Formulation And Evaluation Of Antimicrobial Gel Of *Clitoria Ternatea*(Flowers)

Miss. Gauri V.Vaishnav[1*], Gitanjali C.Chavan[2], Mr. Mrunal K. Shirsat[3]

[1*]M-Pharm Scholar, SBSPM B-Pharmacy College Ambajogai
[2]Faculty of Pharmacy, SBSPM B-Pharmacy College Ambajogai
[3]Principal, SBSPM B-Pharmacy College Ambajogai

***Corresponding Author:** Miss. Gauri V. Vaishnav
*SBSPM B-Pharmacy College Ambajogai Dist-Beed-431517, E-mail: gaurivaishnav001@gmail.com

Abstract
To formulate novel topical herbal gel prepared by *Clitoria ternatea* flowers and evaluate their antimicrobial activity. Dried, powdered flowers were extracted with ethanol using maceration method for 24 hrs. Drug-excipients compatibility study were performed for the selection of formulation excipients. Topical formulations like gel containing *C. ternatea* extract were formulated using combination of gelling agents such as HPMC K100M and Carbopol 934 P. Prepared gels were subjected to various evaluation parameters such as physicochemical parameters, pH, viscosity, spreadability, homogeneity, drug content uniformity, in-vitro drug diffusion, permeability and antimicrobial activity. A topical gel was successfully formulated containing bioactive ethanolic extract of *C. ternatea* flowers. The gel was found to be very effective as antimicrobial formulations. Prepared topical gel of *Clitoria ternatea* was shown pH range 6.2 to 6.6, viscosity range 480 to 603cp, spreadability range 20.62 to 24.33g.cm/s and homogeneous. Prepared gel was shown uniformity in drug content which ranges from 94.32 to 96.62%, zone of inhibition ranges 12mm for Bacillus spp. Prepared gel formulations were shown drug release, not more than 85% in 6 hr. As the concentration of Carbopol 934 P and HPMC K100M was increased from 0.5 to 2.0gm and 5.0 to 6.5gm respectively in the gel, antibacterial activity was synergistically improved against Bacillus spp.

Key words: Topical gel, *Clitoria ternatea* , Drug diffusion study, Antimicrobial activity.

INTRODUCTION

Herbal medicines used since ancient times by saints and munis. From ancient times, nature has been the only source of medicinal plants. These medicinal plants are the gift of the Goddess, to treat a huge number of diseases in human beings and other living organisms[1]. Plants and herbal extracts are well known for their capability to have therapeutic potential and have been accomplished worldwide to cure various severe conditions. Plants are the basement of mainly Ayurvedic medicine, Unani form of medication and Siddha. There are hundreds of effective drugs and biologically active compounds designed from traditional medicinal plants[2] .

Clitoria Ternatea Linn –first named by Breyne, butterfly pea[3], Aparajita[4], Shankhpushpi[5], bluepea and cordofan-pea[6] is its common name. *Clitoria ternatea* (Butterfly pea) is a member of family Fabaceae. Approximately 60 *Clitoria ternatea* species are found within the tropical belt, while a few species are distributed in temperate areas[7]. It has most likely initiated from tropical Asia and later spread widely in South and Central America, China, and India, where it has become naturalized. The most noticeable phenotypic difference across the butterfly pea variety is the color of the corolla. *Clitoria ternatea* corollas range in color from dark blue, to white with diverse blue and white shades in between[8]. It is a perennial legumious climber which is used since traditional Ayurvedic system of Indian medicine for treating a wide variety of conditions[9]. Its extracts possess a wide range of pharmacological activities including analgesic, antidiabetic, anti-inflammatory, antimicrobial, diuretic, hepatoprotective, insecticidal, and for use as a vascular smooth muscle relaxing properties. All plant parts such as seed, root, stem, leaves, flower, legume were used medicinally[10].

Herbal medicines possess many bioactive constituents for many diseases but the proper knowledge must be necessary for the preparation of herbal formulation. In a market, the oral antimicrobial drugs are available but the disadvantages of the oral formulations of drugs are more as compared to the topical formulation. Hence, to overcome the above problems novel topical gel formulation of Clitoria ternatea blue flower extracts was prepared. The proposed topical antimicrobial gel can avoid drawbacks of drug administration by the oral route. In this study, CTFE extracts gel showed acceptable antimicrobial activity against Bacillus spp. microbes. The major phytoconstituents responsible for antimicrobial activities found are anthocyanins such as ternatin and pentacyclic triterpenoids such as taraxerol and taraxerone. Optimized formulations showed acceptable pH, spreadability, in vitro diffusion and drug release. This study revealed that this CTFE gel was very effective as an antimicrobial agent.

Image 1 Fresh *Clitoria ternatea*

Image 2 Dried *Clitoria ternatea*

MATERIALS AND METHODS

1.1 Selection of Plant Materials And Preparation of Extract:

The plant *Clitoria ternatea* blue flowered were grown at the home garden, Ambajogai. The herbarium sample sheet for the *Clitoria. ternatea* plant with visible of seeed, legumes, stem, leaves and flowers was prepared and authenticated by Dr S. W. Bhivgade Head of Botanical Department, Yogeshwari Mahavidyalaya, Ambajogai. *Clitoria ternatea* flowers were collected regularly ,dried in the shady place and kept in the air tight zipper pouch for storage as well as further studies.

The extract of the *Clitoria ternatea* flowers further called as (CTFE) were prepared by maceration process. Firstly the collected *Clitoria ternatea* flowers were washed with tap water followed by deionized water. The dried *Clitoria ternatea* flowers was blended into the grinder until fine chopped pieces formed and then sieved with a 120 sized mesh. 50 g of *Clitoria ternatea* flowers powder were extracted with 500 mL 98% ethanol in a maceration apparatus for 24 h. After 24 hr, the filtrate was then evaporated under rotary vacuum evaporator. The extracts were collected into a sterilized bottle and stored in refrigerator until further use[11,12,13].

1.2 Characterization of Extract:

FTIR studies were performed for the *Clitoria ternatea* flowers extract in ethanol solvent. The calibration curve of the CTFE was performed. UV-Visible spectra also performed for the CTFE. The analytical studies for the *Clitoria ternatea* flowers done. The phytochemical analysis tests for the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenols, tannins, saponins, sterols, terpenoides and quinones were performed successfully.

1.3 Characterization of Gelling Agents:

FTIR studies were carried out for the gelling agents HPMC K-100M and Carbopol-934P using FTIR spectrophotometer at the Government College of Pharmacy, Chatrapati Sambhajinagar.

1.4 Microorganisms culture:

Bacterial culture of Bacillus spp. were used to check the antimicrobial activity.

METHODS

Preparation of Gel Formulation

Gel formulations of different batches were formulated by a cold mechanical method as per the composition is given in Table 1.

The 0.6ml of *Clitoria ternatea* extract (active drug) was dissolved in 15 ml of glycerin with the aid of mild heat (solution A). Weighed amount of HPMC K100M and Carbopol 934 were dispersed in 75 ml of distilled water. The mixture was stirred continuously by using magnetic stirrer for around 30-45min; until uniform suspension obtained to prevent no lump in the dispersion. Propylparaben was added in it (solution B). Now, Solution A was added into the solution B and mixed thoroughly with constant stirring and homogeneous dispersion was obtained. For the neutralization of the gelling agents, dropwise 10 % sodium hydroxide solution was added in it up to gel get formed. Finally, the required quantity of distilled water was added at room temperature to make up the volume of gel upto 100ml and formulation were evaluated[14].

Image 3 *Clitoria ternatea* gel formulation

Table 1: Formulation development of *Clitoria ternatea* topical gel

Sr. no.	Ingredients	Quantity
1.	*Clitoria Ternatea* Extract	0.6ml
2.	HPMC K100M	6.0gm
3.	Carbopol-934P	1.5gm
4.	Propyl Paraben	0.2gm
5.	Glycerin	15ml
6.	10% NaOH	Q.S.
7.	Distilled Water	Up to 100ml

EVALUATION

Gel formulations were evaluated for various parameters such as appearance, color, grittiness, pH, viscosity, drug permeablity, drug content, in-vitro drug diffusion, spreadability and stability.

1. Appearance

Physical appearance of the formulated gels were evaluated by visual perception by observing the sample in light at white background and colour observed is noted[15] .

2. Homogeneity

All developed gel formulations were allowed to set in a suitable container and homogeneity of formulated gels was examined by visual inspection for the presence of any aggregates. The gel appearance was reported[14].

3. Grittiness

All developed gel formulations were observed microscopically for the presence of particles. The developed each gel formulation was seen under light microscope. If no particulate matter was observed under light microscope, the gel fulfills the requirement of free from grittiness[14,16].

4. pH

For pH determination a standard digital pH meter was used. 1.0g gels was weighed and dissolved in 100ml of distilled water and it was stored for 2 h. The pH of each gel formulation was noted[14].

5. Viscosity

The viscosity of the gel was measured using a Brookfield Digital Viscometer (Ametek company). The spindle no. 6 was dipped in gel sample rotated at 10 rpm and 20 ± 1°C temperature for 15 min. The reading was done in in triplicate, average value and ± standard deviation was calculated. Viscosity in centipoise (cp) was measured[14].

6. Spreadability

Spreadability is determined by Parallel plate method. In this method the spreadability of all developed formulations were determined by using 'Wooden block' and 'Glass' slide apparatus. In this Parallel plate method, two glass slides of were used for determination of spreadability. 1.0 g of the formulation was weighed and placed on fixed one slide on this wooden block (i.e. ground slide) and the approximate diameter of the prepared formulation applied was measured in mm. Then the second slide was placed on top of that ground slide. Now, the applied gel was sandwiched between these two slides. Place 100g of weight on the top glass slide for 5.0 min which showed removal of the air bubbles and provided a uniformed film of sample gel and removed the excess of gel from edges. After 5 min, the diameter of the formulation was measured. The increase in diameter of the formulation noted. The spreadability calculated by using the following equation[14,17].

$$\text{Spreadability} = \frac{\text{Weight tide to upper slide sample} * \text{Length of glass slides}}{\text{Time taken to separate both slides}}$$

7. Drug content

The drug content of the herbal gel was determined using UV-Visible spectrophotometer. 100 mg of gel from each developed formulations was dissolved in 100 ml of Phosphate buffer pH 6.8 and placed it on a mechanical shaker for 2 hrs to dissolve drug completely. Then this solution of prepared gel formulation was filtered and determined drug content on UV spectrophotometer at 266 nm wavelength using a phosphate buffer pH 6.8 as a blank solution. Finally the drug content was measured in the triplicate and average value was determined[14].

8. In-vitro Drug Diffusion Study

In-vitro Drug Diffusion studies of all developed gel formulations were determined by using Franz-diffusion cell. The diffusion cell apparatus was fabricated locally as open-ended cylindrical tube with 3.14 cm2 area and 100 mm height having a diffusion area of 3.8 cm2 .The Phosphate buffer pH 6.8 was used as receptor media. The egg membrane was prepared by dipping the egg into the 1M con. HCl for 30 min. After half hr, egg was removed from con. HCl and membrane was withdrawn form the egg . The egg membrane was used as dialysis membrane in drug diffusion study and

mounted in between the receptor and donor compartment of the Franz-diffusion cell such that the egg membrane side was in intimate contact with the release surface of the formulation in the donor cell. The donor compartment contained 10.0 ml of isotonic phosphate buffer pH 6.8 mounted on the diffusion cell and maintained the temperature at 37 ± 1°C. The assembly was fixed on a magnetic stirrer. 1.0 g quantity of gel sample was placed over the egg membrane and solution of phosphate buffer pH 6.8 in the receptor compartment was stirred constantly using magnetic bead at 50.0 rpm. Then withdrawn the 1.0 ml of sample at 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min and diluted with 10.0 ml of blank solution and analyzed the withdrawn sample by spectrophotometer at 266 nm. After each withdrawal, the diffusion medium was replaced with an equal volume of fresh diffusion medium to maintain the sink condition. The cumulative percent drug release was calculated for each time (in min) interval. Diffusion study of formulations was measured[14,18].

9. Stability

The stability studies were carried out for each of the formulations to check the quality of the gel formulation varies with time under the influence of temperature and humidity. The developed formulations were kept at two different temperatures 4 ± 2°C and 30 ± 2°C, 65 RH, for 3 months. The pH and the viscosity of each developed formulations at the initial and after 3 months, were compared. Samples at an initial, first, second and third months were withdrawn and evaluated for changes observed in color, odor, homogenity, pH, microbial growth and viscosity[16,18].

Determination of Antimicrobial Activity

The antimicrobial activity of optimized herbal gel and standard gel formulations was tested against Bacillus spp. by using disk diffusion method. The re-cultured bacterial strains were used for antibacterial evaluation. The strains were streak on the Mueller Hinton media to form lawn cultures and the drug entrapped discs were placed at room temp. The disc of optimized gel was prepared having 10µg/ ml concentration. The petri plates were kept in incubator for 24 hrs. After 24 hrs, the petri-plates were checked for the degree of antimicrobial activity against tested bacteria by measuring the diameter of zone of inhibition. The zone of inhibition diameter was recorded with the help of zone reader scale. Marketed Clotrimazole gel(MCC) 1% w/w IP was used as a standard. The more the zone of inhibition the more will be antimicrobial activity[15,19].

RESULTS AND DISCUSSION

Table 2: Phytochemical screening

Phytochemical	***Clitoria ternatea* flower extract(CTFE)**
Alkaloids	Positive
Carbohydrates	Positive
Flavonoids	Positive
Glycosides	Positive
Phenols	Positive
Quinones	Negative
Saponins	Negative
Steroids	Negative
Tannins	Positive
Terpenoides	Positive

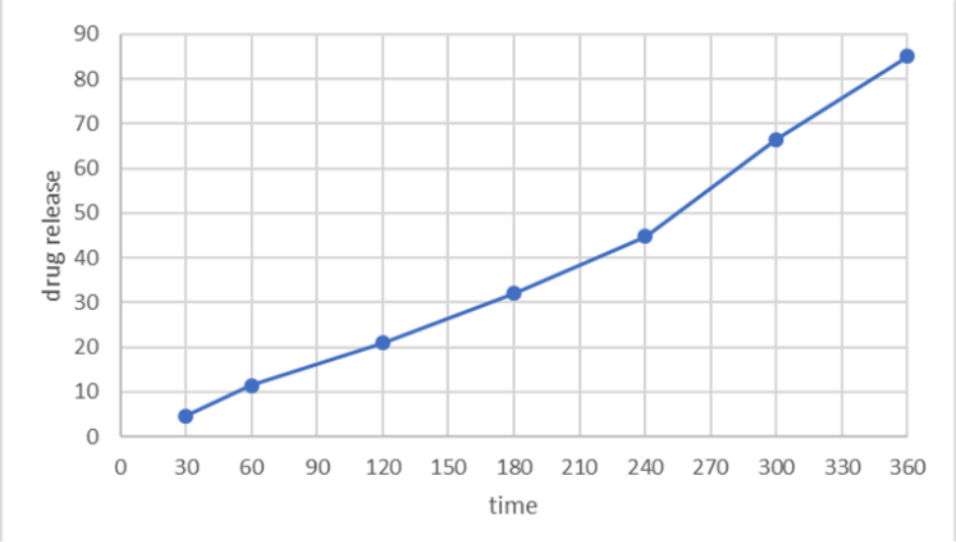
Table 3: Appearance of gel as and clarity of formulations

Formulation	**Appearance**	**Clarity**
batch	Pearl White	Clear

Table 4: Evaluation of topical gel for pH, Viscosity, Spread ability, Homogeneity, and Drug Content

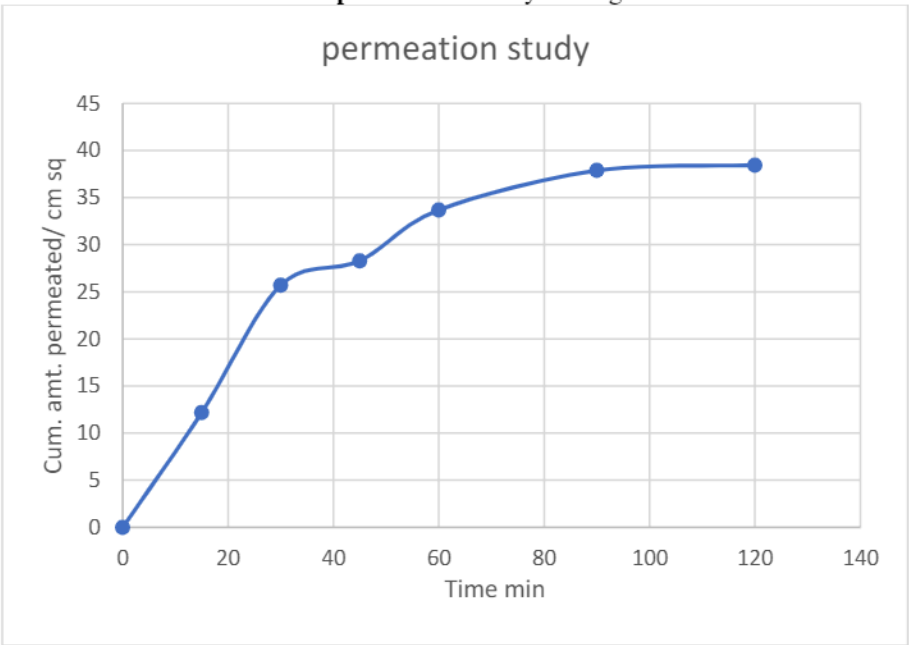
Formulation	**PH**	**Viscosity cp**	**Spread ability**	**Homogeneity**	**Drug Content %**
Batch	6.6	603	20.62	Homogenous	96.62

Graph 1: In vitro drug release



Drug release

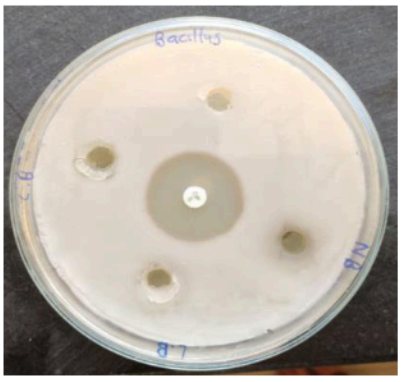
Graph 2: Permeability of drug



Drug permeability

Table 5: Evaluation of topical gel for antimicrobial activity (zone of inhibition)

Sr. No.	Microbial Strain	Zone of Inhibition
1.	Bacillus spp.	12mm



Antimicrobial Activity of Bacillus spp.

CONCLUSION

The antimicrobial topical gel of *Clitoria ternatea* formulation showed better antibacterial activity . As the concentration of Carbopol 934 P and HPMC K100M was increased from 0.5 to 2.0gm and 5.0 to 6.5gm respectively in the gel, antibacterial activity was synergistically improved against Bacillus spp. The prepared gel formulation was found stable and useful for topical application due to its negligible diffusion, good spreadability, neutral pH, and low viscosity.

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