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Formulation, Optimization and *In- Vitro* Antibacterial Efficacy of Polyherbal Nanogel against Propionibacterium acne

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ABSTRACT
Pimple, acne, sunburn mark and pigmentation are issues that affected every individual at least once during life time. Consumers have begun to search for a product that can cure the skin issue and grant them with a good and healthy skin such as anti-acne cream or anti acne gel. The gel based oil free formulation is always preferred. Nevertheless, most of the anti- acne creams available in the market contain lots of chemicals that may have some kinds of side effects to the consumers. The present study was conducted to formulate and evaluate the anti-acne nanogel containing extracts of garlic and aloe Vera. The antibacterial activity of the extract and nanogel formulation in different concentrations was investigated using Propionibacterium acne through disc diffusion method. Cutibacterium acnes (formerly Propionibacterium acnes) is the relatively slow-growing, typically aerotolerant anaerobic, gram-positive bacterium (rod) linked to the skin condition of acne The antibacterial potential of the extract was studied with extract of garlic alone and in combination with aloe vera. The nanogels of garlic extract alone and in combination with aloe vera were formulated by emulsion/solvent evaporation method using Pluronic F127 and Polyethyleneimine polymer. The formulated Nanogel was also stable after two months. This formulated Nanogel can be successfully used for skin infections which including acne vulgaris, after the confirmation of clinical and toxicity studies in future.
Keywords: Garlic, Aloe Vera, anaerobic, nanogel, antiacne, Propionibacterium acne.

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INTRODUCTION

Plants are the natural reservoir of various medicinal agents and thus, they have been used to cure different diseases and disorders for thousands of years. Medicinal plants are extensively studied as a source of safe and effective medicine because they possess bioactive compounds (secondary metabolites) which are mainly responsible for different healing effects. Various bioactive components are present in the medicinal plants, which are the key source of new pharmaceuticals and health care products [1,2].Acne is a multifaceted skin disorder, affecting more than 85% of young individuals worldwide. It is the most common skin disease, and although it usually manifests during puberty and worsens throughout adolescence, epidemiological studies suggest that it can arise at any age.[3] Acne treatment aims to lessen the inflammatory or non-inflammatory acne lesions, improve appearance, prevent or minimize potential adverse effects, and minimize any scarring.[4] Pharmacological therapy is not always desirable because of the development of antibiotic resistance or the potential risk of adverse effects. Non-pharmacological therapies can be viable alternatives for conventional therapies. It is noteworthy that acne severity and scarring have been related to Propinobacterium acne inflammatory factors, bacterial growth metabolites such as allergens, toxins, or porphyrins, and enzymes. Presence of follicular bacteria, specifically Proprionibacterium acnes is a normal colonizer of human follicles but seems to be present in excessive numbers in patients with acne and plays a role in the induction of inflammation in association with the follicular plugs.[5] The main aim of acne treatment is to control and treat existing acne lesions, prevent permanent scarring as far as possible, limit the duration of the disorder and to minimize morbidity. Acne may be treated topically or systemically (with oral drugs) such as retinoids, salicylic acid and derivatives, antibiotics like clindamycin etc. Other treatment options include the use of natural products or the use of non-drug treatments like optical therapy..[6, 7]

Garlic possesses a defense mechanism against attack from the soil-borne organisms. It has been found that invasion of growing garlic cloves by fungi and other soil pathogens causes the alliin and allinase to react, rapidly producing localized bursts of allicin which deactivates the invaders. This ability underlies

the exceptional capacity of allicin to kill unwanted organisms 8 the moisturizing and anti-bacterial and wound healing activity of aloevera helps to improve the quality of formulated nanogel. Among most of the topical formulations, Nanogels can deliver the active moiety in controlled and sustained manner. Release of therapeutics can be regulated by crosslinking densities. Crude garlic extract cannot be applied directly on skin due to its irritant nature & may sometimes produce burns on the applied area. Therefore slow delivery of the extract can reduce the irritation & prolong the therapeutic action of garlic extract. The moisturizing, cooling anti-bacterial and wound healing activity of aloevera helps to reduce irritation potential of garlic and also improves the quality of formulated nanogel. Being cross linked structure, nanogels are not easily washed off the applied site, which also helps in maintaining the prolonged action of therapy. Formed nanogel containing garlic extract was tested for the activity against Propionibacterium acnes for its antiacne activity.

MATERIAL AND METHODS

Collection and authentication of plant and Extraction:

Garlic used for the extraction of garlic powder was purchased from the local market. Aloe vera used for the extraction of aloe vera powder was collected from the Botanical garden of Ideal College of Pharmacy and Research, Kalyan. The plants were authenticated by Department of Botany, Ramniranjan Jhunjhunwala College Ghatkopar, and Mumbai. Voucher Specimen Number: ICPR/2019-20/140.Rest of the materials for synthesis of nanogels, and characterization, analysis, etc. was purchased from different vendors. Preparation of garlic powder was done by temperature controlled extraction process as described by British Pharmacopoeia [9] and from the book 'Allicin – The Heart of [10].

Aloe vera extract was prepared from Aloe Vera leaf gel according to the published procedure 11 with slight modifications. Mature, healthy and fresh leaves of Aloe Vera having a length of approximately 60 to 100 cm were washed with fresh water. The leaves were cut transversely into pieces. The thick epidermis was selectively removed. The fleshy solid gel in the center of the leaf was scratched with a spoon, collected and homogenized. The resulting mucilaginous, thick and brownish green colored homogenate was lyophilized. Then the lyophilized sample was extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator at 60°C. The residue was stored in dry sterilized small containers at 4°C until further use.

PREPARATION OF DRUG-LOADED F127/PEI NANOGEL12

Preparation of drug-loaded Pluronic F127/PEI nanogel was done as per the method given by which is a 3 step process:

1. Activation of Pluronic F127 by 1, 1 – carbonyldiimidazole,
2. Preparation of F127/PEI nanogel,
3. Drug- loaded F127/PEI nanogel.

Preparation of CDI-activated Pluronic F127

A solution of Pluronic F127 (1.25 g, 0.1 mmol) in anhydrous THF (15 mL) was added drop-wise (during 2 hr.) to an excess amount of CDI (0.81 g, 5 mmol) in THF (15 mL) at room temperature under nitrogen atmosphere. After the addition, the mixture was kept stirring for an additional 6 hr. The solution was concentrated to a small volume under vacuum and poured into ethyl ether (150 mL), and the precipitate was collected by filtration to get CDI-activated Pluronic F127. This process was repeated three times to remove the unreacted CDI. The CDI-activated Pluronic F127 was obtained as white powder after drying under vacuum at room temperature for 12 hr.

Preparation of F127/PEI nanogel

The F127/PEI nanogel was prepared by an emulsification/solvent evaporation method. The activated Pluronic F127 was dissolved in chloroform and added drop- wise to an aqueous solution of PEI under stirring. The mixture was sonicated for 3min and the organic solvent in the emulsion was removed by rotary vacuum evaporation at 50°C for 45 min. The remaining solution was centrifuged at 3000 rpm for 30 min to remove adhesive fragments. After neutralizing with hydrochloric acid, the solution was dialyzed in a dialysis bag with 14,000 – 16,000 Da molecular weight cut-off against water at pH 4.0. The purified nanogel samples were freeze dried to obtain F127/PEI nanogel.

Drug loading

Marketed formulation contains 180 mg of allicin. So, from the calculation it was found that 3.82 g of garlic powder used can provide 180 mg of allicin. The F127/PEI nanogel was prepared by an emulsification/solvent evaporation method using different proportion of polymers as described by Li et al. [13]. The activated Pluronic F127 were dissolved in chloroform and added drop-wise to an aqueous solution of PEI under stirring. After neutralizing with hydrochloric acid empty nanogel samples were freeze dried. Then drug and lyophilized empty nanogels were dissolved separately in mixture of methanol

and water, mixed it and the organic solvent was subsequently removed by rotary vacuum evaporation. The resulting film formed was further hydrated with a suitable amount of phosphate buffered saline pH 7.4 to obtain yellowish liquid nanogel formulation.

Optimization of polymer concentration

For the present work, factorial design was applied to obtain optimized formulation. The optimization was done by using Design Expert software (v8.0.7.1)In this experiment, the concentration of Pluronic F127 and concentration of PEI may have impact on the quality of nanogel and hence were selected as independent variables. In this design (Table 1), two factors were evaluated each at three level in such a way that low level was -1, medium level 0 and high level +1 (table 1).

Table 1: 32 Factorial Design Layouts

Batch	Coded value	Actual values	Coded values	Actual values
	X1	Pluronic F127 (mg)	X2	PEI (mg)
F1	-1	100	-1	100
F2	0	200	-1	100
F3	+1	300	-1	100
F4	-1	100	0	200
F5	0	200	0	200
F6	+1	300	0	200
F7	-1	100	+1	300
F8	0	200	+1	300
F9	+1	300	+1	300

Table No 2: Formulation Table for Garlic Alone

Batch	Garlic (g)	Concentration of Pluronic F127 (mg)	Concentration of PEI (mg)	Water (ml)	Chloroform (ml)
F1	3.82	100	100	20	2
F2	3.82	200	100	20	2
F3	3.82	300	100	20	2
F4	3.82	100	200	20	2
F5	3.82	200	200	20	2
F6	3.82	300	200	20	2
F7	3.82	100	300	20	2
F8	3.82	200	300	20	2
F9	3.82	300	300	20	2

Table No 3: Formulation Table for Garlic in combination with Aloe vera

Batch	Drug 1 Garlic (g)	Combination of garlic and Aloe Vera	Concentration of Pluronic F127 (mg)	Concentration of PEI (mg)	Water (ml)	Chloroform (ml)
G1	3.82	3.7	100	100	20	2
G2	3.82	3.7	200	100	20	2
G3	3.82	3.7	300	100	20	2
G4	3.82	3.7	100	200	20	2
G5	3.82	3.7	200	200	20	2
G6	3.82	3.7	300	200	20	2
G7	3.82	3.7	100	300	20	2
G8	3.82	3.7	200	300	20	2
G9	3.82	3.7	300	300	20	2

Evaluation for optimization

On the basis of preliminary screening study, the following variables were selected.

Independent variables

1. Concentration of Pluronic F127 (X_1) 2. Concentration of PEI (X_2)

Dependent variables

1. Particle size (Y_1)
2. % entrapment efficiency (Y_2)
3. % cumulative drug release (% CDR) (Y_3)

Particle size

The droplet size of the nanogel loaded with garlic powder as well as in combination with aloevera was measured by using Malvern zeta sizer according to the method described by Singka *et al*. [15].

Drug entrapment efficiency

Measurement of drug entrapped into the prepared nanogel was done by the method described by Azadi *et al*. [14].

***In-vitro* release study optimized formulations**

In vitro drug release study of prepared nanogels was performed according to the method given by Azadi *et al*. [15].

A 3 level 2 factors factorial design (3^2) was employed to design sustained release nanogel formulation of garlic powder alone and in combination with aloe vera. The design was employed to study the effect of independent variables, i.e. concentration of Pluronic F127 (X_1) and concentration of PEI (X_2) on dependent variables particle size (Y_1) % entrapment efficiency (Y_2) and % CDR (cumulative percentage Drug Release) (Y_3) (Table 4 and 5).

Table 4: 3^2 Full factorial design

Sr. No.	Batch Code	Actual Value		Responses		
		Factor 1 Conc of Pluronic F127 (mg) (X_1)	Factor 2 Conc of PEI (mg) (X_2)	Particle Size (Y_1) (nm)	% Entrapment Efficiency (Y_2) (%)	% cumulative drug release (Y_3) (%) (12 hr.)
1	F1	100	100	131.8	70.83	95.58
2	**F2**	**200**	**100**	**81.97**	**76.67**	**96.42**
3	F3	300	100	56.18	46.38	95.84
4	F4	100	200	714.6	48.81	93.28
5	F5	200	200	711.6	67.78	94.68
6	F6	300	200	520.2	46.15	94.06
7	F7	100	300	331.3	45.07	93.24
8	F8	200	300	290.4	68.42	95.21
9	F9	300	300	144.1	58.4	95.73

Table 5 3^2 Full factorial design

Sr. No.	Batch Code	Actual Value		Responses		
		Factor 1 conc of Pluronic F127 (mg) (X_1)	Factor 2 Conc of PEI (mg) (X_2)	Particle Size (Y_1) (nm)	% Entrapment Efficiency (Y_2) (%)	% cumulative drug release (Y_3)(%)(12hr.)
1	G1	100	100	142.8	71.51	94.11
2	**G2**	**200**	**100**	**79.52**	**77.15**	**98.46**
3	G3	300	100	54.16	45.31	95.85
4	G4	100	200	615.6	51.81	92.56
5	G5	200	200	681.6	69.74	93.68
6	G6	300	200	526.6	44.25	94.66
7	G7	100	300	336.9	44.11	93.69
8	G8	200	300	299.2	70.42	95.80
9	G9	300	300	154.6	68.2	95.88

Evaluation Of Optimized Nanogel

F2 and G2 formulations batches were well optimized batches , which were taken for further evaluation.

pH

The pH of nanogel formulation was determined using digital pH meter as per the method described in

Indian Pharmacopoeia [19].

Viscosity

The viscosity of the prepared formulations was determined using Brookfield viscometer as the method described by shah *et al*. [11].

Particle size

The particle size of the optimized nanogel was measured during optimization studies.

Drug entrapment efficiency

Measurement of drug entrapped into the prepared nanogel was done by the method described by Azadi *et al*. [14].

***In-vitro* release study**

In vitro drug release study of optimized nanogel was performed measure during optimization studies.

Drug release kinetics

Drug release kinetics to investigate the mechanism of drug release from nanogel was performed as described by dash *et al*. [17].

Anti-Acne study

Disc diffusion method was used for antibacterial activity. A stock solution of extract was prepared by dissolving 0.1 g of formulation with 100 mL of their respective to produce a final concentration of 100 mg/ml. Distilled water was used as negative controls Discs were fully dried before the application on bacterial lawn. The positive controls used were clindamycin. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs. The assay was repeated trice. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by garlic alone and in combination with aloe Vera. As P. acne is anaerobic organism, strict anaerobic conditions were maintained.

Stability study

The stability study of optimized formulation was performed as per the ICH guideline Q 1 C [20].

RESULT

Characterization of optimized nanogel formulation (f2).

pH

The pH of optimized nanogel was determined using digital pH meter. The optimized F2 formulation was pH 5.51 ± 0.0152 and G2 was 5.55 ± 0.0111, which is similar to the pH of skin i.e., 4 – 7.

Viscosity

The viscosity of optimized nanogel was determined using Brookfield viscometer. Using spindle no S63 at 100 rpm, the viscosity of optimized nanogel formulation F2 and G2 was found to be **2.68 ±** 0.13 and 2.43 ± 0.15 respectively**.** As the concentration of polymer solution increases viscosity also increases.

Particle size

The particle size of optimized nanogel was measured by using Malvern zeta sizer. Particle size of optimized nanogel was found to be 81.97 nm and 79.52 respectively which is desired for the nanogel

Drug entrapment efficiency

Drug loaded amount of optimized nanogels was measured by HPLC after centrifugation at 12,000 rpm for 10 min. The % entrapped drug in optimized nanogel was found to be **76.67 %** and 77.15 respectively.

***In-Vitro* Drug Release profile**

In-vitro drug release study of optimized nanogel was performed using franz diffusion cell. From the release study, it was found that optimized nanogel shows initial burst release of drug in first half hours and afterward provides sustained release of drug The optimized nanogel was shown release of more than 90 % (96.42 %) and (98.46 %) of drug in 12 hr.

Stability study

Stability study of the optimized nanogel was performed as per ICH guideline. After 1 month of stability study formulation was evaluated for its particle size and % entrapment efficiency. From the results it was found that formulation was shown increase in particle size and decrease in % Entrapment efficiency at refrigeration condition (4 ±0.05°C) While formulation stored at 40 ± 2°C/75% ± 5% RH (relative humidity) was being precipitated and degraded. So, it was concluded that optimized nanogel formulation was stable at refrigeration condition and unstable at room temperature.

Anti-acne study

An agar well diffusion method was used for determination of antibacterial activity of optimized formulation against one of the acne inducing bacteria Propionibacterium acnes. The four samples, Clindamycin gel (standard), garlic optimized nanogel formulation (F) garlic in combination with Aloe Vera optimized nanogel formulation and negative control(Water) were used for this study (Fig. 1). Zone of inhibition of F2, G2, Standard marketed gel and Negative contro was as given in Table No 6.So, from the

result of anti-microbial study it should be concluded that optimized nanogel formulation having inhibitory effect on one of the acne inducing bacteria Propionibacterium acnes.

Figure no 1. .Zone of inhibition of optimized formulations against P. Acne

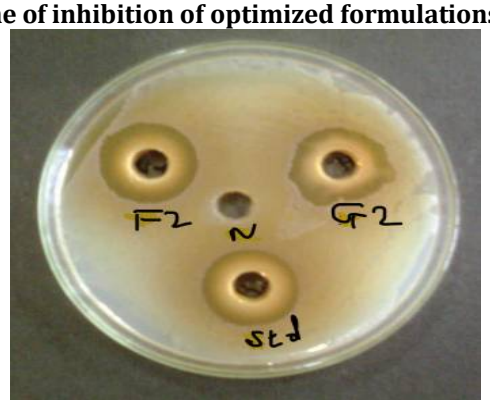

Table no 6.Zone of inhibition of optimized formulations against P. Acne

Bacterial culture	F2	G2	Positive Control (Clindamycin gel)	Negative control
Zone of inhibition in mm 1	22	23.5	25.9	Nil
Zone of inhibition in mm 2	24	24.8	26.1	Nil
Zone of inhibition in mm)3	25.3	25.4	26.9	Nil
Average Zone of inhibition in mm	23.76	24.56	26.9	Nil

CONCLUSION

The present work has shown that nanogel containing garlic extract alone and in combination with Aloe vera can be prepared by emulsion/solvent evaporation method. The particle size of optimized formulations was less than 200 nm which desire for nanogel formulation. The *in-vitro* diffusion study was shown that optimized formulations seems to be a sustained and controlled release dosage form. The pH of optimized formulation was suitable for the skin and can be applied topically. Anti-microbial study was shown that garlic extract nanogel alone and in combination with Aloe vera having good inhibitory effect on acne inducing bacteria Propionibacterium acne. The stability study of optimized formulation was shown that the nanogel having good stability at refrigeration condition but unstable at room temperature. The results of present study supports the traditional usage of Garlic and Aloe vera and suggests that optimized nanogel formulations possess good anti-acne properties that can be used in for the therapy of infectious diseases caused by Propionibacterium acne.

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