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FORMULATION AND EVALUATION OF ANTIBACTERIAL HERBAL GEL CONTAINING ETHANOL EXTRACT OF LANTANA CAMARA AND NEEM

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ABSTRACT
formulation and Evaluation of the herbal gel made from Azadirachta indica and Lantana camera ethanol extract to determine the gel's antibacterial and antimicrobial activities against Staphylococcus aureus bacteria for this, the Agar Cup plate method was used. The diameter of the zones of inhibition (in millimetres) was measured and compared in order to test the antibacterial activity. The area surrounding the well that is clear and contains an antimicrobial agent is known as the zone of inhibition. The antibacterial activity of the herbal gel formulation was assessed against conventional organisms like Staphylococcus aureus and compared to gentamicin (10-mcg). According to the results, there was a good zone of inhibition, but it was smaller than standard. 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. The ethanol extract of Lantana camera and Azadirachta indica good antimicrobial and antibacterial properties against the Staphylococcus aureus. It also contains various phytoconstituents, which may be helpful in various health-related Problem.

KEYWORDS: Azadirachta indica, Lantana camera, Herbal gel formulation, Evaluation, antimicrobial, antibacterial activity.

INTRODUCTION
There has been a focus on safer, innovative, and safe substitute antimicrobial compounds in antimicrobial therapy. Therefore, certain steps must be taken to address the problems with the current antibacterial treatment. These steps include learning how to use antibiotics or looking into how resistant different antibiotics are, as well as creating new antibacterial products or formulations from natural sources that will have very few or non existent side effects in comparison to antibiotics. Topical medicine administration is recognised as the most efficacious approach to treating skin problems. The rate and amount of medication release primarily dictate how effective topical therapy is a topical medicine delivery device that works by diffusing various pharmaceuticals into the epidermal layers to reach the body Because Azadirachta indica and Lantana camera are known to have antibacterial qualities, they were combined to create a gel for this investigation. Staphylococcus aureus germs were used to test the gel's antibacterial properties. Using the agar cup plate method, the zone of inhibitions was evaluated to determine the antibacterial activity of the gel formulation. The antibacterial activity was measured as the mean of inhibition in diameter (mm) for the well diffusion test, which was run in triplicate.

MATERIAL AND METHOD
Materials: Lanthana Camara, Azadirchta Indica (Neem), Carbapol 934, Methyl Paraben, Propyl Paraben, Propylene Glycol, Triethanolamine, Ethanol etc.

Methods
Collection of the Plant Material
The fresh and clean aerial elements of lantana camara and Azadirchta indica (neem) were collected from A/p Dighanchi, Tal-Atapdi, Dist-Sangli in Maharashtra(India). These aerial parts were then dried for 8-10 days under the shade.

EXTRACTION PROCESS

Preparation of Lantana Camera leaf extract

For eight to ten days, the leaves of lantana and Azadirchta indica (neem) were shed and dried at 28°C room temperature. Using a mortar and pestle, the coarse powder was created after drying. Carefully fill the Soxhlet apparatus with coarse powder, then top it off with ethanol, the solvent, in an adequate amount. Assemble the extraction assembly and continue the procedure for 48 hours at 60°C.Follow these steps for six to seven cycles to obtain the whole extract of the leaves: collect the extract, evaporate it, and then strain the mixture to obtain a semisolid mass similar to the extract. To make the gel, use these final or semisolid extracts as an active ingredient.

Determination of the Phytochemical Constituents Present in the Ethanolic Extract

This investigation involved qualitative chemical analysis of ethanol extract to identify several phytochemical elements, including proteins, carbohydrates, alkaloids, glycosides, terpenoids, saponins, tannins, phytosterols, and flavonoids. The test used to identify the phytochemical elements is presented in Table 1.

Table No. 1: Phytochemical Test.

SR. NO.	Constituents	Test
1	Alkaloid	Dragendorff's test
		Mayer test
		Hager test
2	Flavonoids	Lead acetate
		Alkaline test
3	Cardiac glycoside	Legal test
4	Coumarins	Coumarins
5	Resins	Turbidity test
6	Saponins	Foam test
7	Steroids	Salkowski test

Method of preparation of Gel: Combine 5 ml of distilled water with methyl and propyl parabens. Warming In a water bathNext, add lantana camara and Neem extract at various concentrations. Finally, well combined materials were added to the carbopol 934 gel. Tri-ethanolamine is added dropwise while swirling continuously to adjust the pH (6.8–7).

Table No. 2: Formulation table of Lantana Camara and Neem.

Sr.no	Ingredients	F1	F2	F3	Use
1	Lanthana Camara	1ml	0.5ml	1ml	Antimicrobial
2	Azadirchta Indica (Neem)	1ml	1ml	0.5ml	Antimicrobial
3	Carbapol 934	1gm	1gm	1gm	Gelling agent
4	Methyl Paraben	0.1gm	0.1gm	0.1gm	Preservative
5	Propyl Paraben	0.3gm	0.3gm	0.3gm	Preservative
6	Propylene Glycol	5ml	5ml	5ml	Drug Solubilizer
7	Triethanolamine	1ml	1ml	1ml	pH adjuster
8	Dist.Water	q.s	q.s	q.s	Solvent
9	Rose Water	q.s	q.s	q.s	Perfume

Fig. No. 1: Lantana Camera and Azadirchta indica (neem) Extract and herbal gel.

The process for making cultural media is as follows
Use distilled water to suspend nutritional agar powder and other materials in the necessary amount. Bring this liquid to a boil and whisk to dissolve all the ingredients. Fill the conical flask with agar media, then cover it with a cotton stopper. Dissolve mixture in conical flask by autoclaving at 121°C for 15 minutes. Allow the nutritional agar to cool after autoclaving so that it can be used later.

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.

The Cup plate method's antimicrobial activity
After adding Simple Agar media to the sterile Petri dishes, test organisms such as Escherichia coli, Bacillus subtili, and Streptococcus faecalis were diluted appropriately. With a sterile borer in each plate, three cylinders or cups were formed in the media. The solvent control, standard disc, and designed gel were ready. The cup was filled evenly with 0.2 ml of solution, and it was incubated for 24 hours at 37°C. The antibacterial activity was measured as the mean of inhibition in diameter (mm) for the well diffusion test, which was run in triplicate.

RESULTS AND DISCUSSION
Qualitative Chemical Analysis: The phytochemical components found in the extract were listed in this investigation. In conclusion, Table 3 presents the study's findings.

Table No. 3: List of phytochemicals constituents found in the Lantana Camara and Neem extract.

SR. NO.	Constituents	Test	End point	Result
1	Alkaloid	Dragendorff's test	Orange red Ppt	+++
		Mayer test	White or yellow ppt	++
		Hager test	Yellow crystalline Ppt	+++
2	Flavonoids	Lead acetate	Yellow ppt	-
		Alkaline test	Yellow ppt	+++
3	Cardiac glycoside	Legal test	Deep red colour	+++
4	Coumarins	Coumarins	Yellow colour	++
5	Resins	Turbidity test	Turbidity present	+
6	Saponins	Foam test	Stable foam	+++
7	Steroids	Salkowski test	Red colour	+++

[+++ -Strongly present, ++ -Moderate present, +Weakly present, - -Completely absent]

Evaluation Parameters for Gel Formulations: The created gel formulation was subjected to both physical and microbiological assessments, as indicated in Table 4. Gels were found to have a translucent appearance and were green in hue. The gels' pH ranged between 6.45 and 6.50. It was discovered that the gel did not cause any irritation when it was administered to the healthy volunteers' skin. Gentamicin was used as the standard medication, and the microbiological evaluation was quantified in terms of creating a zone of inhibitions.

Table No. 4: Evaluation Parameters for Gel Formulations.

SR. NO.	Parameter	Duration					
		15 Days			30 Days		
		F1	F2	F3	F1	F2	F3
1.	Color	Green	Green	green	Green	Green	green
2.	Appearance	Semisolid	Semisolid	Semi solid	Semisolid	Semisolid	Semisolid
3.	pH	6.5	6.6	5.8	6.6	6.5	5.8
4.	Viscosity(mPa.s)	55	67.6	67.3	55.1	67.7	67.6
5.	spreadablility(cm/5min)	4.2	4.4	4.6	4.4	4.8	5.1

Antibacterial Assay of Formulation Prepared

For every formulation, three duplicates of the antibacterial assay were taken, and the mean was extracted at the conclusion. Table No. 5 displays the antimicrobial assay results. The antimicrobial activity testing was performed by measuring and comparing the diameter of zones of inhibition (in mm). The zone of inhibition can be defined as the clear regionaround the well that contains an antimicrobial agent. It is known that the larger the zone of inhibition, the more potent the antimicrobial agent. The formulated herbal gel was observed for its antimicrobial property towards the organism such as Staphylococcus aureus (ATCC-6538P) and it was also compared with standard such as Gentamicin (10- mcg). From the result it was observed that it showed good zone of inhibition but lesser when compared to standard.

Table No. 5: Zone of inhibition of the formulated herbal gel.

Organism				
	Staphylococcus aureus(ATTC-6538P)			Mean
	1	2	3	
Herbal gel	12.2	12.4	12.2	12.3±0.1
entamicin(10 mcg)	21.1	23.2	21.1	22.2±0.06

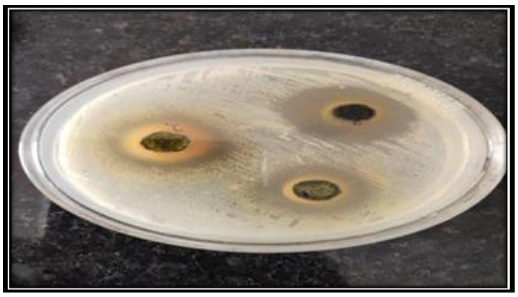

Fig. No. 2: Zone of inhibition of Lantana camera extract towords staphylococcus aurieus.

CONCLUSION

All these investigations have brought out ultimate factors which leads to the following conclusions.

This study targets the skin condition bacterial infection with the aim of formulating an effective and safe herbal gel by using lantena camara and azadirachta indica.

The ethanolic extract of lantana camara and collected Neem leaves gel were incorporated in to optimized Carbopol gel base.

The combination of these two herbal constituents may produce an effect to minimize theantibacterial infection.

Antimicrobial study shows that there was no microbial contamination observed and it showed good zone of inhibition and in skin irritation study results showed that there was no skin lesions like defatting of skin, adverse skin reactions, local systemic change. Overall, this study reports concluded that the formulation herbal gel may offer an effective and safe dosage form which leads to patient adherence and compliance to the therapy.

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