

# FORMULATION AND EVALUATION OF THE HERBAL GEL FOR STAPH INFECTION

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## **ABSTRACT: -**

*With the advancement in medication there has also been an evolution of new-new diseases, new pathogens which are multiple drug resistant have come into existence. Nature has blessed us with a vast treasure of medicinal plants. The manufactured herbal topical gels of various concentrations (0.2 percent, 0.4 percent, and 0.6 percent) were examined at three different time intervals, namely day-0, 14th day, and 28th day, immediately after preparation. The above-mentioned parameters were assessed in all of the gels of various concentrations. Physical measurements revealed that perhaps the formulations were light Yellow in colour, with no odour and a sour taste. The consistency and greasiness results were good and non-greasy, while homogeneity, extrudability, and spreadability were also good.*

**KEYWORDS: -** Herbal gel, *S. aureus*, Gram positive, Extrudability.

## INTRODUCTION

The WHO released its first assessment on resistant bacteria surveillance in 2014, demonstrating that this is a growing worldwide concern that is jeopardizing our ability to treat prevalent nosocomial or community-acquired infections<sup>1</sup>. Contagious disorders produced by drug-resistant Gram-negative bacteria have been known as the ESKAPE pathogens, and they are posing an increasing threat to public health policy around the world. This word refers to their ability to evade the antibacterial agents' effects or the lack of newer, more appropriate drugs<sup>2</sup>.

Because of the number and variety of infectious diseases it causes, as well as the significant mortality and morbidity it causes, *S. aureus* is by seems to be the most important toxigenic pathogen in the genus *Staphylococcus*<sup>3</sup>. These Gram-positive bacteria can thrive in harsh conditions and generate several antimicrobial resistance mechanisms. The creation of chromosomal-encoded Amp C-lactamase, the decrease of porin channels, the creation of extended-spectrum-lactamase, and the mutation of topoisomerase II and IV are the most prominent among them<sup>4</sup>. It's important to remember that a single strain can have multiple resistance mechanisms, and any one of them can be effective against a wide range of antimicrobials<sup>5</sup>.

*S. aureus* resistance mechanisms have been linked to an increase in the death rate of individuals infected with this infection<sup>6</sup>. Furthermore, the increasing indiscriminate need for antimicrobials in health centers or by persons who self-medicate could expose vulnerable individuals to multidrug-resistant bacteria<sup>7,8</sup>. Antibiotic susceptibility & associated toxicity issues have reduced the use of such medications, resulting in a resurgence in phytotherapy research<sup>9</sup>. Scientists are becoming engaged in discovering and evaluating antibacterial elements in selected plants as a local alternative to drugs and therapy options to fix this concern<sup>10</sup>. The majority of them are native to Peru or grow in the Amazon. Despite this, it's likely that less than 1% of the varieties have been investigated for chemicals with possible medicinal benefits<sup>11</sup>.

Antibacterial compounds can be engineered using gels as a starting point. They are really a type of highly hydrophilic biomaterial that is often made of synthetic polymer. Natural polymers which create excellently Gels include polysaccharides like oxalate, dextran, and chitin, as well as the protein jelly and fibrin. Gel cream synthetic polymers include poly(vinyl alcohol) (PVA), polyethylene oxide (PEO), and poly(acrylic acid) (PAA). Gels could also be made from peptide and polypeptide synthesised synthetically<sup>12-15</sup>.

Numerous gels are bioactive and can be intended to have mechanical characteristics exposure to normal tissues, and have thus been used in a variety of applications such as drug delivery, wound repair, prosthetic polymeric materials, cell encapsulation for three-dimensional cultured cells, and regenerative medicine, to name a few<sup>16-18</sup>. Gels with antibacterial characteristics, which are relevant to this review, have been created, extending the utility of this significant class of biomaterial<sup>19</sup>. The usage of Gels to impart antibacterial action will be discussed in this article<sup>20</sup>.

## METHODOLOGY:

### Sample collection and preparation:

The samples were collected and then washed with double distilled water. Further the samples were allowed for drying. The dried samples were grinded and converted into powder<sup>21</sup>.

### Extraction of bioactive compounds:

The powdered samples were dipped into the respective polar and non polar solvents and then allowed for incubation at room temperature for 48 hours. After the completion of incubation the samples were filtered and then collected in bottle for the further analysis<sup>22</sup>.

### Phytochemical tests:

For the identification of the phytochemicals responsible for the medicinal activity in the respective plants, the extracts were analysed for the presence and absence of the phytochemicals such as alkaloids (Mayer's test), flavonoids (Alkaline reagent test), carbohydrates (Fehling's test), Phenolic compounds (Ferric chloride test) and amino acid test (ninhydrin test)<sup>23</sup>.

### Screening for antibacterial activity:

The screening of the plant extracts against the gram positive *Staphylococcus aureus* was carried out by performing the agar well diffusion method and the broth dilution method<sup>24</sup>.

### Gel formation:

Various concentrations of Sodium CMC & Carbopol 934 were spread in purified water using a vortex mixer, while other beaker contained measured and needed quantities of extracted oil, that were then mixed in polyethylene glycol and homogenised for 15 minutes. After 15 minutes of sonication, the mix was introduced to the first solution, which included a mixture of Sodium CMC & Carbopol 934, and stirred continuously. But at the other hand, 5 ml distilled water was used to dissolve the needed amount of sodium benzoate using a water bath. After cooling the solution, polyethylene glycol was added and blended with it. Finally, all of the components were thoroughly combined in the Carbopol 934, and the gel was continuously stirred to get the desired thickness. This procedure was used to create three herbal gel formulations with varied concentrations of the parts of plants, namely 0.2 percent, 0.4 percent, and 0.6

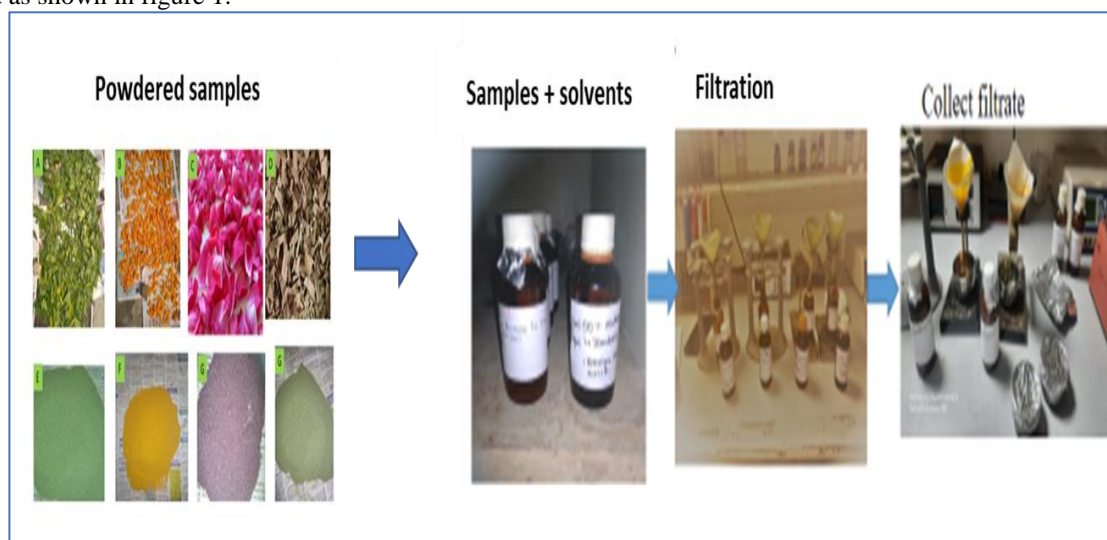
percent (Table 1, 2, 3 & 4). Coding of gels are done as follows S1 this contain 0.2% of extracts S2 contain 0.4% of extract S3 This contain 0.6% of each extract <sup>25</sup>.

**Gel evaluation:**

- **Color:** The compositions' colour was tested with white and black backgrounds.
- **Odour:** The smell of the gels was tested by dissolving a little amount of gel in water and sniffing it.
- **Consistency:** The gel's texture was tested by rubbing it to the epidermis.
- **Greasiness:** By rubbing the solution to the skin, the greasiness of the compositions was noticed.
- **Homogeneity:** After the gel had been set in the container, all of the formulated gels were visually inspected for homogeneity. They were examined for the presence of any aggregates and their manifestation.
- **pH:** A digital pH metre will be used to measure the pH of various ointment compositions. Dilute one gramme of ointment in 100 ml of distilled water and set aside for two hours. Each formulation's pH will be measured three times and the average values will be determined.
- **Extrudability study:** The amount in % of gels extruded when finger pressure was applied was used to determine extrudability. Extrudability improved as the quantity extruded increased.
- **Non irritancy test:** The effect of the herbal gels was examined visually after they were applied to the skin of a human being.
- **Stability studies:** Stability tests were performed on all three formulations at various times, including day 0, 15th day, and 30th day. All evaluation investigations and skin irritation studies were carried out during these time intervals to ensure the stability of the topical herbal gel compositions<sup>26</sup>.

**RESULTS:**

The collected sample were initially washed to remove the dirt and then allowed for air drying. The samples were grinded and then converted to powder. Further these powders were used for the extraction process by maceration method as shown in figure 1.



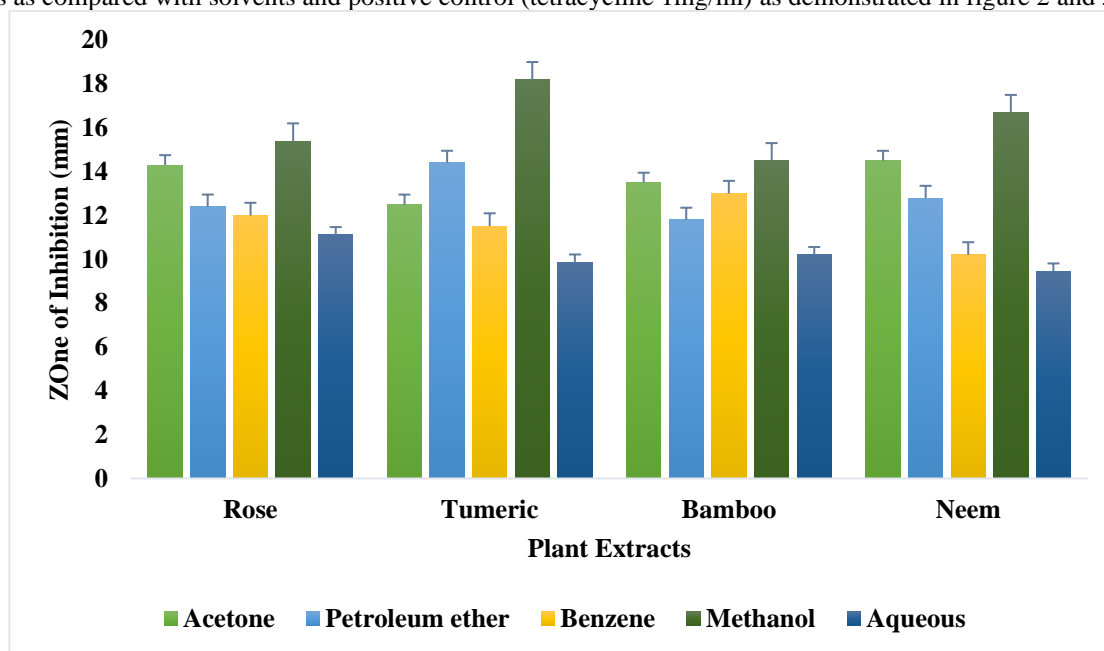
**Figure 1: Sample preparation and extraction of phytochemicals**

Different-different activities are possessed by plants in due the presence of certain bioactive components or the secondary metabolites. Trease and Evans et al., gave a standard procedure for identification these secondary metabolites as given in table 1.

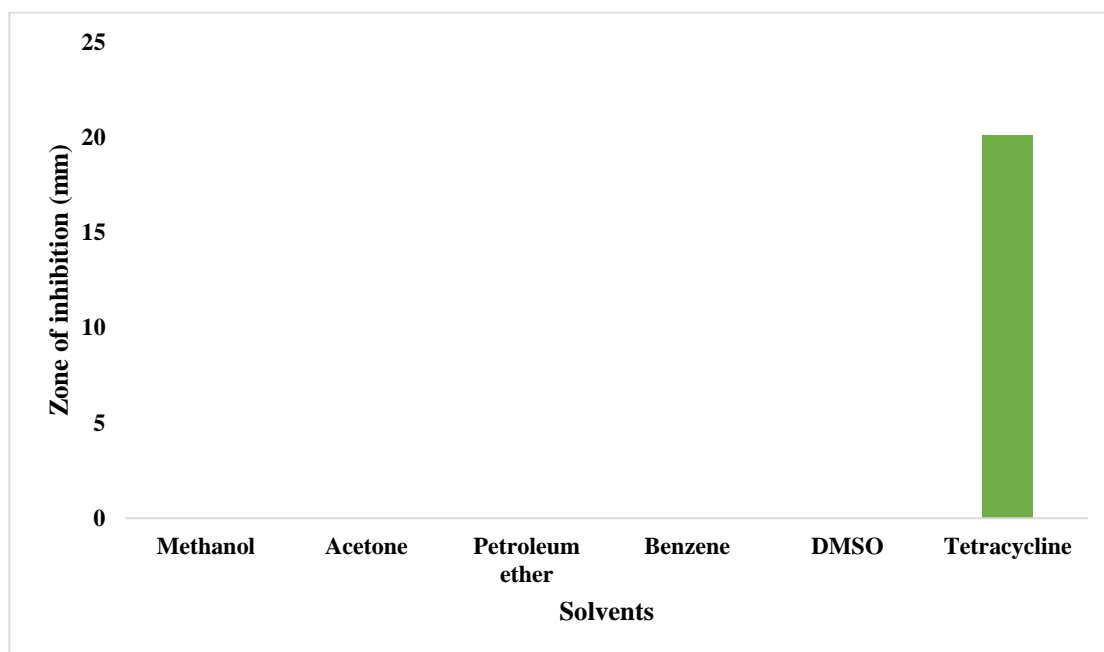
**Table 1: Phytochemical analysis of methanolic extracts of Neem leaves, Rose petals, Turmeric and Bamboo leaves**

Secondary metabolite	Neem leaves	Rose petals	Turmeric	Bamboo leaves
<b>Alkaloids</b>	+	+	+	+
<b>Flavonoids</b>	+	+	-	+
<b>Carbohydrates</b>	+	+	+	+
<b>Tannins</b>	+	+	-	+
<b>Coumarin</b>	+	+	+	-
<b>Saponins</b>	-	-	+	-
<b>Steroids</b>	-	+	-	-
<b>Fatty acids</b>	+	-	+	+

The extracts were analyzed for the antibacterial screening against *S. aureus* and found that the effective results were obtained from all the plants samples (Neem leaves, Rose petals, Turmeric and Bamboo leaves. Hence the samples were selected for the herbal formulation. The best antibacterial activity was obtained by methanolic extracts of the respective samples as compared with solvents and positive control (tetracycline 1mg/ml) as demonstrated in figure 2 and 3.



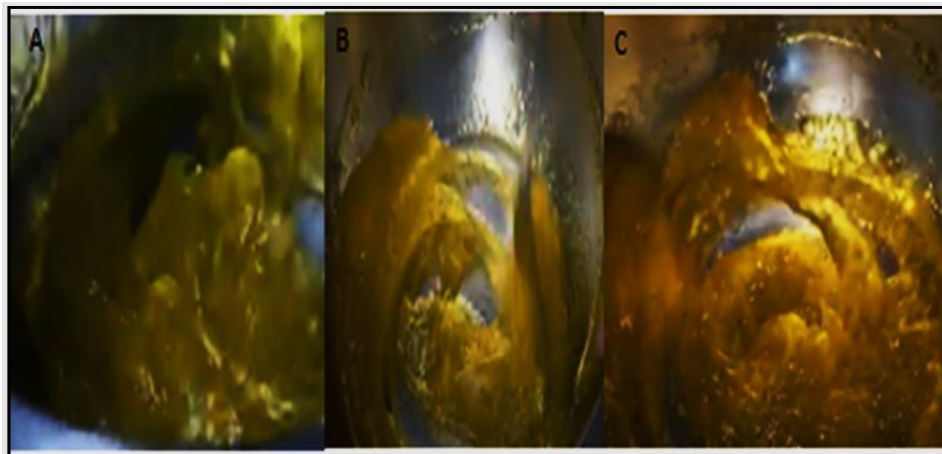
**Figure 2: Antibacterial analysis of the extracts against *S. aureus*.**



**Figure 3: Antibacterial analysis of the solvents and the tetracycline against *S. aureus*.**

**Formulation of herbal gel:**

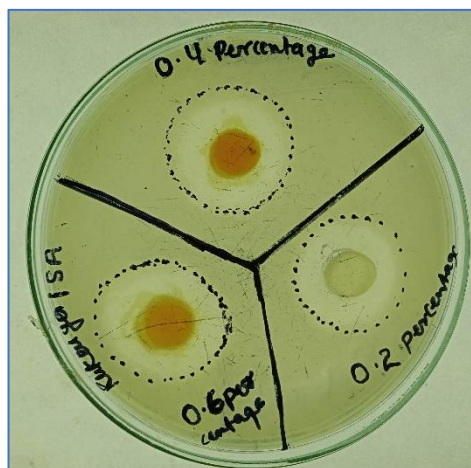
The herbal gel formulated by using these extracts, and then analyzed for the antibacterial activity found that the S1, S2 and S3 showing the effective results. Hence further analysis was carried out by using these formulated gel.



**Figure 4:** Three formulated gel were prepared (A) S1 (0.2%) gel, (B) S2 (0.4%) gel, (C) S3 (0.6%) gel.

**Table 2: Representation of the evaluation of the formulated gel along with their stability**

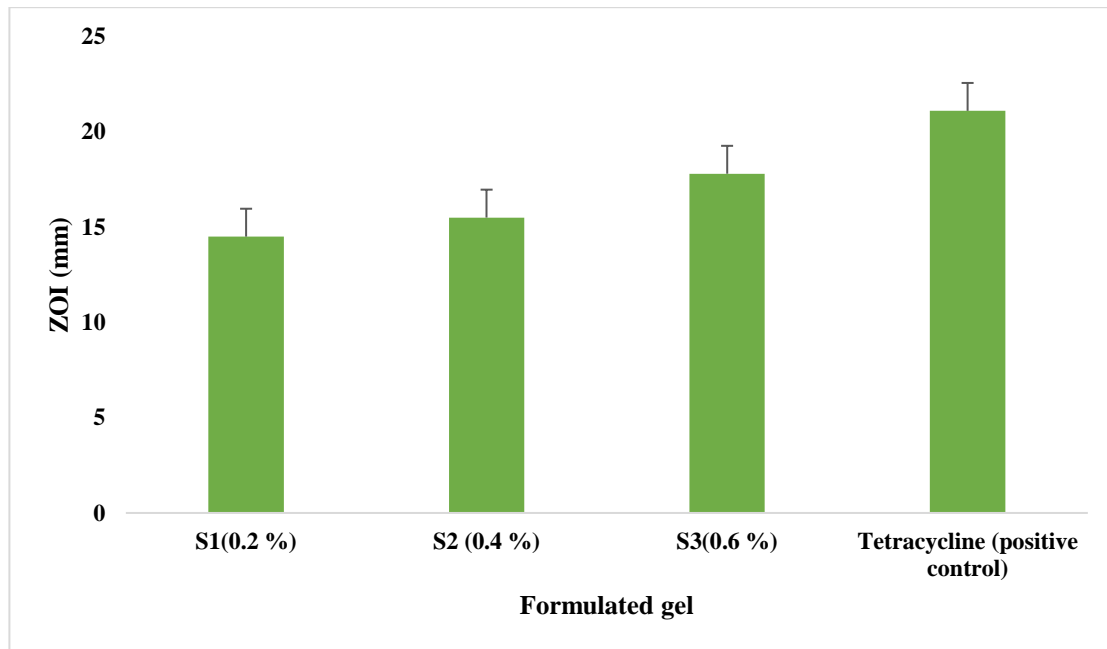
Parameters	Day -0			DAY-14			Day 28		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Physical Parameters									
Spreadablely	+++	+++	+++	+++	+++	+++	+++	+++	+++
Color	Light Yellow	Dark yellow	Dark yellow	Light Yellow	Dark yellow	Dark yellow	Light Yellow	Dark yellow	Dark yellow
Irritation on skin	no	no	no	no	no	no	no	no	no
Consistency	better	better	better	better	better	better	better	better	better
pH	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
Odour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour
Greasiness	no	no	no	no	no	no	no	no	no
Homogeneity	++	++	++	++	++	++	++	++	++
Extrudeability	+++	+++	+++	+++	+++	+++	+++	+++	+++



**Figure 5:** Antibacterial activity of all three formulated gel.

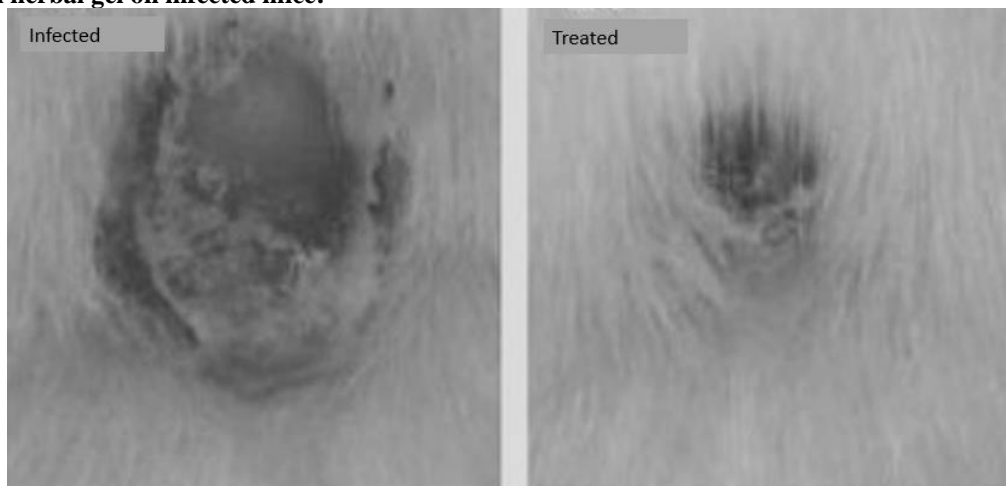
**Table 3: Extracts of plants formulations have antibacterial properties.**

Herbal Formulations	Name of the culture strain	ZOI (mm)
S1(0.2 Percent)	<i>S. aureus</i>	14.5±0.12
S2 (0.4percent)	<i>S. aureus</i>	15.5±0.32
S3(0.6 percent)	<i>S. aureus</i>	17.8±0.11
Tetracycline (positive control)	<i>S. aureus</i>	21.1±0.01



**Figure 6: Antibacterial analysis of formulated gel against *S. aureus***

**Application herbal gel on infected mice:**



**Figure 7: Treated mice with herbal gel**

Formulated herbal gel accelerate thermal burn wound healing in setting infection: Day 15 following full thickness thermal burn and infection with *Staphylococcus aureus*. Burn injuries of Balb/c mice untreated and treated with herbal gel, day 0, 1, 5, 10, and 15. Five animals per group were used. These experiments were done twice with similar results.

**DISCUSSION:**

The last few years have witnessed a change. A change in the thinking of the people. A change in their approach towards life. With the modernization of the world and advancements in technology we have a great increase in medicines. The diseases which were non-curable some years back are now no longer a threat. In fact human race is totally immune to some of diseases. With the advancement in medication there has also been an evolution of new-new diseases, new pathogens which are multiple drug resistant have come into existence. To combat such a scenario we consume a lots of medicines and antibiotics though they show instant results and we get cured in minimum possible time, but these medicines have an adverse effect on us. Prolonged use of such high dose medicines have harmful effects on kidney and heart. And in one way or the other these medicines which cure us today make us ill tomorrow. Thus, there arises a need to focus on herbal medication. Medication from the arms of our mother nature. Nature has blessed us with a vast treasure of medicinal plants. Despite giving us fruits to eat, woods for fuel lowers for aesthetic beauty all what nature gives us is the gateway to herbal medication it is we who need to discover it and implement in the benefit of human beings. And entering into this gateway I chose the parts of plants oil, the to evaluate its antibacterial activity.

The manufactured herbal topical gels of various concentrations (0.2 percent, 0.4 percent, and 0.6 percent) were examined at three different time intervals, namely day-0, 14th day, and 28th day, immediately after preparation. The above-mentioned parameters were assessed in all of the gels of various concentrations. Physical measurements revealed

that perhaps the formulations were light Yellow in colour, with no odour and a sour taste. The consistency and greasiness results were good and non-greasy, while homogeneity, extrudability, and spreadability were also good. The pH of all formulations of various concentrations was 6.9, and skin irritancy tests revealed that the created gels were devoid of dermatological reaction. The antibacterial action of herbal gel formulas against *Staphylococcus aureus* was demonstrated in a dose-dependent ZOI in an exponential manner, with the S3 formulation (0.6%) showing a zone of inhibition of  $17.8 \pm 0.11$  mm, which is greater than the other two formulations. The conventional Fluconazole revealed the  $19.0 \pm 0.01$  mm zone of inhibition, while 2% showed  $14.5 \pm 0.12$  mm and 4 percent showed  $15.5 \pm 0.23$  mm.

**CONCLUSION:**

After reviewing all of the study done so far on this research, we have reached the conclusion that. The parts of plants plant, flowers are a good source of antibacterial chemicals and can be used as a herbal medication. Antibacterial compound yields can be increased by employing more sophisticated techniques and these can be evaluated in a variety of solvents. It has been shown to be effective in the fight against bacteria and can be utilized at high temperatures.

As a result, we can assume that medications (herbal gel) made from plant extract are not affected by the conditions in which they are stored, providing an additional advantage in managing the plant's drugs. Future components of my project work would include, first and foremost, a focus on the precise compounds that give it the numerous qualities it has. Secondly, a more advanced procedure for extracting secondary metabolites from plant sources is being developed. I'd also like to investigate the plant's other latent magical properties.

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**CONFLICTS OF INTEREST:**

The authors have no conflicts of interest regarding this investigation.

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