VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 1179-1187 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

An Investigation into Development and Testing of Herbal Acne Gel Containing *Vigna radiata* and *Aloe barbadensis*

Sethi Pranshul[1], Thilagam E.[2], Bhaskar Reddy K.[3], Varalaxmi S.[4], Sudhahar Dharmalingam[5], Varghese Nina[6] and Jamal Roohi[7]*

[1]Department of Pharmacology, College of pharmacy, Shri Venkateshwara University, Gajaraula, Uttar Pradesh 244236, India
[2]Department of Pharmacognosy, JKKMMRF's Annai JKK Sampooorani Ammal College of Pharmacy, Komarapalayam 638183, India
[3]Apollo Institute of Pharmaceutical Sciences, The Apollo University, The Apollo Knowledge City Campus, Saketa, Murukambattu, Chittoor 517127, Andhra Pradesh, India
[4]Department of Pharmaceutics, MB School of Pharmaceutical Sciences (Sree Vidyanikethan College of Pharmacy) MB University, Rangampet, Tirupati, Andhra Pradesh 517102, India
[5]Department of Pharmaceutical Chemistry and Analysis, Nehru College of Pharmacy (affiliated to Kerala University of Health Sciences, Thrissur) Pampady, Nila Gardens, Thiruvilwamala, Thrissur Dist, Kerala 680588, India
[6]Spurthy College of Pharmacy, No 328, Marasur Gate, Anekal Main Road, Bangalore 562106, Karnataka, India
[7]Allana College of Pharmacy Pune, DPOs Nett College of Pharmacy, Thane West, Maharashtra 411001, India

**Corresponding Author*

Received: 2nd November, 2023; **Accepted:** 24th November, 2023; **Published online:** 28th November, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.131

Abstract: Long-term inflammation of the pilosebaceous unit caused by acne vulgaris can result in inflammatory lesions, seborrhea, comedones, and other conditions. It has been established that *Staphylococcus epidermidis* and *Propionibacterium acnes* are the pus-forming bacteria that cause acne inflammation. The bacteria *Staphylococcus aureus* contributes to acne inflammation. Natural medications are more readily accepted than their synthetic counterparts because it is generally believed that natural medicines have fewer adverse effects. On the global stage, there has been a recent uptick in inquiries for herbal preparations. The purpose of the present investigation was to develop and evaluate a herbal anti-acne gel that includes *Aloe barbadensis* and *Vigna radiata* ethanolic extract as active ingredients. Three formulations (F1, F2, F3) utilizing extracts of aloe barbadensis and vigna radiata, as well as a combination of these two extracts, were created in order to optimize the herbal anti-acne gel. A number of factors, including the formulation's physical appearance, pH, drug content, spreadability, and extrudability, were assessed. It was successfully researched how anti-acne activity assay against *S. aureus* works. Out of all the formulations examined, batch F3 was determined to be the most optimal for every parameter. When combined, the ethanolic extracts of *Aloe barbadensis* and *Vigna radiata* have the potential to be effective against *Acne vulgaris* and to work in concert with the bacteria.

Keywords: *Acne vulgaris*, *Vigna radiata*, *Aloe barbadensis*, Acne gel, Herbal

Citation: Sethi Pranshul, Thilagam E., Bhaskar Reddy K., Varalaxmi S., Sudhahar Dharmalingam, Varghese Nina and Jamal Roohi: An investigation into development and testing of herbal acne gel containing *Vigna radiata* and *Aloe barbadensis.* Intern. J. Zool. Invest. 9(2): 1179-1187, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.131

Introduction

Acne can be inherited or acquired; its name comes from the Greek word "akme," which can be translated as "peak" or "apex." Acne affects the pilosebaceous units of the skin. Acne vulgaris is the medical term that should be used when referring to acne. Acne is the most common skin ailment seen in people of childhood age, which is commonly between the ages of 18 and 25 (Chellathurai *et al.*, 2023). Acne vulgaris is a common kind of acne that manifests on the skin and is caused by an overactive sebaceous gland. In addition to the formation of seborrhea, comedones, inflammatory lesions, and the production of sebum, it is defined by the presence of the bacteria *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Staphylococcus aureus* in the follicular canal (Ramya, 2019). Seborrheic dermatitis is a common form of atopic dermatitis that affects the skin. It is a disease that affects practically everyone, since 95 per cent of males and 83 per cent of females of all racial groupings are affected by it. It has been asserted that *P. acnes* is a microorganism that must be anaerobically adapted to survive (David *et al.*, 2022). It is associated to the beginning of inflammatory acne because of its potential to activate complements and convert sebum triglycerides into fatty acids, both of which chemoattract neutrophils. On the other hand, infections of a superficial nature in the sebaceous unit are often brought on by the anaerobic pathogen *S. epidermidis* (Nandagopal *et al.*, 2011). *Staphylococcus aureus* flourishes and contributes to the development of acne lesions as a result of the production of chemicals by *P. acnes* that disrupt the cellular structure of skin cells (Ahire *et al.*, 2020). These constituents have the opportunity for a therapeutic target. *P. acnes*, *S. epidermidis*, and *S. aureus* are the bacteria that are targeted by acne medicines. Acne-causing bacteria like *S. epidermidis*, *S. aureus*, and *P. acnes* have become more resistant to antibiotics as a result of prolonged and improper usage of antibiotics (Kora, 2023). Antibiotic resistance is the result of a complex interaction between a number of factors, including the type of bacteria that are associated with antibiotics, the manner in which antibiotics are employed, the characteristics of the host, and the conditions of the environment (Pawar *et al.*, 2023). In an effort to combat the problem of antibiotic resistance, a great number of studies have been carried out on medicinal plants as possible alternatives to the current standard medical treatments for diseases (Ahire *et al.*, 2023). The antibacterial and anti-inflammatory characteristics of medicinal herbs that have been used for a long time, such as garlic and neem, were tested against bacteria that typically cause acne inflammation, such as *Staphylococcus aureus*. The results showed that medicinal herbs such as garlic and neem were effective against these bacteria. Neem and garlic juice should not be applied directly on acne because doing so can cause irritation (Shukla *et al.*, 2023). As a result, it is necessary to produce topical dosage forms such as a gel. The gel is simple to absorb, it imparts a chilly sensation to the skin, and it generates a film that is straightforward to remove (Dlova and Ollengo, 2018). The fundamental objective of this investigation was to formulate a herbal anti-acne gel containing *Aloe barbadensis* and *Vigna radiata* and evaluate its efficacy through the application of preliminary research methods (Dhama *et al.*, 2018).

Materials and Methods

Aloe barbadensis leaves were gathered from a medicinal garden and *Vigna radiata* seeds were procured from local market of Thane.

Vigna radiata extraction:

After the *Vigna radiata* seeds were collected, they

were further broken down into extremely minute pieces using a pulverizer. The powder of roughly 500 g of crushed *Vigna radiata* was subjected to a hot extraction procedure, during which ethanol was used as the solvent and a Soxhlet equipment was utilized (Upadhyay *et al.*, 2010). After several repetitions, the operation was carried out until the solution in the thimble became transparent. After that, a vacuum desiccator was utilized in order to dry the extract (Kumari *et al.*, 2022).

Aloe barbadensis gel collection:

The *Aloe barbadensis* plant's fresh leaves were collected. The leaf's inner, gel-like pulp was carefully removed while the thick epidermis on its surface was painstakingly peeled away. The pulp was then divided, diced, and homogenized with a mortar and pestle. To achieve a translucent liquid, muslin fabric was used as a filter (Byamukama *et al.*, 2021).

Evaluation of extract of the Vigna radiata:

Characterization of extracts of the Vigna radiata:

The ethanolic extracts of *Vigna radiata* were observed in terms of their shape, color, smell, and taste. The color was also taken into account (Tshikhudo *et al.*, 2023).

Standardization of Inoculum:

Agar plates with steps of dilution were used for this. Dilution of the culture ten times, from 10-1 to 10-10, was done by carefully moving 1 milliliter (ml) of the bacterial suspension tube to a set amount of sterile water. After the bans were watered down, they were slowly turned into Muller Hinton Agar media. After being kept at 37°C for 24 hours, a colony clock was used to count them (Ahire *et al.*, 2023). Plates that can be counted must have at least 30 colonies on them. To get the total count of the suspension, increase the number of cells on each plate by the dilution factor, which is the opposite of the dilution (Ahire *et al.*, 2022).

Cup plate method for evaluation of Antimicrobial activity:

Test organisms, such as *Candida albicans*, *Escherichia coli*, or *Staphylococcus aureus*, were diluted correctly before being introduced to the Muller Hinton Agar medium that had been applied to the sterile petri dishes. With the use of a sterile borer, the medium on each plate has been fashioned into the shape of four cylinders or cups. The *Vigna radiata* extract was prepared at different concentrations, as well as a standard disc, a solvent control, and a sample of each (Ribeiro *et al.*, 2017). After the cup had been evenly stuffed with 0.2 ml of the solution, it was placed in an incubation chamber and heated to 37 °C for 24 h. The level of antibacterial activity was determined by taking the mean of the inhibition in diameter (mm) for the well diffusion test, which was performed three times (Augustine and Hasan, 2020).

Optimization and formulation of gelling agent:

It is possible to make clear gels with carbopol, which dissolves in water and works well as a thickener for gels. To get the gel consistency and spreadability that was wanted, several different amounts of Carbopol 940 were tried out and analyzed. Some of these amounts were 1%, 1.5%, and 2%. The concentration was then got just right (Qadir and Raja, 2021).

Gel base Formulation:

The gelling powder was spread out with enough water. It was mixed with propylene glycol-400, which is a humectant or softener. Extra ingredients, such as propyl and methyl paraben, were added while the mixture was being mixed all the time. TEA (Triethanolamine) was used to make the medium in Carbopol gels (pH 7). It was possible to get the gel's weight down to 50 g by adding pure water (Singh *et al.*, 2016).

After that, a rotor was used to stir the mixture for 2 h at 500 rpm. This gel did not seem to have any bubbles after it was stirred. To test how stable and consistent it was, the gel was left at room temperature for a whole day (Surana and Mahajan, 2022). The carbopol gel mixture is shown in Table 1.

Table 1: Formulation of Carbopol gel

Excipients	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)
Carbopol (940)	1.0%	1.50%	2.0%
Propylene glycol (PG)	5.0 ml	5.0 ml	5.0 ml
Methyl paraben (Preservative)	0.20 g	0.20 g	0.20 g
Propyl Paraben (Preservative)	0.30 g	0.30 g	0.30 g
Triethanolamine (TEA)	5.0 ml	5.0 ml	5.0ml
Vehicle (Water)	Quantity sufficient	Quantity sufficient	Quantity sufficient

Table 2: Formulation of polyherbal gel

Formulation ingredients	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)
Vigna radiate extract	1.0%	1.50%	2.0%
Aloe barbadensis gel	5.0 ml	5.0 ml	5.0 ml
Carbopol (940)	2.0 %	2.0 %	2.0 %
Propylene glycol (PG)	5.0 ml	5.0 ml	5.0 ml
Methyl paraben (Preservative)	0.20 g	0.20 g	0.20 g
Propyl Paraben (Preservative)	0.30 g	0.30 g	0.30 g
Triethanolamine (TEA)	5.0 ml	5.0 ml	5.0 ml
Vehicle (Water)	Quantity sufficient	Quantity sufficient	Quantity sufficient

Polyherbal gel Formulation containing Aloe barbadensis and Vigna radiata:

To make a polyhedral gel, *Aloe barbadensis* juice and *Vigna radiata* ethanolic extract were added to better Carbopol gel. The liquid *Aloe barbadensis* and the water needed to thin out the carbopol are both included (Pal *et al.*, 2022). On the other hand, the Carbopol dispersion had the exact amounts of propylene glycol-400, methylparaben, propyl-paraben, and ethanolic extracts of *Vigna radiata* (1.0%, 1.50%, and 2.0%). Drop by drop, triethanolamine was added to the mixture to get it to the right pH for skin (6.8–7) and the right gel consistency. After that, a rotor was used to stir it for 2 h at 500 rpm. After being stirred, the glue that was made looked smooth and free of air bubbles. The gel that was made was kept at room temperature for a whole day (Caruso *et al.*, 2022). Table 2 illustrates the formulation of polyherbal gel.

Evaluation of formulated polyherbal gel:

Factors like the color, look, pH, viscosity, and ability to spread were tested for the polyherbal gel that was made. It was also tested for its ability to kill germs, its stability, and its ability to cause skin irritation in living things (Vanlalveni *et al.*, 2021).

Standardization of Inoculum:

The agar plate method with stepwise reduction was used to do this. The method was to carefully move 1 ml of the bacterial solution from the tube to a known amount of clean water.

The society stayed 10 times less concentrated, from 10^{-1} to 10^{-8}, because of how this was done. In order to make the solutions less concentrated, they were slowly poured into nutrient agar media. After being left to grow at 37°C for 24 h, a colony clock was used to keep track of how many colonies there were. Plates can only be counted if they have at least 30 colonies and no more than 300 colonies. To find out how many cells are in the suspension as a whole, take the amount of cells on each plate and multiply it by 1 (Fadiji *et al.*, 2020).

Cup plate method for evaluation of Antimicrobial activity:

A small amount of a test organism either *Escherchia coli*, *Staphylococcus aureus*, or *Candida albicans* was mixed with Muller Hinton Agar in clean Petri dishes. A sterile borer was used to make four spheres or cups in the medium for each plate. The normal disc, the solvent control, and the polyherbal gel formulation were all ready. Equal amounts of 0.2 ml of fluid were put into the cup, and it was kept at 37 ℃ for 24 h. The mean of the inhibition in width (mm) for the well diffusion test, which was done three times, was used to measure the antibacterial activity (Haleem, 2019).

Results and Discussion

Evaluation of extracts of Vigna radiata:

Characteristics of the extracts:

Table 3 listed the ethanolic extract of *Vigna radiata*'s physical condition, color, flavor, and taste.

Table 3: Characteristics of the extract

Properties	Observation
Physical Nature of the extract	Jelled
Color of the extract	Greenish
Odor of the extract	Pleasant
Taste of the extract	Specific

Phytochemical study of the extract:

Phytochemistry was used to look at the extract of *Vigna radiata* and found flavonoids, phenolics, and other parts. It has been shown that some of these chemicals are very good at killing bacteria, lowering blood sugar, and lowering high cholesterol.

Anti-microbial activity of the extract:

In order to evaluate the effectiveness of the antibacterial treatment, the diameter of the zones of inhibition (expressed in millimeters) was measured and compared. The term "zone of inhibition" refers to the area that encompasses the well and has been treated with an antimicrobial agent. It is well knowledge that the effectiveness of an antimicrobial agent rises along with the size of the zone it can inhibit. The antibacterial capabilities of an extract of *Vigna radiata* were examined using a variety of standard organisms, such as fluconazole (25 mcg) and gentamicin (10 mcg). Additionally, the extract was examined using *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. According to the findings, there was no indication that the product had been contaminated by microorganisms, and the zone of inhibition was satisfactory, although being smaller than the standard. In order to evaluate the effectiveness of the antibacterial treatment, the diameter of the zones of inhibition (expressed in millimeters) was measured and compared. The term "zone of inhibition" refers to the area that encompasses the well and has been treated with an antimicrobial agent. It is well knowledge that the effectiveness of an antimicrobial agent rises along with the size of the zone it can inhibit. The antibacterial capabilities of an extract of *Vigna radiata* were examined using a variety of standard organisms, such as fluconazole (25 mcg) and gentamicin (10 mcg). Additionally, the extract was examined using *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. According to the findings, there was no indication that the product had been contaminated by microorganisms, and the zone of inhibition was satisfactory, although being smaller than the standard.

Gelling Agent Optimization:

In order to manufacture gel with the desired physical qualities, a number of different carbopol-940 concentrations, including 1.0%, 1.50%, and 2.0%, were tested and optimized. When ethanolic extracts of *Aloe barbadensis* and *Vigna radiata* are added to a 2.0% concentration of carbopol gel, the gel has favorable physicochemical features.

Polyherbal gel Formulation containing Aloe barbadensis and Vigna radiate:

Aloe barbadensis and *Vigna radiata* were both included in the polyherbal gel that was mixed into the optimum 2.0% Carbopol gel foundation. Carbopol gel basis was formulated with varying percentages of ethanolic extract of *Vigna radiata*,

Table 4: Physical nature of formulated gel

Properties	**F1 (1.0%)**	**F2 (1.50%)**	**F3 (2.0%)**
Physical Nature of gel	Yellow gel	Yellow gel	Yellow gel
Color of gel	Yellow	Yellow	Yellow
Homogeneity of gel	No aggregation	No aggregation	Less aggregation

Table 5: pH Measurement

Formulation	**pH**
Formulation F1	5.80
Formulation F2	5.60
Formulation F3	5.70

Table 6: Viscosity Measurement

Formulation	**Viscosity [cps]**
Formulation F1	1429±0.10
Formulation F2	1426±0.76
Formulation F3	1359±0.26

including 1.0%, 1.50%, and 2.0% respectively. The concentration of *Aloe barbadensis* was maintained at a fixed level (5 ml) throughout the Carbopol gel base.

Evaluation of Antiacne Gel:

Physical Evaluation:

The results of a visual inspection of the gel after it had been produced regarding its color, appearance, and homogeneity are presented in Table 4.

pH Measurement:

The pH of all the formulations that were made was between 5.60 and 5.80. The pH of the developed gel mixture was thought to be just right so that it wouldn't irritate the skin when it was applied. The results are shown in Table 5.

Viscosity Measurement:

The basic property of a fluid that determines how slowly it moves and is linked to friction inside the fluid is called viscosity. This rheological property helps figure out how thick the gel is and how fast the medicine is spreading. The prepared gel's viscosity was checked with a Brookfield viscometer with spindle number 62. The outcomes are displayed in Table 6. It is possible to attain the benefits of more aesthetically pleasing properties and easier application accuracy through better flow and pourability by maintaining the viscosity below roughly 15,000 cps.

Spreadability of gel:

The term "spreadability" refers to the region that the gel easily covers when applied to the skin or the affected portion. The spreading was measured in terms of the number of seconds it took for two slides to separate from the gel that was positioned in between them when subjected to a specific force. Better spreadability results from a shorter time required to separate the two slides. Standard-sized glass slides were taken in two sets. One of the slides was covered with the gel formulation. The spreadability of several gel formulations was investigated. In comparison to the other formulation, the formulation F2 produced better spreadability. The outcomes are displayed in Table 7.

Table 7: Spreadability Measurement

Formulation	Spreadability (g.cm/sec)
Formulation F1	19.40
Formulation F2	21.36
Formulation F3	22.15

Table 8: Gel strength of formulation

Bloom value (Gel strength) (g)	Force at target (kg)	Gradient to positive peak kg/sec
0.700	0.020	0.005

Table 9: Extrudability of gel

Firmness (g)	Force at target (kg)	Force at 5mm (kg)
3169.700	3.370	3.230

Table 10: formulated Polyherbal gel Zone of inhibition

	Organisms											
	S. aureus			Mean (mm)	*E. coli*			Mean (mm)	*C. albicans*			Mean (mm)
	a	b	c		a	b	c		a	b	c	
F2	11.9	11.8	11.9	11.9±0.1	12.9	12.9	12.9	12.9±0.1	12.4	12.3	12.4	12.4±0.1
Gentamicin	20.1	20.2	20.1	22.1±0.1	21.2	22.2	22.2	22.2±0.1	-	-		
Fluconazole	-	-			-	-			22.1	22.2	22.2	22.2±0.1

Optimized Formulation Selection:

Gel needs to have the perfect properties and be stable over time in order to have effective skin penetration. It was determined that formulation F2 was optimal based on the outcomes of the stability studies and physical parameters like viscosity, pH, and spreadability. It was selected for additional characterization, including texture analysis, antimicrobial activity testing.

Gel strength:

The gel strength of a colloidal dispersion shows how well it can make and keep a gel shape. In the world of gelatin, gel strength is often called "Bloom." Based on a standard 0.5" diameter cylinder probe, this is how much force, in grams, is needed to press down on the top of a gelatine gel by 4 mm. This amount of force is often called "the force needed to break the gel." The Polyherbal gel mixture had a value of 0.0160 kg. The bloom strength of a gel shows how hard it is to penetrate. Table 8 mentions the gel's graph and bloom strength value.

Extrudability:

Rheological properties, such as spreadability and hardness have a role in determining how formulations act *in vivo* in the skin. In addition to this, the viscosity of the gel had an effect on how easily it could be extruded. A test was also done on the extrudability of gel to find out how much compression force the piston needs to push a

product through a standard-sized opening and into the base of the container. In Table 9, the information about the extrudability numbers and the graph is added. According to the data, an amount of force equal to 3.401 kilograms is required to force the gel through the outlet. The peak or maximum force was used to calculate the firmness measurement; the higher the value, the more substantial the sample's consistency was. When the probe was brought back, there were no negative spots to suggest that the graph was inconsistent or resistant to flow off the disk. The extrudability of the gel that was produced is excellent.

Cup plate method for evaluation of Antimicrobial activity:

It was measured and compared the width of the inhibition zones (in millimeters) to see how well they killed bacteria. The clear area around the well that has an antimicrobial drug in it is called the zone of inhibition. It is well established that an antimicrobial agent's potency increases with its zone of inhibition. The antibacterial properties of the prepared polyherbal gel (F2) were assessed against conventional organisms like Fluconazole (25 mcg) and Gentamicin (10 mcg), as well as organisms like *Escherchia coli*, *Staphylococcus aureus* and *Candida albicans.* According to the results, the zone of inhibition was good although smaller than the standard. Table 10 listed the formulated gel's zone of inhibition.

Conclusion

With the help of *Vigna radiata* and *Aloe barbadensis*, a safe and effective multiherbal gel is being made to treat acne, which is a skin problem that doesn't go away. Extra gel from *Aloe barbadensis* and an ethanolic extract of *Vigna radiata* were added to the better Carbopol gel base to make it even stronger. These two plant ingredients may help lessen the severity of acne by working together in a way that makes them stronger. An antibacterial test was done, and the results showed that there were no microbial contaminants and that the zone of inhibition was good. In the same way, an *in vivo* study of skin irritation did not find any skin ulcers. These skin blemishes could be loss of skin fat, bad skin reactions, or changes that only happen in one area of the body. The study's results suggest that the formulation of polyherbal gel may provide a safe and effective dose form that makes patients more likely to follow through with their treatment. All of the study's data were taken into account to come to this result.

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