

A Novel Antibacterial Topical Gel From *Nigella Sativa* And *Achyranthes Aspera* Against Acne Causing Microorganisms

Abstract Inflammation in sebaceous follicles leads to skin related maladies more specifically, acne vulgaris. Bacterial species involved in causing such maladies involve *P. acnes*, *S. epidermidis*, *S. aureus*. These microbes have negatively affected the currently available remedies for acne vulgaris. In this research herbal *Nigella sativa*, *Achyranthes aspera* seeds were used instead of using any chemical based product on skin. The present study aims to develop novel antibacterial gel using *Nigella sativa*, *Achyranthes aspera* seeds to target pathogens that cause dermal acne. To extract the antibacterial property of the seed, they were soaked in desired chemicals individually in a shaker and then evaporated by using a rotary evaporator. After the extraction the extract was screened using the agar gel well diffusion method against *P. acnes* as well as *S. aureus*. From the diffusion method it was found that the extract is loaded with antibacterial properties. Now, the extract of *N. sativa* and *A. aspera* seeds was further divided into gels of 2 different concentrations and subjected for stability evaluation of antimicrobial activity. In this research it was found that the antibacterial property of the 10% of seed extract used was surpassed the commercial synthetic product. In addition, it was found that there were no changes in colour, pH, odor, consistency, homogeneity, and washing capacity, although the antibacterial potential was physiologically stable during storage time. The strong antimicrobial property produced from the extract in topical gel formulations suggests that the formulation can be a potential alternative to current remedies in the treatment of acne vulgaris.

Keywords Acne Vulgaris, *Achyranthes aspera*, *Nigella sativa*, Topical Gel.

Introduction

The most common dermatological disorder, Acne vulgaris affects more than 70% of teenagers and more than 10% of adults. The pathological characteristics of the disorder includes severe inflammatory response in sebaceous follicle leading to formation of seborrhea, comedones, skin lesions and nodules. Because of versatility and wider surface area, skin epidermis has been considered a promising route for drug administration. Topical drug delivery system designed to distribute a variety of drugs by diffusion through the layers of the skin to the body [1]. Gels are transparent to opaque

semisolids and have a gelling component that interferes with the creation of a colloidal 3D network structure. This phenomenon prevents resistance to deformation and imparts visco-elastic properties to the material. As a tool, gels have a greater potential to administer drug topically than ointment due to their non-stickiness, low energy based formulation, durability and aesthetic value.

Topically dermal administration of compounds such as benzoyl peroxide, retinoids and antibiotics (clindamycin), or oral antibiotics (retinoids, tetracycline), are some of the current treatments for acne vulgaris. Combination therapies are usually required in the case of extreme acne [2]. While antibiotic treatments are known for inhibiting inflammation caused by acne along with targeting *P. acnes*. The growing problem of antibiotic drug resistance in *P. acnes* and other bacterial species has posed the need for the production of novel therapeutic agents. Thus, there is an undeniable need to evaluate antimicrobial, anti-acne components from natural sources such as plants, traditionally used minerals and spices to develop a novel formulation against acne vulgaris.

Spices have been valued for several antiseptic properties like anti-inflammatory, analgesic, bacteriostatic, and anti-tumorigenic. Specially, spices mustard, cinnamon, saffron have been evaluated for their cosmetology and pharmacology applications. In amongst spices, *Achyranthes aspera* L. and *Nigella sativa* L. is of cast relevance because of its utility in dermatology and pathologic treatments such as various bioactivities like antioxidant, anti-tumor, antimicrobial and immuno-modulatory activity have been testified in *N. sativa* and *A. aspera* seeds [3]. These seeds have been used for several decades, with different compositions in the various skin treatments such as burns, wound, and acne vulgaris. A clinical research revealed *N. sativa* oil extract (20%) used in lotion development had improved efficiency and relatively less negative effects as compared to benzoyl peroxide treatment, which is commonly used for beginner to medium state of dermal acne. Hence the present research develops topical cosmetic dermal formulations including *Nigella sativa* L. and, *Achyranthes aspera* L. and evaluates the antiseptic property against the pathogens causing dermal acne. Thus, the present study is expected to provide potential justification for the utility of *Achyranthes aspera* and *Nigella sativa* as an anti-acne agent.



Figure 1: *Achyranthes aspera*[4]



Figure 2: *Nigella sativa* seeds[5]

RESEARCH QUESTION

How to prepare an herbal formulation possessing anti-bacterial properties to treat acne vulgaris and also cure allergies related to it?

The literature study revealed the topical cosmetic formulations developed from *Achyranthes aspera* and *Nigella sativa* with antiseptic property against microbes responsible for causing acne.

A study conducted by Nawarathne et al. (2019), showed the development of gel from the seeds of *Nigella sativa* L. along with its antibacterial properties evaluated against the acne causing microorganisms. Antibacterial extracts were screened for their effect on *S. aureus* as well as *P. acnes* using the gel well diffusion method. Results concluded that antibacterial property of topical gel formulation prepared using ethyl-acetate extract of *Nigella sativa* proved its applicability as an alternative to existing remedies against dermal acne [1].

Upasna Bhalani et al (2015), in a study revealed the efficacy of *Nigella sativa* against the *Propionibacterium acnes* species and developed a formulation of *Nigella sativa* for acne vulgaris treatment. Carbopol 940 was the active constituent of the gel. Developed gel was tested for physicochemical properties such as pH, viscosity and physical appearance [6].

Dr. AR. Mullaicharam (2012), study showed the anti-acne activity of herbal extracts and developed formulation to treat acne, evaluated by in vitro method of antibacterial activity. Anti-acne property of formulation was evaluated by sub-culturing method and broth dilution method. Results showed the formulation having effective acne properties [7].

G S Kumar et al (2007), study revealed the antimicrobial activity of medicinal plants against the acne causing bacteria. Ethanolic extracts of such as *Eclipta alba*, *Symplocos racemosa* were tested for antimicrobial activity by broth dilution method and diffusion method [2]. Kate Nelson et al (2016), study showed the anti-acne activity of medicinal plants of Italy used for the skin infection and characterization of extracts by chemical method and evaluate their suitability for future purpose [8]. Mohamed A. Toama et al. (1974), in this study the antimicrobial property of oil developed from *Nigella sativa* L. was evaluated [9].

In the prior research conducted, although the gel formulation prepared using *Nigella sativa* seeds were found to be effective against dermal acne but takes a long time to recover which may render the skin exposed to contaminants for long duration of time. This exposure for long duration due to delayed recovery increases the chance of infection which is a serious cause of issue. Therefore, there is need to develop gel with enhanced recovery time for the better treatment of acne vulgaris and thus prevent chances of contamination thereby providing a safe and healthy life to people. In the present research combination of 2 different plants (*Nigella sativa* and *Achyranthes aspera*) were used to develop the gel with enhanced efficiency to control acne vulgaris. *Achyranthes aspera* traditionally used to control acne vulgaris progression, therefore combination of *Nigella sativa* and *Achyranthes aspera* have been used to develop a novel remedy against acne vulgaris.

METHODOLOGY

Design:

To prepare a topical gel to control acne vulgaris progression, *Nigella sativa* and *Achyranthes aspera* seeds were selected and prepared the gel by using the plant extracts, different ingredients and MIC test was performed to examine the antibacterial property for dermal infections caused by *S. aureus* and *P. acnes*. Then, physical parameters of the prepared formulation were evaluated.

Sample:

Nigella sativa and *Achyranthes aspera* Plant extract

The seeds of *A. aspera* and *N. sativa* were collected. 30g of dry seeds were incubated in ethyl acetate, hexane, methanol (400-500 ml) individually in a shaker for 24 hours, and

seed extracts were concentrated using a rotary evaporator. Crude extracts were tested against *Propionibacterium acnes* and *Staphylococcus aureus* for antibacterial activity.

Preliminary extracts prepared from *A. aspera* and *N. sativa* seeds were tested for antibacterial property against *P. acnes* and *S. aureus*. The method of gel well diffusion was employed to examine the bacteriostatic property against *S. aureus* in the manner as suggested by Soyza et al. [3]

Bacterial inoculums of *P. acnes* and *S. aureus* were introduced in separate Mueller Hinton Agar (MHA) plates. McFarland 0.5 standard was used to adjust the bacterial suspension turbidity. In these cultivation plates, then wells (4mm in depth and 5mm in diameter) were prepared by using the sterilized cork borer. 40ml of each solution (Dichloromethane extract (25 mg/ml), hexane, ethyl acetate in 3% DMSO) were filled in wells separately. Inhibition zone was measured after incubation overnight at 37°C. DMSO and Co-amoxiclav were used as test controls. Due to the substantial antibacterial activity, > 5 mm zones were identified as inhibitions. Experiments were conducted as duplicates and mean value of inhibition zone diameter was calculated.

On the basis of zone inhibition test results, solvent for extraction scale-up was chosen. The culture broth dilution method was used to detect the minimum concentration of inhibition. To prevent bacterial growth lowest concentration of a measuring agent was used and its concentration was classified by resazurin. MIC values determined by microbial sub-culturing recommended MBC values also. Antibacterial activity was evaluated against *P. acnes*, *S. aureus* and ethyl acetate was chosen for scale-up extraction using *N. sativa* and *A. aspera* seeds.

600 gm of the dried seeds were extracted with ethyl acetate in a shaker for the 24 hours. Then, solvents evaporated by using the rotary evaporator. With this extract, topical gel was formulated.

Optimization of Topical Gel Formulation

Two formulations were optimized by combining *N. sativa*, *A. aspera* seeds extracts in ethyl acetate at varying concentrations (Table 1). The compositions were considered by studying amount of *N. sativa* and *A. aspera* used in herbal compositions traditionally used.

Table 1: Composition of gel formulations abbreviated as F1 and F2

Constituent name	Weight of constituents (in grams/100 grams)	
	F1	F2
Carbopol 940	1.20	1.20
Sodium benzoate	1.10	1.10
Glycerin	4.00	4.00

Poly-ethylene glycol (PEG)	0.06	0.06
Tri-ethanol-amine	Sufficient Amount	
Fuller's earth	0.09	0.09
EDTA	0.10	0.10
Cucumber water	Sufficient Amount	
<i>N. sativa</i> extract	6.00	12.00
<i>A. aspera</i> extract	6.00	12.00

The combination of ethyl acetate extract concentrations for making therapeutic gels was ensured as an added advantage for bacteriostatic effect, by the agar well diffusion method. Antibacterial efficiency of extract at different concentrations were assessed against *P. acnes* and *S. aureus* (erythromycin -positive test control).

Anti-acne gel was developed using carbopol 940, EDTA, glycerin, cucumber water, sodium benzoate, fuller's earth, triethanolamine, PEG. Carbopol amount was liquefied by incubating it in cucumber water until it was completely hydrated. Glycerin, EDTA, sodium benzoate, cetyl alcohol, cucumber water, polyethylene glycol (PEG), earth fuller, and tri-ethanol-amine were mixed with carbopol solution using vortex. The seed extract was subsequently added in this solution at varying concentration.

Studies for antibacterial activity in gel formulations

To assess the antibacterial activity against *S. aureus* and *P. acnes*, the formulations were prepared by using methanol as solvent. The process of agar gel well diffusion was used, wherein a synthetic commercial anti-acne gel was used as positive test control, and the gel base, methanol solvent were used as negative test controls. The MIC was then calculated in 96-well micro-titre plates by the process of broth micro-dilution. The test was carried out in triplicate.

Under anaerobic conditions, the agar gel well diffusion assay was employed to assess antibacterial activity against *P. acnes*. Gel wells (6 mm X 5 mm as diameter X depth) were prepared in the blood agar plates using a steam sterilized cork borer aseptically inoculated with *P. acnes* culture. The wells were loaded with test gel formulations, and plates were incubated anaerobically at 37 ° C for 48 hours, and the inhibition zones were calculated after the incubation.

Examination of long term physical and antibacterial stability of developed gels

The reliability of the physical attributes (color, odor, wash ability, purity, and pH) of both formulations was measured on 30th day after the gel was made (storage conditions: temperature 28 ± 3 degree Celsius and relative humidity 76 ± 4%). Similarly, the antibacterial property against *S. aureus* was determined on 30th day after formulation was made, to decide if gel formulations can maintain antibacterial potency during storage.

RESULT

Preliminary Crude Extract screening for Antibacterial

activity

The maximum effect on *S. aureus* was observed in the ethyl-acetate sample, with an inhibition zone of 10 ± 0.0 mm in diameter, among the three crude extracts evaluated. For both hexane and methanol extracts the inhibition zones of 6 ± 0.0 mm in diameter were detected. Co-amoxiclav, the positive control exhibited an inhibition zone of 29 ± 0.0 mm while no inhibition zone was found for negative test control, 3% DMSO. In addition, the MIC value of $32.36 \mu\text{g mL}^{-1}$ represented this ethyl acetate extract's high antibacterial potency which is somewhat similar to that of the positive test control co-amoxiclav with MIC value as $6.7 \mu\text{g mL}^{-1}$.

The extract of ethyl acetate introduced into the gel base at two different concentrations, was found capable of inhibiting *S. aureus* and *P. acnes* growth as shown by characteristic inhibition zones around the wells agar plates.

Gel-formulated antibacterial activity

Formulation F2 demonstrated the maximum activity against both species with 10% of the seed extract. Negative test controls, i.e. gel base and methanol, showed no inhibition while inhibition zones with a diameter of 7.8 ± 1.5 and 9.1 ± 0.5 were observed for *S. aureus* and *P. acnes*, respectively in case of positive test control synthetic commercial anti-acne gel product.

Table 2: Antibacterial activity of two gel formulations against the *S. aureus* and *P. acnes* evaluated through zone inhibition method. Formulation F2 displays the maximum antibacterial activity in comparison with other formulation.

Microorganism name	Diameter of inhibition zone (mm)			
	F1	F2	Positive test control	Negative test control
<i>Staphylococcus aureus</i>	7.0 ± 0.5	8.6 ± 0.8	7.8 ± 1.5	0.0 ± 0.0
<i>Propionibacterium acnes</i>	6.9 ± 0.0	10.2 ± 0.2	9.1 ± 0.5	0.0 ± 0.0

For F1, F2 MIC values of formulations against *S. aureus* were evaluated as 240, $61.8 \mu\text{g/mL}$. Interestingly, the synthetic commercial anti-acne gel showed less MIC value i.e. $120 \mu\text{g/mL}$, suggesting better antibacterial property of F2 as compared to positive test control. Although the same MIC values were observed against *S. aureus* for F2, the diameter of the inhibition zone was higher in F2, as observed in the agar well diffusion.

Long term physical and antibacterial stability of gel formulations

All two gel formulations demonstrated significant stability over an experimental period of 30 days, without any change in the initial physical parameters. Interestingly, the findings showed that, during the storage period, the antimicrobial ability of these gels against *S. aureus* persisted as evidenced by presence of inhibition zones of 10.2 ± 0.5 , 10.9 ± 0.6 mm for formulations F1, F2 respectively.

Table 3: Physical attributes of gel formulation at initial day and 30th day. Prepared formulations showed good stability.

Formulation no.	Number of days after production	Color	Odor	Homogeneity	Water washability	Consistency	pH
F1	1	Pale yellow	Pleasant	✓	✓	Semisolid	4-5
	30	No change	No change	✓	✓	Semisolid	No change
F2	1	Dark Yellow	Pleasant	✓	✓	Semisolid	5-6
	30	No change	No change	✓	✓	Semisolid	No change

DISCUSSION

In the present research, combination of *Nigella sativa* and *Achyranthes aspera* was used to optimize a gel formulation for an effective remedy against *acne vulgaris*. *Nigella sativa* and *Achyranthes aspera* possess significant antibacterial properties. Although topical gels were prepared but takes a long time to recover which may render the skin exposed to contaminants for long duration of time. Therefore, in the present research it was found that combination of *Nigella sativa* and *Achyranthes aspera* showed larger inhibition zone in comparison with available commercial gel and the physical evaluation of gel showed significant result.

CONCLUSION

For various parameters including the antibacterial activity, the topical gel formulations of *N. sativa* and *A. aspera* seed extracts were successfully formulated and evaluated. The results indicate that the formulations prepared have a strong antibacterial potency against acne-causing bacteria and that the activity was prominent in the formulation of 10% extract composition. Interestingly, this formulation's antibacterial potency efficiently surpassed that of the synthetic commercial anti-acne formulation. The present investigation thus revealed the potential for the development of commercial products with *N. Sativa* and *A. aspera* to manage *acne vulgaris*.

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