	Extrudability	Physical appearance
F1	0.5	6.2	4145±1.77	8.2	Good	Yellowish, smooth and liquid consistency
F2	1	6.3	4260±0.80	6.1	Excellent	Yellowish, smooth and semisolid consistency
F3	1.5	6.1	4540±0.91	4.4	Good	Yellowish, smooth and semisolid consistency
F4	2	6.0	4930±1.50	4	Good	Yellowish, smooth and semisolid consistency

Table 5: Evaluation parameters for topicalherbal nanoemulgel of vitex negundo oil with Carbopol 934

Nanoemulgels were homogeneous in nature without any sort of grittiness. F1 formulation has liquid like consistency whereas other formulations have semisolid consistency. The pH values of all the formulations lies in the close range of skin pH (6-6.3) and hence it does not cause any skin irritation.

Figure 8: Nanoemulgel of vitex negundo oil

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Carbopol 934 polymer was used to formulate topical nanoemulgel formulation to provide a prompt release of drug within thetherapeutically effective range. F2 formulation of 1% w/v Carbopol 934 polymer gives semisolid consistency with viscosity of 4260±0.80 CPs with Excellent extrudability. Spreadability study revealed that nanoemulgel formulations were easily spreadable.

Stability study:

For ensuring the quality, safety and efficacy throughout the shelf life, stability study was performed as per ICH guidelines for F2 formulation (Prepared by1% w/v Carbopol 934). No change in colour, odour, homogeneity, pH, viscosity of the topical herbal nanoemulgel formulation was observe after three months of stability study revealed that the formulated topical nanoemulgel F2 is found to be stable (Table 6).[23]

Table 6: Stability studies for topical herbal nanoemulgel of Vitex negundo oil

Parameters	**Topical herbal nanoemulgel (F2) prepared by 1 % Carbopol 934**											
	Storage conditions											
	25±2°C/60±5%RH				**32±2°C/60±5%RH**				**40±2°C/75±5%RH**			
	Months				**Months**				**Months**			
	0	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Colour	No change in colour				No change in colour				No change in colour			
Odour	No change in odour				No change in odour				No change in odour			
Homogeneity	Smooth				Smooth				Smooth			
pH	6.39	6.37	6.31	6.28	6.33	6.30	6.27	6.18	6.31	6.23	6.15	6.08
Viscosity	4260	4260	4255	4251	4260	4254	4250	4247	4260	4251	4248	4241
Sterility test	No microbial growth was observed at 24,48 and 72 h				No microbial growth was observed at 24,48 and 72 h				No microbial growth was observed at 24,48 and 72 h			

IV. CONCLUSION

In the present study, an attempt was made to develop a single novel antimicrobial nanoformulation containing vitex negundo oil. From the result we can conclude that the formulated nanoemulsion possesses promising antimicrobial activities. This nanoemulsion further convered into a nanoemulgel formulation by incorporating it into a Carbopol 934 hydrogel.

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