

RESEARCH ARTICLE

Formulation and Evaluation of Antibacterial Gel containing Ethanol Extract of Thorns of *Bombax ceiba*

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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 concentrations of extract were formulated that is 2, 4, 6 and 8%, respectively and antibacterial activity of the gels were measured against the bacteria *S. aureus* and *P. acnes*. In this clindamycin gel was used as the standard for comparative analysis. It was concluded from evaluation results that a formulation of 8% showed better antibacterial activity than other formulated preparations. In addition to this, gel formulations were evaluated considering various parameters such as pH, appearance, viscosity, spreadability and homogeneity and the result were calculated.

Conclusion: The ethanol thorn extract of *B. ceiba* possess good antibacterial property against the *S. aureus* and *P. acnes*. It also contains various phytoconstituents which may be helpful in various health-related problems.

Keywords: Antibacterial activity, *B. ceiba*, Evaluation, Herbal gel formulation, Thorn.

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INTRODUCTION

Acne vulgaris is one of the most frequent skin¹ illnesses in teenagers, with a frequency of 80–90%, and in cases of acute disfigurement, 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 the *Bombax ceiba* plant (Figure 1) were used to be formulated into gel, as it is known to have antibacterial properties (Figure 2).

The gel's antibacterial activity was observed on the bacteria: *Staphylococcus aureus* and *Propionibacterium acnes*. The antibacterial activity of the gel formulated was measured through agar well diffusion method, 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 *B. ceiba* were collected from the local area of the north-west Delhi region and soon after, that collected material

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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's test
Glycosides	Brontrager's test
Terpenoids	Liebermann-buchard's test
Saponins	Froth formation test
Tannins	Ferric chloride test
Phytosterol	Liebermann-buchard's test
	Salkowski's test
Flavonoids	Shinoda test
Carbohydrates	Barfoed's test
Proteins	Ninhydrin test
	Biuret test

Table 2: Different compositions of the gel formulation prepared.

Ingredients	F1	F2	F3	F4
<i>B. Ceiba</i> 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.

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, and proteins (Table 1).⁷ Following tests are performed for the identification of phytochemical constituents

Isolation and Identification of Acne Causing Bacteria from the Human Skin

Bacteria that are responsible for causing acne (*S. aureus*,



Figure 1: Thorns of *B. ceiba* plant.

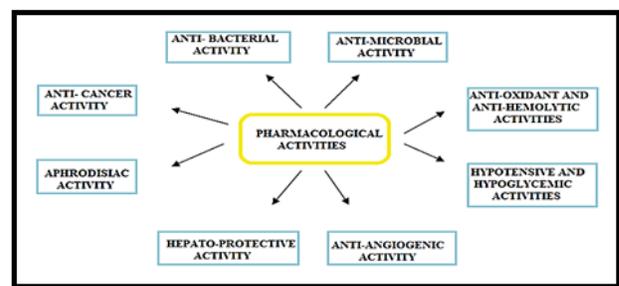


Figure 2: Thorns of *B. ceiba* plant known to have various pharmacological activities.

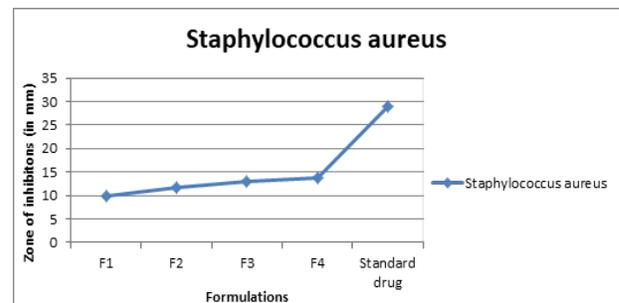


Figure 3: Graphical representation of zone of inhibitions against *S. aureus*.

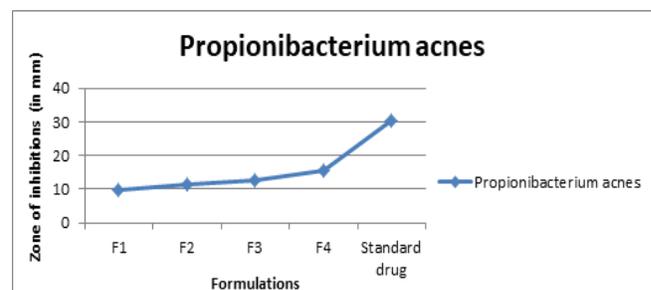


Figure 4: Graphical representation of zone of inhibitions against *P. acnes*.

Preparation of antibacterial herbal gel formulation for acnes

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

Phytoconstituents	Test performed	Observations	Interference
Alkaloids	Mayer's test	Yellowish white or creamy precipitate formed.	Present
	Wagner's test	Reddish brown precipitate formed.	Present
	Hager's test	Yellow colored precipitate formed.	Present
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

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.

Table 5: Antibacterial assay of the gel formulation prepared against the acne-causing bacteria*

Bacteria	Formulations	Zone of inhibitions (in mm)
<i>S. 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>P. 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.

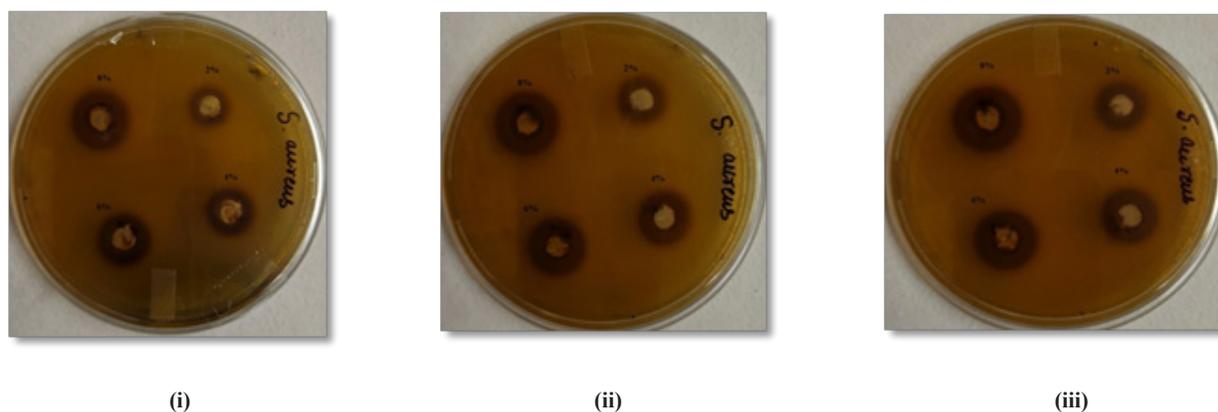


Figure 5: Zone of inhibitions against the *S. aureus*.

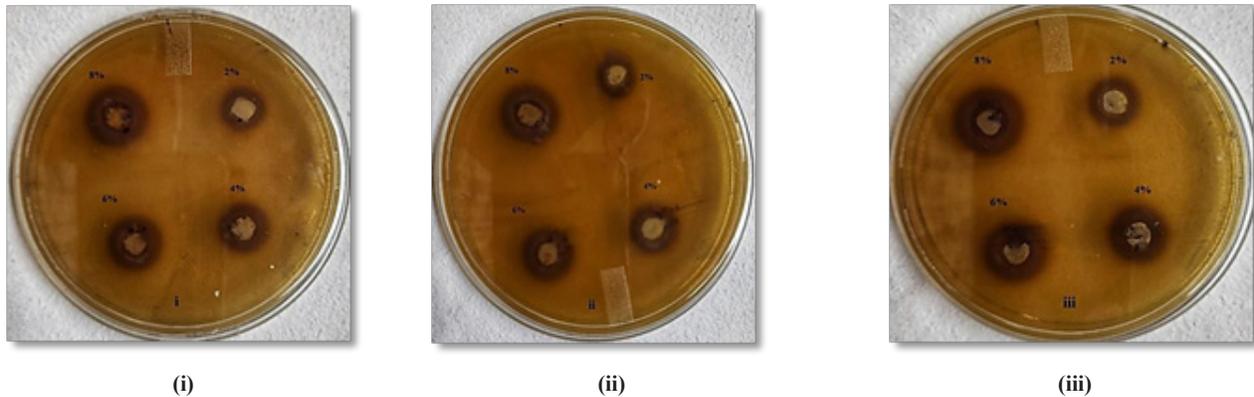


Figure 6: Zone of inhibitions against *P. acnes*.

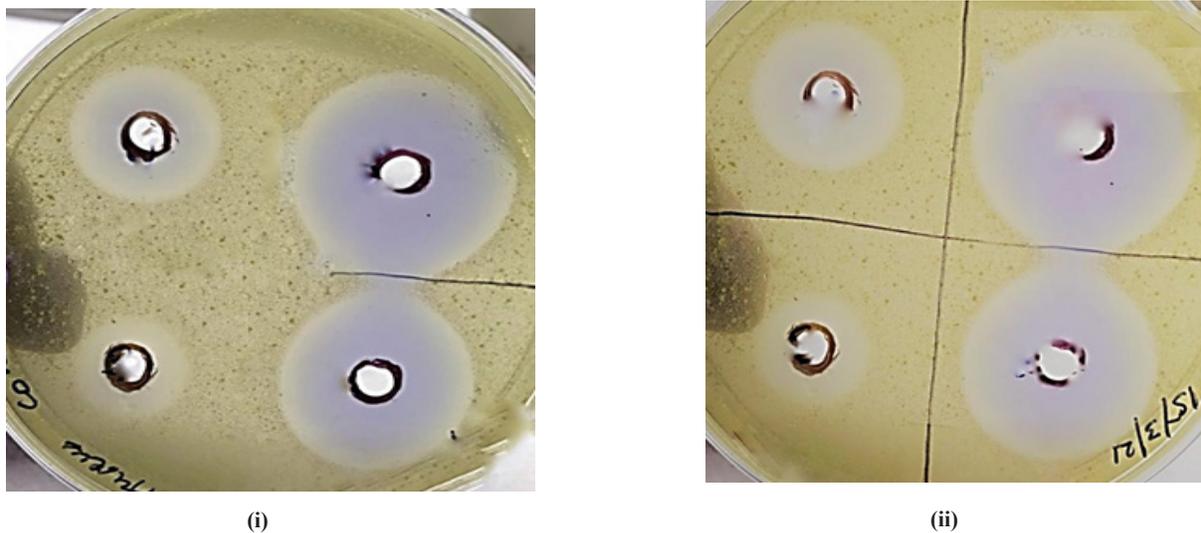


Figure 7: (i) Inhibitions Zone of standard drug clindamycin against *S. aureus* (ii) Inhibitions zone of standard drug clindamycin against *P. acnes*.

P. 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 24 hours the bacterial growth was clearly visible and two different colonies were observed. Identification tests were performed for the identification of the cultured bacteria. Polymerase chain reaction (PCR) technique and some biochemical test were performed for the identification.⁸

Formulation of Gel

All the ingredients were collected as per the required amount to formulate the 50 g gel preparation. For this, the formulation ingredients were mixed in two beakers. Water was divided equally in two beakers, in the first beaker the required amount of plant extract was added and dissolved, to a 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 (Table 2).⁹

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 1-g of gel dispersed between two glassed slides.

Skin Irritation Test

It was performed on 10 healthy volunteers comprise of both male and female. About 1-gm 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.¹⁰

Antibacterial Evaluation

Agar well diffusion method was used for this purpose. *S. aureus* and *P. 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, with the help of sterile cork borer wells were made in the petri dishes of 6 mm diameter each, to which the prepared formulations were added and allow the drug to spread in the media.¹⁰ Then it was incubated for 24 hours at 37°C. The diameter of the inhibition zone was observed and measured with the help of the ruler (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 comparison.

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 in Table 3.

Evaluation Parameters of Gel Formulations

Both physical and microbial evaluations of the prepared gel formulation were performed. Gels were found to be have a transparent appearance and were light brown. The pH range of the gels were in the range of 6.45–6.50. When the gel was applied to the healthy volunteers' skin, it was found to be non-irritant. A microbial evaluation was measured in terms of the formation of zone of inhibitions and Clindamycin was taken as the standard drug (Table 4).

Antibacterial Assay of Formulation Prepared

Antibacterial assays were taken in triplicates for each formulation and the mean was taken out at the end. Results for the antibacterial assay are shown in Table 5.

Zone of Inhibitions (Figures 3–7)

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

- *S. aureus*

- *P. acnes*
- Zone of inhibition of standard drug (clindamycin)

CONCLUSION

The study concluded that the ethanol thorn extract of *Bombax ceiba* possess good antibacterial properties against the *S. aureus* and *P. acnes*. It also contains various phytoconstituents which may be helpful in various health-related problems. Different formulations containing 2, 4, 6 and 8% extract were prepared, 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.

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