IJPSR (2022), Volume 13, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 February 2022; received in revised form, 25 March 2022; accepted, 24 April 2022; published 01 October 2022

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

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Keywords:

Bombax ceiba, Thorn's, Herbal gel formulation, Antibacterial activity, evaluation

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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 was employed for this 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 was 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 the formulation of 8% showed better antibacterial activity as compared to other formulated preparations. In addition to this, an 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* possesses good antibacterial properties 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 on 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].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(10).4199-04

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(10).4199-04

As a result, in antimicrobial therapy, emphasis has been placed on safer, novel, and harmless alternative antimicrobial ingredients. So, some actions need to be taken to address the issues regarding the current antibacterial treatment, which include understanding the use of antibiotics or investigating the resistance of various antibiotics, and developing the new antibacterial products or formulations from natural sources which will show very less or negligible side effects as compared to antibiotics [3].

As it is known, 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 were used to be formulated into a gel, as it is known to have antibacterial properties. The antibacterial activity of the formulated gel was observed on the bacteria: *Staphylococcus aureus* and *Propionibacterium acnes.*

The antibacterial activity of the gel formulated was measured through the agar well diffusion method, the zone of inhibitions was measured in triplicates, and the mean value was calculated.

MATERIALS AND METHODS:

Materials: Carbapol 940, methylparaben, 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 northwest 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 a 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 the 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 h with continuous stirring for the initial few hours. Then plant material was filtered out through Whatman filter paper, and the collected portion was kept in a hot air oven for drying; 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 [7]. **Table 1** shows the test 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's 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 (*Staphylococcus aureus, Propionibacterium acnes*) were isolated from the human skin.

Sample from the skin was taken by using a sterile swab and toothpick and allowed to grow in a freshly prepared media.

After the incubation period of 24 h, the bacterial growth was 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 [7].

Formulation of Gel: All the ingredients were collected as per the required amount to formulating the 50g gel preparation, as shown in **Table 2**. For this, mixing of the formulation ingredients was done in two different beakers.

Water was divided equally into two beakers; in the first beaker, the required amount of plant extract was added and dissolved. A calculated amount of propylene glycol 400 was added, and in another beaker, Carbopol 940 was added and dissolved. It, EDTA, and methylparaben was added and dissolved.

After that, both the solutions in the beaker were mixed in a single beaker. Finally, 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 based on the following parameters:

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

Appearance and Homogeneity: Visual inspections were done to check the physical appearance and the homogeneity of the prepared formulations.

Viscosity: It was measured using a Brookfield viscometer with spindle no. 6 at 100 rpm.

Spread Ability: 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 comprised of both males and females. About 1gm of gel preparation were applied to the hand of all the volunteers and held for a particular period. After 2 h, the test area was observed for any visible signs resulting from 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 into the freshly prepared nutrient media and stirred properly so that there would be a uniform distribution of the culture all over the media. The media was poured into 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 allowed the drug to spread in the media [9]. Then it was incubated for 24 h at 37 °C. The diameter of the zone of inhibitions was observed and, with the help of a 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 was identified. The result of the study has been concluded in **Table 3**.

TABLE 3: LIST OF PHYTOCHEMICALS CONSTITUENTS FOUND IN THE THORN'S EXTRACT

Phytoconstituents	Test Performed	Observations	Interference
Alkaloids	Mayer's test Wagner's test Hager's test	Yellowish white or creamy precipitate formed. Reddish-brown precipitate formed. 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

Evaluation Parameters of Gel Formulations: Both physical and microbial evaluations of the prepared gel formulation was performed as shown in **Table 4**.

Gels were found to have a transparent appearance and were light brown in color. Ph range of the gels was in the range of 6.45-6.50. When the gel was applied to the skin of the healthy volunteers it was

found to be a non-irritant. The microbial evaluation was measured in terms of forming a zone of inhibitions, and Clindamycin was taken as the standard drug.

TABLE 4: EVALUATION PARAMETERS OF THE PREPARED GEL FORMULATIONS

Formulations	pH	Appearance	Viscosity	Spread ability 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 the three readings taken for each formulation.

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 shown in **Table 5.**

TABLE 5: ANTIBACTERIAL ASSAY OF THE GEL FORMULATION PREPARED AGAINST THE ACNE-CAUSING BACTERIA

Bacteria	Formulations	Zone of Inhibitions (in mm)
Staphylococcus aureus	F1	9.8±0.2
	F2	11.6±0.13
	F3	13±0.4
	F4	13.8±0.1
	Clindamycin	28.9±0.15
Propionibacterium acnes	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 the zone of inhibitions for the antibacterial activity.

Staphylococcus aureus:

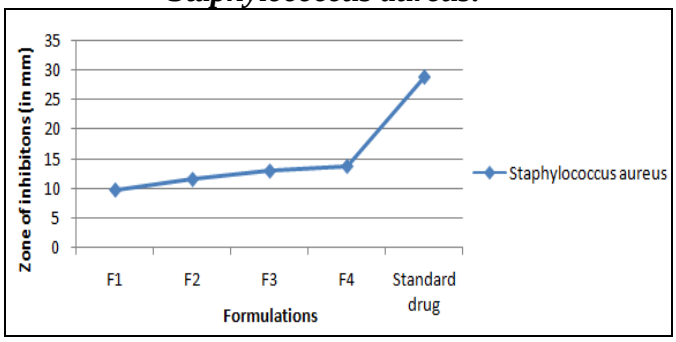

FIG. 1: GRAPHICAL REPRESENTATION OF ZONE OF INHIBITIONS AGAINST *S. AUREUS*

Propionibacterium acnes:

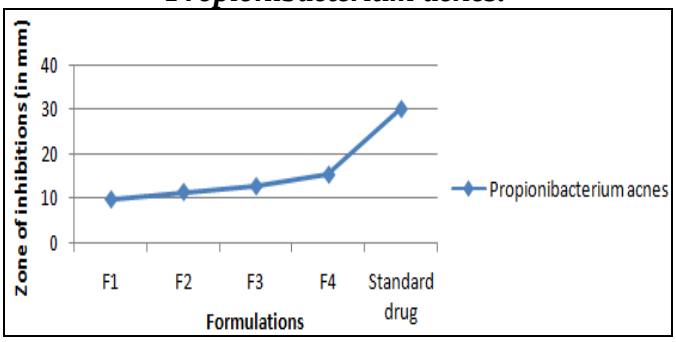

FIG. 2: GRAPHICAL REPRESENTATION OF ZONE OF INHIBITIONS AGAINST *P.ACNES*

Zone of Inhibitions: Zone of inhibitions for both the bacteria are measured in triplicates and the results are shown in **Fig. 3** and **Fig. 4**.

***Staphylococcus aureus*:**

FIG. 3: (I), (II), (III) SHOWS THE ZONE OF INHIBITIONS AGAINST THE *STAPHYLOCOCCUS AUREUS*

***Propionibacterium acnes*:**

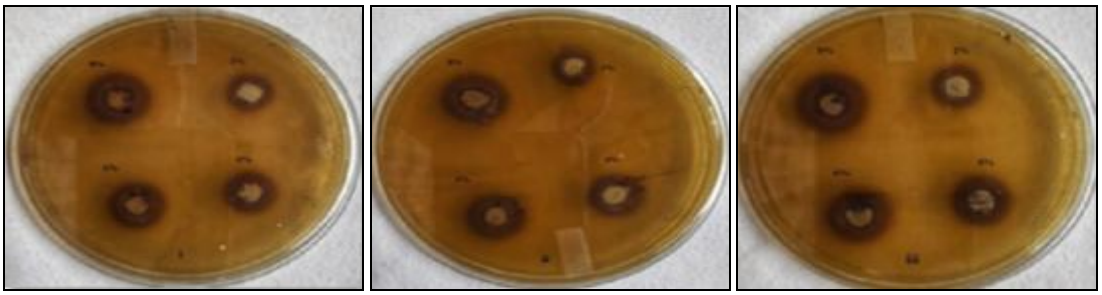

FIG. 4: (I), (II), (III) SHOWS THE ZONE OF INHIBITIONS AGAINST *PROPIONIBACTERIUM ACNES*

Zone of Inhibition of Standard Drug (Clindamycin): Shown in Fig. 5:

FIG. 5: (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* possesses good antibacterial properties 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 contained 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 than all other preparations.

ACKNOWLEDGMENT: I am thankful to the Noida Institute of Engineering and technology for providing the necessary facilities. I am also grateful to Dr. Monika for guiding and supporting me throughout the process.

Financial Support: None.

CONFLICT OF INTEREST: None.

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How to cite this article:
Chauhan P and Monika: Formulation and evaluation of antibacterial gel containing ethanol extract of thorns of *Bombax ceiba*. Int J Pharm Sci & Res 2022; 13(10): 4199-04. doi: 10.13040/IJPSR.0975-8232.13(10).4199-04.