Formulation and evaluation of antibacterial gel containing ethanol extract of thorns of *Bombax ceiba*

prashant chauahn (✉ chauhanprash8@gmail.com)

noida institue of management & technology

Dr. Monika

noida institue of management & technology

Research Article

Keywords: Bombax ceiba, Thorn's, Herbal gel formulation, Antibacterial activity, Evaluation.

Posted Date: January 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1260062/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Purpose

Formulation and evaluation of the herbal gel preparation from the thorn's extract of *Bombax ceiba* to check its antibacterial activity against the bacteria *Staphylococcus aureus* and *Propionibacterium acnes*

Methods

Agar well diffusion method were employed for the purpose.

Results

Gel formulation of different concentration of extract were formulated that is 2%, 4%, 6% and 8% respectively and antibacterial activity of the gels were measured against the bacteria *Staphylococcus aureus* and *Propionibacterium acnes*. In this clindamycin gel was used as the standard for comparative analysis. From evaluation results it was concluded that formulation of 8% showed better antibacterial activity as compared to other formulated preparations. In addition to this, evaluation of gel formulations was performed considering various parameters which were pH, appearance, viscosity, spreadability and homogeneity and the result were calculated.

Conclusion

The ethanol thorn' extract of *Bombax ceiba* possess good antibacterial property against the *Staphylococcus aureus* and *Propionibacterium acnes*. It also contains various phytoconstituents which may be helpful in various health related problems.

Introduction

Acne vulgaris is one of the most frequent skin⁽¹⁾ illnesses in teenagers, with a frequency of 80-90 percent, and in cases of acute disfiguration, it can have serious effects for young people's personality development, which is linked to a high psychological distress⁽²⁾. Many patients do not cure with present anti-acne therapy due to high costs, side effects that cause non-compliance, or a lack of therapeutic benefits from current antibiotics, all while clinically beneficial drugs face substantial hurdles like liver functioning problems, kidney damage, ear poisoning and many more⁽²⁾. As a result, in the area of antimicrobial therapy, emphasis has been placed on safer, novel, and harmless alternative antimicrobial ingredients. So, some actions needs to be taken to address the issues regarding the current antibacterial treatment which include the understanding the use of antibiotics or investigating the resistance of various antibiotics and development of the new antibacterial products or formulations form the natural

sources which will show very less or negligible side effects as compared to antibiotics⁽³⁾. As it is known that, Topical medication administration is the most effective method for treating skin disorders⁽⁴⁾. The efficiency of topical treatment is mostly determined by the pace and extent of drug release. A topical drug delivery system designed to deliver a range of medications to the body through diffusion throughout the skin layers⁽²⁾.

For this study thorns of *Bombax ceiba* plant were used to be formulated into gel, as it is known to have antibacterial property.

The antibacterial activity of the gel formulated were observed on the bacteria: *Staphylococcus aureus* and *Propionibacterium acne*. The antibacterial activity of the gel formulated was measured through agar well diffusion method and the zone of inhibitions were measured in triplicates and the mean value was calculated.

Materials And Methods

Materials: Carbapol 940, methyl paraben, propylene glycol 400, EDTA, triethanolamine, distilled water, ethanol

Methods:

Collection of the plant material: Thorns of *Bombax ceiba* were collected from the local area of the north-west Delhi region and soon after, that collected material was washed to remove the dirt and foreign particles present on it⁽⁵⁾. After washing the plant material was converted into minute pieces by cutting down and shade dried. After that, the plant material was collected and converted into fine powder form with the help of mechanical grinder and passed through the sieve of 40 to get the desired powder size⁽⁶⁾.

Preparation of the extract: The extract was prepared by maceration process. The powdered plant material was weighed accurately 5 g and to it 100 ml of ethanol was added (ratio 1:20) in a beaker⁽⁵⁾. The beaker was kept for 72 hours with continuous stirring for initial few hours. Then plant material was filtered out through Whatman filter paper and the collected portion was kept in hot air oven for drying and after drying the ethanolic extract of the plant material was collected which is the main ingredient of the gel formulation⁽⁶⁾.

Determination of the phytochemical constituents present in the ethanolic extract:

In this study ethanol extract was subjected to qualitative chemical analysis for various phytochemical constituents like alkaloids, glycosides, terpenoids, saponins, tannins, phytosterols, flavonoids, carbohydrates, proteins⁷. Following tests are performed for the identification of phytochemical constituents:

Table 1
List of phytochemical constituents and the test performed for the identification

Phytochemical constituents	Test performed
Alkaloids	Mayer's test
	Wagner's test
	Hager'sb test
Glycosides	Brontrager's test
Terpenoids	Libermann-buchards's test
Saponins	Froth formation test
Tannins	Ferric chloride test
Phytosterol	Libermann-buchard's test
	Salkowski's test
Flavonoids	Shinoda test
Carbohydrates	Barfoed's tets
Proteins	Ninhydrin tets
	Biuret test

Isolation and identification of Acne Causing Bacteria From The Human Skin:

Bacteria that are responsible for causing acne (*staphhylococcus aureus*, *propionibacterium acnes*) were isolated from the human skin. Sample from the skin were taken by using sterile swab and tooth pick and allowed to grow in a freshly prepared media. After the incubation period of 24hours the bacterial growth was clearly visible and two different colonies were observed. Identification tests were performed for the identification of the cultured bacteria. PCR (polymerase chain reaction) technique and some biochemical test were performed for the identification⁽⁷⁾.

Formulation of Gel:

All the ingredients were collected as per the required amount to formulate the 50g gel preparation. For this, mixing of the formulation ingredients were done in two different beakers. Water was divided equally in two beakers, in the first beaker the required amount of plant extract was added and dissolved, to it

calculated amount of propylene glycol 400 was added and in another beaker, Carbopol 940 was added and dissolved and to it EDTA and methyl paraben were added and dissolved. After that both the solutions in the beaker were mixed in a single beaker and at last triethanolamine was added drop by drop to obtain the consistency of the prepared formulation⁽⁸⁾.

Table 2
Different compositions of the gel formulation prepared

INGREDIENTS	F1	F2	F3	F4
B.Ceiba Thorn's Extract (%)	2	4	6	8
Carbapol 940 (%)	1	1	1	1
Methyl Paraben (%)	0.2	0.2	0.2	0.2
Propylene Glycol 400(%)	5	5	5	5
EDTA (%)	0.03	0.03	0.03	0.03
Triethanolamine (%)	1.2	1.2	1.2	1.2
Distilled Water	Q.S.	Q.S.	Q.S.	Q.S.

Evaluation of the prepared gel formulation:

The evaluation of the prepared gel formulations was done on the basis of following parameters:

pH determination:

A pH meter was used for the determination of the pH of the prepared gel formulations.

Appearance and homogeneity:

Visual inspection were done in order to check the physical appearance and the homogeneity of the prepared formulations.

Viscosity:

It was measured using as Brookfield viscometer with spindle no. 6 at 100 rpm.

Spreadability:

It was measured by measuring the diameter of 1g of gel dispersed between two glassed slides.

Skin irritation test:

It was performed on 10 healthy volunteers comprise of both male and female. About 1gm of gel preparation were applied on the hand of all the volunteers and held for particular period of time. After 2 hours, the test area was observed for any visible signs which might be the result of skin irritation⁽⁹⁾.

Antibacterial evaluation:

Agar well diffusion method was used for this purpose.

Staphylococcus aureus and *Propionibacterium acnes* strains were used for the study. Bacterial cultures were poured to the freshly prepared nutrient media and stirred properly so that there would a uniform distribution of the culture all over the media. The media was poured in sterilized petri dishes and the media was stand still and allowed to solidify. Then, wuth the help of sterile cork borer wells were made in the petri dishes of 6mm diameter each, to which the prepared formulations were added and allow the drug to spread in the media⁽⁹⁾. Then it was incubated for 24 hours at 37⁰C. The diameter of zone of inhibitions were observed and with the help of ruler was measured (in mm). Each formulation's antibacterial activity was measured in triplicate form and their mean value was recorded. Here, in the study clindamycin gel was used as the standard drug for the comparision.

Results And Discussion

Qualitative Chemical Analysis:

In this study the list of phytochemical constituents that are present in the thorn's extract were identified. The result of the study have been shown below:

Table 3
List of phytochemical constituents found in the thorn's extract.

PHYTOCONSTITUENTS	TEST PERFORMED	OBSERVATIONS	INTERFERENCE
Alkaloids	Mayer's test	Yellowish white or creamy precipitate formed.	Present
	Wagner's test	Reddish brown precipitate formed.	
	Hager's test	Yellow colored precipitate formed.	
Glycosides	Brontrager's test	Light pink to red tint appeared	Present
Terpenoids	Libermann-buchard's test	Dark green tint	Present
Saponins	Froth formation test		Absent
Tannins	Ferric chloride test	Formation of brown tint	Present
Phytosterols	Libermann-buchard's test	Color change appeared	Present
Flavanoids	Shinoda's test	Reddish pink tint	Present
Carbohydrates	Fehlings test	Red precipitate formed	Present
Proteins	Biuret's test	Deep purple color obtained	Present

EVALUATION PARAMETERS OF GEL FORMULATIONS:

Both physical and microbial evaluation of the prepared gel formulation were performed. Gels were found to be have a transparent appearance and were light brown in color. Ph range of the gels were in the range of 6.45-6.50. When the gel were applied to the skin of the healthy volunteers it was found to be non-irritant. Microbial evaluation was measured in terms of formation of zone of inhibitions and Clindamycin was taken as the standard drug.

Table 4
Evaluation parameters of the prepared gel formulations*:

FORMULATIONS	pH	Appearance	Viscosity	Spreadability diameter after 1 min(mm)	Homogeneity
1	6.45	Light brown	4456	42	Good
2	6.47	Light brown	4478	40`	Good
3	6.42	Light brown	4478	43	Good
4	6.50	Light brown	4514	45	Good
*results are based on the mean value of of the three readings taken for each formulations.					

ANTIBACTERIAL ASSAY OF FORMULATION PREPARED:

Antibacterial assays were taken in triplicates for each formulation and at the end mean was taken out. Results for the antibacterial assay are as follows:

Table 5
Anti bacterial assay of the gel formulation prepared against the acne causing bacteria*:

Bacteria	Formulations	Zone Of Inhibitions (In Mm)
<i>Staphylococcus aureus</i>	F1	9.8±0.2
	F2	11.6±0.13
	F3	13±0.4
	F4	13.8±0.1
	Clindamycin	28.9±0.15
<i>Propionibacterium acnes</i>	F1	9.83±0.5
	F2	11.3±0.2
	F3	12.6±0.14
	F4	15.3±0.3
	Clindamycin	30.16±0.5
*this data contains the mean value of the triplicates of zone of inhibitions for the antibacterial activity.		

Staphylococcus aureus:

See Figure 3

Propionibacterium acnes:

See Figure 4

Zone of inhibitions: Zone of inhibitions for both the bacteria's are measured in triplicates and the results are as follows:

Staphylococcus aureus:

See Figure 5

Propionibacterium acnes:

See Figure 6

Zone of inhibition of standard drug (clindamycin):

See Figure 7

Conclusion

From the study it is concluded that the ethanol thorn' extract of *Bombax ceiba* possess good antibacterial property against the *Staphylococcus aureus* and *Propionibacterium acnes*. It also contains various phytoconstituents which may be helpful in various health related problems. Different formulations were prepared which contain 2%, 4%, 6% and 8% extract and the clindamycin gel was taken as the standard. From the results of antibacterial activity possessed by the gel formulations it was concluded that the gel with 8% of the extract of the total amount of formulation shows better activity among all other preparations.

Declarations

ETHICAL CLEARANCE:

No animals were used during the experiment.

CONFLICT OF INTEREST:

The author declares no conflict of interest.

SOURCE OF FUNDING:

The project was self funded.

References

1. Almekhlafi S, Thabit AAM. Formulation and evaluation of lomefloxacin HCl as semisolid dosage forms. Journal of Chemical and Pharmaceutical Research. 2014;6(3).
2. Harahap NI, Nainggolan M, Harahap U. Formulation and evaluation of herbal antibacterial gel containing ethanolic extract of mikania micrantha kunth leaves. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(3):429–31.
3. Keri JE. No Title [Internet]. Acne Vulgaris (Acne). 2020. Available from: <https://www.merckmanuals.com/professional/dermatologic-disorders/acne-and-related-disorders/acne-vulgaris>
4. Jaliman D. No Title [Internet]. 2021. Available from: <https://www.webmd.com/skin-problems-and-treatments/acne/understanding-acne-basics>
5. Patankar SP. Phytochemical Investigation and Pharmacological Evaluation of Aerial Parts of Salmalia malabarica (DC) Schot& Endl. for Antiacne Activity. 2005;(Dc).
6. Mukherjee PK. Quality control herbal drugs: An approach to evaluation of botanicals. Business Horizons, New Delhi. 2002;800.
7. Kishishita M, Ushijima T, Ozaki Y, Ito Y. New medium for isolating propionibacteria and its application to assay of normal flora of human facial skin. Applied and Environmental Microbiology. 1980;40(6).
8. Shahtalebi M, Asghari G, Rahmani F, Shafiee F, Jahanian-Najafabadi A. Formulation of Herbal Gel of Antirrhinum majus Extract and Evaluation of its Anti- Propionibacterium acne Effects. Advanced Biomedical Research. 2018;7(1).
9. Sharma M, Dev SK. PREPARATION AND EVALUATION OF ANTI-ACNE HERBAL GEL Biomedical European of AND Pharmaceutical sciences. 2018;(May).

Figures

Figure 1

Figure 3: Graphical representation of zone of inhibitions against *S.aureus*

Figure 2

Figure 4: Graphical representation of zone of inhibitions against *P.acnes*

Figure 3

Figure 5: (i), (ii), (iii) shows the zone of inhibitions against the *Staphylococcus aureus*

Figure 4

Figure 6: (i), (ii), (iii) shows the zone of inhibitions against *Propionibacterium acnes*

Figure 5

Figure7: (i) zone of inhibitions of standard drug clindamycin against *Staphylococcus aureus*

(ii) zone of inhibitions of standard drug clindamycin against *Propionibacterium acnes*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [floatimage1.jpeg](#)