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Research Article

# DESIGN AND EVALUATION OF NOVEL TOPICAL GEL OF TINOSPORA CORDIFOLIA AS ANTIMICROBIAL AGENT

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**ABSTRACT**

**Objective:** The present study deals with topical formulations of a bioactive extract of *Tinospora cordifolia* and its evaluation.

**Methods:** Dried, powdered stem was extracted with chloroform using ultra-sonication method for 3 hrs. Topical formulations like gels containing chloroform extract were formulated using various gelling agents. These gels were evaluated for physicochemical parameters, viscosity, spreadaibility, and antimicrobial activity.

**Results:** A topical gel was successfully formulated containing bioactive chloroform extract of *T. cordifolia*. The gel was very effective as antimicrobial formulations.

**Conclusion:** These kinds of formulations can be very promising wound healing medicines with ease of use and no side effects.

**Keywords:** Topical gel, *Tinospora Cordifolia*, Anitmictobial

## INTRODUCTION

Herbal medicines have been always in demand because of their potentials and minimal side effects, and thus the research on plant-based medicines for their activity and formulations is constantly in interest. For topical treatment of dermatological disease as well as skin care, a wide variety of vehicles ranging from solids to semisolids and liquid preparations is available to clinicians and patients. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. Gels are defined as semi-rigid systems in which movement of the dispersing medium is restricted by an interlacing three dimensional network of particles or solvated macromolecules of the dispersed phase. Gels have better potential as a vehicle to administer drug topically in comparison to ointment, because they are non-sticky, require low energy during formulation, are stable and have esthetic value [1].

Traditionally, *Tinospora cordifolia* (Willd.) Hook. F. and Thomson (Menispermaceae) commonly known as rasayana plant, Guduchi, is one of the commonly used medicinal plants in India for curing ailments ranging from common cold, skin diseases, and dental infections to major disorders such as diabetes, hypertension, jaundice, and rheumatism, and its rejuvenating property is well-reported in Ayurvedic and other ancient literature. This plant is used as stomachic, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, and in fracture of bones in animals [2-6].

In past, many herbal gels have been formulated but gel containing *T. cordifolia* extract as an antimicrobial agent has not been explored. Thus, the present study deals with the investigation of antimicrobial activity of gel containing bioactive extract of *T. cordifolia* commonly known as Gulvel, Guduchi.

## METHODS

### Solvents and chemicals

Guduchi stem was collected from the local region of Devrukh, Ratnagiri. All chemicals and reagents used were of analytical grade and purchased from Rankem and S. D. Fine Chemicals, India.

### Preparation of extract

Guduchi stems cleaned (with 70% ethanol), shade dried, and powdered. Dried, powdered stem was extracted with chloroform using ultra-sonication method for 3 hrs.

### Formulation of gel [7-12]

Weighed amount of Carbopol 940P was soaked in distilled water overnight. The bioactive extract was accurately weighed and uniformly suspended in ethanol. The suspension is then added to the soaked Carbopol. The mixture was stirred using an overhead stirrer for around 1-1.5 hrs; until a uniform suspension was obtained. Care was taken to prevent air entrapment during stirring. This was followed by neutralization of the gelling agent by NaOH or 50% triethanolamine; pH was adjusted to 7.3-7.5. The composition of gel formulations is given in Table 1.

### Evaluation of gel [7-12]

Formulations were evaluated for various parameters such as appearance, color, pH, viscosity, homogeneity, spreadability, extrudability, extract content, uniformity of extract content.

### Viscosity

The viscosity of the formulations was checked using a Brookfield Viscometer (DV-I PRIME, USA).

### Homogeneity

Homogeneity of formulated gels was examined by visual inspection for the presence of any aggregates.

### Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides, and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spreadability was calculated using the formula S = ML/T, where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

**Table 1: Combination of ingredients for formulation of gel**

| Ingredients | Formulation code | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Aqueous extract | 5% w/w | 5% w/w | 5% w/w | 5% w/w | 5% w/w |
| Carbopol 940P | 1.5% w/w | 1.5% w/w | 2% w/w | 1.5% w/w | 2% w/w |
| Tween 80 | - | 5%v/w | 5% v/w | 5% w/w | 5% v/w |
| Flavor | q. s | q. s | q. s | q. s | q. s |
| Water q. s | 100 g | 100 g | 100 g | 100 g | 100 g |
| 50% triethanolamine | - | - | - | pH 7.3-7.5 | pH 7.3-7.5 |
| 10% sodium hydroxide | pH 7.3-7.5 | pH 7.3-7.5 | pH 7.3-7.5 | - | - |

**Primary dermal irritation index (PDII)**
This test is done by applying the formulated gel onto the skin and then observed for any reversible damage to the skin within 4 hrs. Based on their PDII score, the formulation can be graded as irritating or non-irritating.

**Microbial contamination**
Microbial contamination of gel with bacteria and moulds, respectively, was determined by spreading a thin loop full of the material withdrawn from the depth of the bulk product on a nutrient and Sabouraud agar, and incubating for 24-48 hrs, at 37°C.

To assess the degree of contamination, 1 g of material was dispersed in 4 ml of sterile Ringer solution, containing 0.25% Tween 80. Appropriate dilutions were made in the same dispersion vehicle and 0.5 ml was plated out on the appropriate solid medium using the surface viable method. Emergent colonies were counted after necessary incubation.

**Antimicrobial evaluation [13-17]**

*Test organism*

The microbial cultures (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*) were procured from National Center for Industrial Microorganisms (NCIM), Pune, India.

**Antibacterial activity**
*In-vitro* antibacterial activity was evaluated using the agar well diffusion technique. Muller-Hinton agar was used as the medium. The sterile agar was inoculated with the bacteria culture for 48 hrs, at 37°C. Wells were bored by using a sterile borer, and standard formulations (1000 µg/ml was prepared by dissolving the test sample in methanol and the solvent control) were placed into them. Plates were kept for 2 hrs in the refrigerator to enable pre-diffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hrs) at 37°C.

**Stability**
The stability studies were carried out for all the formulations. The 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 the formulations, which were determined after 3 months, were compared with the initial pH and viscosity.

**Statistical analysis**
All experimental measurements were carried out in triplicate and were expressed as an average of three analyzes ± standard deviation. Statistical analyzes were performed by the t-test.

## RESULTS AND DISCUSSION

**Evaluations of formulations**
The aqueous extract of guduchi was formulated into a gel into different combination of excipients; formulation E did not show a considerable change in characters such as color, odor, and consistency, and there was no phase separation observed during the course of the study.

The results of pH, spreadability, and viscosity of the formulations are recorded in Table 2. The result depicted that formulation E is compatible with the skin, well viscous to spread and to retain onto the skin. The extrusion from the tube and spreadability of the topical formulation is important during the application, as also patient acceptance. The formulation E showed acceptable spreadability along with good extrusion. As, the formulation E was found to be the best among all the combination; it was carried forward for microbial contamination test and antimicrobial study.

**Table 2: Evaluation parameters of formulated gel**

| Sr. no | Parameters | Observations |
|---|---|---|
| 1. | Appearance | Lustrous |
| 2. | Color | Pale yellow - white |
| 3. | pH | 7.3-7.5 |
| 4. | Viscosity | 6015±171 cps |
| 5. | Spreadability | 6.96±1.56 seconds |

All the experiments were performed in triplicates

**Table 3: Antimicrobial study of formulation gel E**

| Organisms | Zone of inhibition (mm) (for containing 50 mg extract/well) |
|---|---|
| *S. aureus* | 9.5±1.2 |
| *B. subtilis* | 10.2±2.01 |
| *E. coli* | 8.9±0.99 |
| *P. aeruginosa* | 10.5±1.11 |

*S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa*, All the experiments were performed in triplicates

**Microbial contamination**
The microbial contamination of the gel E after 24 hrs was found to be 1.22 colony forming a unit (CFU) and 1.71 CFU for fungi, at a temperature of 37°C, and 2.99 and 2.50 CFU for bacteria, at the end of 5 days.

**Antimicrobial activity**
The antimicrobial activities (by measuring the zone of inhibition) of the formulation E was compared with the marketed formulation.

Table 3 shows the antimicrobial activity of the formulation E. The Table 3 represents that the formulations had antimicrobial activity against both all four bacteria.

**Stability studies**
The stability studies of formulation E showed that it was most stable during the study period as this formulation did not show any physical instability and the pH before and after the study did not show any significant change.

## DISCUSSION

Until date, guduchi gel has not been formulated as an antimicrobial agent. *T. cordifolia* is well-known for its medicinal effects. Thus, this work uplifts one more arena of guduchi and has successfully formulated "formulation E." The formulation E showed acceptable physical

properties, and hence, was compatible with the skin. The *in-vitro* antimicrobial activity showed maximum activity against *B. subtilis* and *P. aeruginosa*, showing promising prospects as an antibacterial gel for open wounds. In addition, the formulation E passed the short-term stability, indicating the physical and chemical stability of the product. Hence, the formulation E of the aqueous extract of *T. cordifolia* is safe and efficient carriers, with potent antimicrobial activity.

## CONCULSION

From the present study, it was concluded gel formulation of the aqueous extract of *T. cordifolia* was successfully formulated. Thus, this study proves to be beneficial as the formulation can be put forward for extensive evaluation to make it as a marketed product.

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