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## FORMULATION AND EVALUATION OF ANTIBACTERIAL GEL CONTAINING ETHANOL EXTRACT OF THORNS OF BOMBAX CEIBA

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### ABSTRACT

The purpose of the study is to formulate and evaluate 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. Agar well diffusion method were employed for the purpose. 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, spreadibility and homogeneity and the result were calculated. From the results it was 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.

**Key words:** Bombax ceiba, Thorn's, Herbal gel formulation, Antibacterial activity, Evaluation.

### 抽象的

该研究的目的是配制和评估木棉刺提取物的草药凝胶制剂，以检查其对金黄色葡萄球菌和痤疮丙酸杆菌的抗菌活性。为此目的采用琼脂孔扩散法。配制不同浓度提取物的凝胶制剂，分别为2%、4%、6%和8%，测定凝胶对金黄色葡萄球菌和痤疮丙酸杆菌的抗菌活性。在这个克林霉素凝胶中用作比较分析的标准。从评估结果可以得出结论，与其他配制的制剂相比，8%的制剂显示出更好的抗菌活性。除此之外，还考虑了各种参数，如pH值、外观、粘度、铺展性和均匀性，并对凝胶配方进行了评估，并计算了结果。结果表明，木棉乙醇刺提取物

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对金黄色葡萄球菌和痤疮丙酸杆菌具有良好的抗菌作用。它还含有各种植物成分，可能有助于解决各种与健康相关的问题。

**关键词：**木棉木棉，Thorn's, 草本凝胶配方, 抗菌活性, 评价。

## INTRODUCTION

Acne vulgaris is one of the most frequent skin 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<sup>2</sup>. 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<sup>2</sup>. 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<sup>3</sup>. As it is known that, Topical medication administration is the most effective method for treating skin disorders<sup>4</sup>. 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<sup>2</sup>. For this study thorns of *Bombax ceiba* plant ( Fig. 1) were used to be formulated into gel, as it is known to have antibacterial property.



Figure 1: Thorn's of *Bombax ceiba* plant

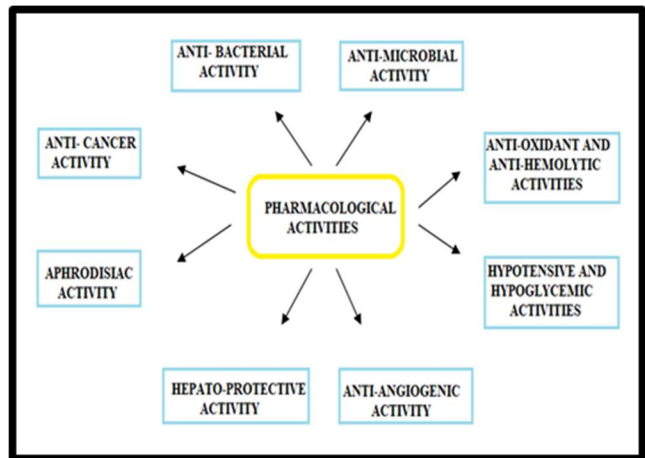


Figure 2: Thorns of *Bombax ceiba* plant known to have various pharmacological activities

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<sup>5</sup>. 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<sup>6</sup>.

**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<sup>5</sup>. 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<sup>6</sup>.

### 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,

proteins<sup>7</sup>. Following tests are performed for the identification of phytochemical constituents:

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

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

Bacteria that are responsible for causing acne (*Staphylococcus 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 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. PCR (polymerase chain reaction) technique and some biochemical tests were performed for the identification<sup>8</sup>.

**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<sup>9</sup>.

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(%)	0.0	0.0	0.0	0.0
Triethanolamine(%)	1.2	1.2	1.2	1.2
Distilled Water	Q.S	Q.S	Q.S	Q.S

Table 2: Different compositions of the gel formulation prepared

#### 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<sup>10</sup>.

#### 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, with 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<sup>10</sup>. Then it was incubated for 24 hours at 37°C. 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 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 below:

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

PHYTO-CONSTITUENTS	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	Brontra ger'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
Flavonoids	Shinoda's test	Reddish pink tint	Present
Carbohydrates	Fehling's 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.

Formulations	pH	Appearance	Viscosity	Spreadability diameter after 1	Homogeneity

				min(m m)	
1	6. 4 5	Light brow n	445 6	42	Good
2	6. 4 7	Light brow n	447 8	40	Good
3	6. 4 2	Light brow n	447 8	43	Good
4	6. 5 0	Light brow n	451 4	45	good

Table 4: Evaluation parameters of the prepared gel formulations\*:

\*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	Formulati ons	Zone of inhibitions (mm)
<i>Staphylococcus aureus</i>	F1	9.8±0.2
	F2	11.6±0.1
	F3	13±0.4
	F4	13.8±0.1

	clindamyc in	28.9±0.15
<i>Propionibacteri um acnes</i>	F1	9.83±0.5
	F2	11.3±0.2
	F3	12.6±0.14
	F4	15.3±0.3
	Clindamy cin	30.16±0.5

\*this data contains the mean value of the triplicates of zone of inhibitions for the antibacterial activity.

Staphylococcus aureus:

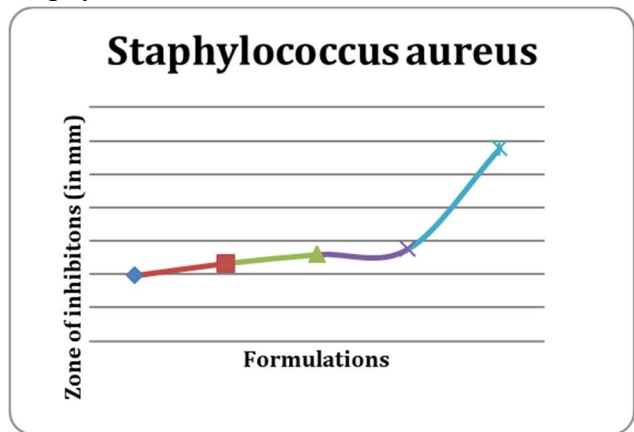


Figure 3: Graphical representation of zone of inhibitions against S.aureus

Propionibacterium acnes:

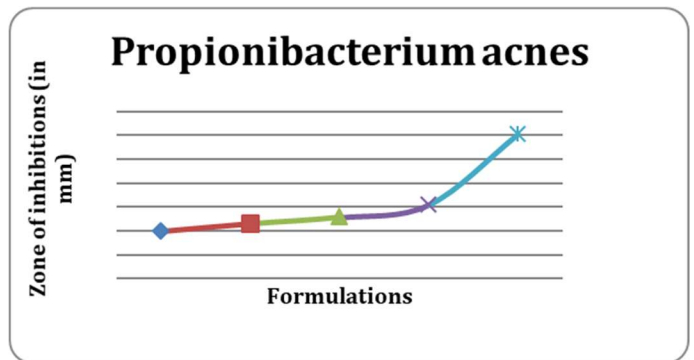


Figure 4: Graphical representation of zone of inhibitions against P.acnes

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

Staphylococcus aureus:

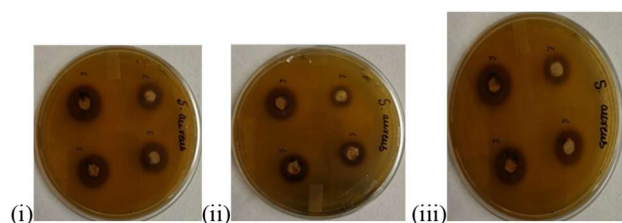


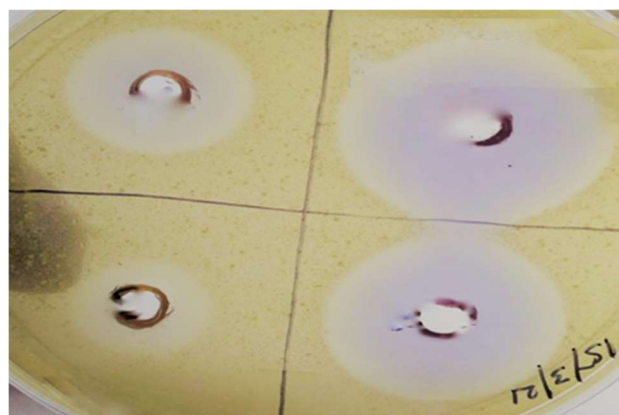
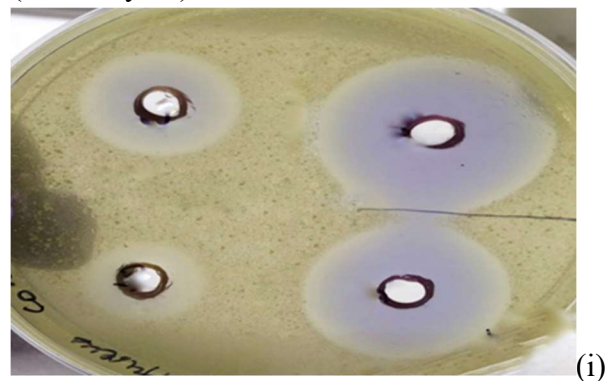
Figure 5: (i), (ii), (iii) shows the zone of inhibitions against the Staphylococcus aureus

Propionibacterium acnes:



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

Zone of inhibition of standard drug (clindamycin):



(ii)

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

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

#### 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.

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