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FORMULATION AND EVALUATION OF NEEM AND ALOE VERA GEL

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ABSTRACT

Several synthetic drugs have been evaluated over the years for their antimicrobial effect in oral cavity; however, all are associated with numerous side effects that prohibit their regular long term use. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Therefore the objective of the current research was to formulate, characterize and evaluate a gel based dental which is more stable in terms of rheological behavior, long term protection and stability in diverse conditions with minimum adverse effects. Antimicrobial activity of the formulated dental gel was carried out by disc diffusion method and was compared for antimicrobial activity with Ciprofloxacin (5µg/disc) as standard. The formulated batches F2 (5%) and F4 (10%) showed significant zone of inhibition. Formulated batch F4 (10%) gel showed maximum zone of inhibition.

KEYWORDS: Neem, Aloe Vera, Antimicrobial, Gel,

INTRODUCTION

Nowadays, herbal remedies are used for the treatment of various health conditions[1]. The aim of department of AYUSH (Ministry of health) is the growth and development of Indian medical systems for the health care delivery[2]. Modern pharmaceutical technology is being combined with traditional health medicines to increase the efficacy[3]. Fungal skin infection has increased in the last two decades[4]. It is now the fourth most common infection in the world. Immobility, mucositis, use of antibiotics, radiation therapy, certain immunosuppressive agents and intensive care units are the various factors responsible for the fungal infections[5]. Candida species are responsible for the variety of the infections ranging from superficial, coetaneous-mucosal to deep seated infections[6]. There are various types of candidiasis or yeast infections which is caused by *C. albicans* out of which reoccurrence rate of coetaneous candidiasis is more and it is rarely cured[7]. In the present study *Candida albicans* is selected to assess the susceptibility patterns against the phytochemical extracts[8].

MATERIAL AND METHODS

Material: Neem seed oil was purchased from Nagarjun Pharmcauticals Ltd.. Tween 80, Carbopol-934 and PEG 400 was obtained from Loba Chemicals, Mumbai. Aloe-vera gel was purchased from Green Pharmacy, Pune. Triethanolamine is obtained from Qualigens. All the chemicals used were of analytical grade and double distilled water was used throughout the study.

Preformulation Studies

The overall objective of preformulation testing is to generate information useful for the development of formulation.

Appearance and color: The Neem seed oil was examined for its organoleptic properties like color and appearance.

Boiling point determination: The boiling point of neem seed oil was determined by open capillary method using boiling point apparatus.

Solubility study: Solubility of Neem oil was determined in various surfactants and co-surfactants by using shake-flask method.

UV lambda max and calibration curve: Determination of lambda max and calibration curve of neem Oil in dichloromethane- 0.01ml of neem oil was dissolved in 10 ml of the solvent to obtain 1000ppm stock solution A. 1 ml from stock solution is further diluted upto 10ml with dichloromethane to obtain 100ppm stock B. Then standard solution in the range of 20-120ppm were prepared and scanned between 200-400 nm using JASCO UV spectrophotometer to determine the maximum wavelength. The absorbance of each standard solution was determined spectrophotometrically at maximum wavelength. The Beer's-Lambert's plot was constructed by plotting concentration Vs its corresponding absorbance.

Fourier Transforms Infrared Spectroscopy (FT-IR): The spectrum of neem oil was evaluated for drug quality. FT-IR was also used as a parameter to determine the incompatibility of any drug-polymer.

Preparation of Herbal Gel

Herbal gel formulations are prepared by low energy emulsification method. In this method oil phase containing neem oil and S_{mix} are mixed together in conical flask according to the formulae mentioned below. Then to this mixture water phase is added drop wise, and the above mixture containing both the phases is homogenized by using laboratory homogenizer at 3500 rpm for 30 minutes. All the formulated herbal gels are kept overnight to check the stability.

Table 1: Manufacturing formula for herbal gel formulations

Formulation code	Oil (%w/v)	SMIX (%w/v)	Distilled water(%w/v)
NE1	10	56.7	33.3
NE2	13.3	53.4	33.3
NE3	20.7	62.7	16.6
NE4	25.3	58	16.7
NE5	26.7	50	23.3
NE6	30.7	46	23.3
NE7	40.7	50	9.3

Preparation of carbopol gel: Carbopol gel was prepared by incorporating 3% w/v of carbopol 934 in distilled water. Weighed amount of carbopol was taken and dispersed over in distilled water for 2 hours till all the carbopol is soaked, triethanolamine is added after soaking and homogenized for 2hr at 600rpm. After homogenization carbopol gel was subjected for two cycles of sonication for 15 min to expel out the entrapped air bubbles from the prepared gel.

Table 2: Manufacturing formula for gel

Carbopol 934 (%)	Triethanolamine (%)	Methyl paraben (%)	Propyl paraben (%)
3	0.2	0.2	0.2

Preparation of Herbal Gel

Herbal gels are prepared by spontaneous emulsification method. Optimized herbal gels are incorporated into the gel base to obtain the herbal gels. Neem Oil herbal gel was then combined with the aloe-vera gel in different concentrations for the synergistic effect of aloe-vera gel against the fungal infection.

Table 3: Formulae for herbal gel formulation

Formulation code	Aloe-vera gel(gm)	Gel base(gm)
NG1	-	Up to 100
NG2	1.5	Up to 100
NG3	2.5	Up to 100
NG4	3.5	Up to 100
NG5	1.5	Up to 100
NG6	2.5	Up to 100
NG7	3.5	Up to 100

Evaluation parameters of herbal gels

Particle size measurement: Particle sizes of herbal gels were measured by scattering light intensity at scattering angle 90^0. Viscosity of the dispersant is 0.8872 and the count rate is 382.1 kcps.

Zeta potential measurement: Zeta potential of the formulations were measured at 25^0C temperature.

Viscosity determination: Brookfield viscometer is used to determine the viscosity of herbal gel formulations at 10rpm for 3 minutes with spindle 62.

Drug content measurement: 0.01 ml of formulation was dissolve in 10 ml of dichloromethane. Make sure to dissolve it completely to obtain the stock solution. 1ml from the stock solution is further diluted with dichloromethane upto 10ml and absorbance was measured spectrophotometrically at 243nm. Drug content was then calculated.

Determination of pH: pH of the formulations was determined by using digital pH meter. The formulation was taken into the beaker, then pH meter is immersed into the formulation and reading was recorded. Same process was repeated three times with the same formulations and average of three was taken as pH. Similar procedure was used for the determination of pH of all formulations.

Spreabability: One of the crieteria for a gel to meet the ideal quantities is that it should possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a 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 lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula:

$$S = M \cdot L / T$$

Where; M = wt. tied to upper slide, L = length of glass slides; T = time taken to separate the slides

Extrudability Study: The formulations were filled in the collapsible tubes after the gels were set in the container. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm. ribbon of gel in 10 second

***In-vitro* release through cellophane membrane**: The *in-vitro* permeation studies were done using Franz Diffusion cell with the help of cellophane membrane. Cellophane membrane was clamped between donor compartment and receiver compartment. 150mg of the herbal gel formulation was kept evenly in the donor compartment. The receiver compartment was filled with the 60ml of phosphate buffer 7.4. It was stirred continuously at 100rpm using Teflon coated magnetic bead and temperature was maintained at $37^0 \pm 0.5\ ^0$C throughout the experiment. 2ml of the receiver fluid was withdrawn at each one hour interval and replace with same amount to maintain sink condition. The samples were analyzed for drug content using UV-spectrophotometer at 243nm.

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continuously at 100rpm using Teflon coated magnetic bead and temperature was maintained at $37^0 \pm 0.5\ ^0C$ throughout the experiment. 2ml of the receiver fluid was withdrawn at each one hour interval and replace with same amount to maintain sink condition. The samples were analyzed for drug content using UV-spectrophotometer at 243nm.

Thermodynamic stability studies- The herbal gels were subjected to following thermodynamic stability tests.

Heating-cooling cycle: The prepared herbal gels were subjected to 6 heating cooling cycles between 4^0c and 45^0c by storing at each temperature for 48 hours. Samples of herbal gels were then observed for separation or precipitation.

Centrifugation: Formulae which were stable after heating-cooling cycle were then subjected to centrifugation at 3500 rpm for 30 minutes. Those which did not show any phase separation were subjected to freeze-thaw cycle.

Freeze-thaw cycle: The temperature range selected was in between -21^0c and $+25^0$c for 48 hours at each temperature then the formulae were observed for phase separation.

Antifungal Activity

Fungal strains The fungal strain employed in the study was *Candida albicans* to assess susceptibility patterns against the formulated neem aloe vera gels.

Preparation of inoculums: Stock cultures were maintained at 4^0C on slant of nutrient agar. Active cultures for experiment were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for fungi that were incubated for 24 hours at 37^0C. The assay was performed by agar disc diffusion method. Susceptibility was measured in term of zone of inhibition.

RESULTANDDISCUSSION

Preformulation Study

Colour: Neem seed oil was found to be yellowish brown in color.

Boilingpoint: Boiling point of neem seed oil was found to be 226ºC.

Solubility Study: Neem oil shows maximum solubility in Tween 80 and PEG400 hence they were selected as surfactants and co-surfactants for further experiments. Ratio of S_{mix} (5:1) is selected as the optimized ratio for the preparation of herbal gel formulations depending on the solubility studies.

Lambda max and calibration curve: lambda max was obtained at 243nm.

Evaluation of herbal gel

Physical appearance: Formulations were examined for appearance which shows transparent yellowish brown colored formulations. They do not show any turbidity or phase separation.

Table No. 4: Droplet size and polydispersity index

Formulation code	Particle Size(nm)	Polydispersity Index	Zeta potential (mV)
NE1	491.5	0.666	-3.26
NE2	488.8	0.534	-15.2
NE3	317.7	0.840	-3.85
NE4	55	0.999	-0.635
NE5	73.96	0.357	-1.58
NE6	144.6	1.000	-15.2
NE7	538.9	1.000	0.144

Values are expressed in mean ± SD, where n=3

Table No. 5: Evaluation of herbal gel formulations

Code	Viscosity (cps)	Drug content (%)	pH	Viscosity (cps)	Spreadability (mm)	Extrudability (gm)	Flux (µg/cm^2/hr)
NE1	10220 ± 3.26	94.5 ± 0.213	6.51	41980	4.87	0.61	30.2
NE2	9829 ± 4.10	96.6 ± 0.314	6.55	45361	5.89	0.63	28.9
NE3	10846 ± 4.62	93.7 ± 0.420	6.58	47820	6.12	0.58	24.4
NE4	10425 ± 2.39	97.1 ± 0.129	6.54	48980	6.34	0.66	37.2
NE5	9692 ± 4.82	95.4 ± 0.265	6.59	49813	6.43	0.54	32.6
NE6	9487 ± 3.96	92.8 ± 0.203	6.48	46546	6.29	0.59	29.5
NE7	9875 ± 5.13	95.9 ± 0.386	6.43	49310	6.84	0.63	30.1

Table No. 6: Thermodynamic stability studies

S. no.	Formulation code	Heating-Cooling Cycle	Centrifugation	Freeze-thaw cycle
1	NE1		×	-
2	NE2			
3	NE3			
4	NE4			
5	NE5			
6	NE6	×	-	-
7	NE7	×	-	-

Antifungal activity study of herbal gel formulations

Table 7: Antifungal activity study of gel formulations against *Candida albicans*

Formulation code	Zone of inhibition (mm)
NG1	161±0.34
NG2	167±0.23
NG3	188±0.42
NG4	196±0.22
NG5	143±0.13
NG6	128±0.26
NG7	158±0.22
Marketed formulation(Ketoconazole 2% cream)	195±0.11

CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It was a very good attempt to establish the Herbal gel containing plant extract.

In present research work, herbal gel of neem oil was formulated by spontaneous emulsification method and characterized for vesicle size, polydispersity index, zeta potential, drug content and viscosity. Droplet sizes of all the formulated herbal gels were found to be satisfactory in the herbal gel range. Polydispersity index indicates homogeneous population of herbal gel droplet in formulation.NE5 formulation showed highest transdermal flux across cellophane membrane. From the characterization study of herbal gels NE5 was selected as the optimized formulation whch was formulated into herbal gel by using carbopol-934 hydrogel and aloe-vera gel in diffeerent concentrations and antifungal activity is determined by petri-plate method and zone of inhibition was calculated. NEG4 shows maximum zone of inhibition which was then compared with marketed (0.2% ketoconazole cream) for various parameters i.e. viscosity, extrudability and drug content. It

was observed that herbal gel formulation (37.23 ± 0.733µg/cm2/hr) show two fold increase in transdermal flux as compared to marketed (161.35 ± 0.52µg/cm2/hr). From the results it can be concluded that herbal gel formulation is potential and effective transdermal drug delivery system for neem oil and aloe-vera gel indicates homogeneous population of herbal gel droplet in formulation.

NE5 formulation showed highest transdermal flux across cellophane membrane. From the characterization study of herbal gels HE5 was selected as the optimized formulation which was formulated into herbal gel by using carbopol- 934 hydrogel and aloe-vera gel in different concentrations and antifungal activity is determined by petri-plate method and zone of inhibition was calculated. NEG4 shows maximum zone of inhibition which was then compared with marketed (0.2% ketoconazole cream) for various parameters i.e. viscosity, extrudability and drug content. It was observed that herbal gel formulation (37.23 ± 0.733µg/cm2/hr) show two fold increase in transdermal flux as compared to marketed (16.35 ± 0.52µg/cm2/hr). From the results it can be concluded that herbal gel formulation is potential and effective transdermal drug delivery system for neem oil and aloe-vera gel. indicates homogeneous population of herbal gel droplet in formulation.

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CONFLICT OF INTEREST

There are no conflicts of interest

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