FORMULATION AND EVALUATION OF HERBAL GEL CONTAINING
TRIDAX PROCUMBENS EXTRACT FOR WOUND HEALING

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ABSTRACT

The aim of present study was to prepare herbal gel formulation containing extract of Tridax Procumbens aerial plant parts for wound healing. Gel was prepared by using Tridax Procumbens extract, carbopel 940, propylene glycol, ethanol, methyl paraben, propyl paraben, EDTA disodium, tiethanolamine and required amount of distilled water. Prepared gel formulations were evaluated for physical appearance, pH, drug content, spreadabilty, viscosity, diffusion study, homogeneity, and grittiness.

KEYWORDS: Tridax procumbens, Carbopol 940, Herbal Gel, Wound Healing.

I. INTRODUCTION

A large number of medicinal plants belonging to the family Asteracea contain chemical compounds exhibiting antimicrobial activity. A number of synthetic drugs produced from pharmaceutical industries from time to time have laid to develop resistant microorganism that becomes major global issue in the treatment of infectious diseases. The antimicrobial formulations of plant origin have been proved to be effective with lesser side effects [2]. The family Asteracea includes about 25 thousands species, many of which are reach in secondary metabolites with biological activity [3]. The aerial part of different species of genus Ashillea are widely used as a folk medicine due to numerous pharmacological properties such as anti-inflammatory, anti-oxidant, anti-plasmodic and antihemorrhoidal [4]. Extract from natural dried leaves of Eupatorium sp. posses with antibacterial and antimalarial activity. Tridax sp. is commonly used in the indian traditional medicine as anti coagulant, hair tonic, antidiarrohea, antidysentry and insect repellant. Both gram positive bacteria (Bacillus Subtilis and Staphylococcus Aureus) and gram negative bacteria (Enterobacter Aerogelles) has been proved to be medical casual organisms of various human infections such as food poisoning, wound infection, nosocomial infection and urinar tract infection.

II. MATERIAL AND METHODS

a) Plant material

The whole plant of Tridax Procumbens was collected form botanical garden of Shiva Trust's Rajesh Bhaiyya Tope College of Pharmacy, Aurangabad.

b) Preparation of plant extract

The aerial parts of plant (leaves, flowers, and stem) were shade dried for a week. The plant material were cut into fine pieces and dried powder (50gm) of each part were extracted sequentially using soxhlet extractor with 250ml of hexane, chloroform, methanol and petroleum ether separately in order to extract non-polar and polar compounds [10]. The crude extract then filtered through whatmann filter paper no. 1 and concentrated extract was subsequently dried aseptically at room temperature.

C) Phytochemical investigation of Tridax Procumbens

1. Detection of steroids: For detection of steroid add 0.5ml of chloroform to the extract. The concentrated sulphuric acid (H2SO4) was added from sides of test tube.

2. Detection of carbohydrate:

2.1. Molisch's test: 1ml of extract was treated with few drops of molisch's reagent and few drops of concentrated H2SO4 were added from sides of test tube.

2.2. Benedict's test: 1ml of extract was treated with Benedict's reagent and boiled for few minutes.
3. Detection of protein:

3.1. Xanthoprotic test: 3ml of extract was treated with few drops of concentrated nitric acid and observe the color change.

3.2. Ninhydrine test: 3ml of extract was treated with 3ml of ninhydrin reagent and allowed to boil for a few minutes.

4. Detection of anthocyanins: 3ml of extract was treated with 3ml of 2N hydrochloric acid and NH₃.

5. Detection of phenols: For detection of phenols 3ml of extract was treated with few drops of alcoholic FeCl₃ solution.

6. Detection of tannins: For detection of tannins 3ml of extract was added to 1% lead acetate.

7. Detection of alkaloids: For detection of alkaloids concentrated extract was treated with 2 ml of dilute HCl and the mixture was gently heated for 20min and allowed to cool and filtered. The filtrate was used the Hagner's test and Wagner's test. In Hagner's test filtrate was treated with Hagner's reagent and in Wagner's test it was treated with Wagner's reagent.

8. Detection of saponins: For detection of saponins the extract was subjected for frothing test. For this 5ml of warm aqueous extract was vigorously shaked and observed for formation of stable foam.

9. Detection of flavonoids: For detection of flavonoids, alkaline reagent test was performed. The extract was treated with 10% of NaOH solution.[10]

c) Formulation of gel:
The gel was prepared by using cabopol 940 (1%), propylene glycol, ethanol, propyl paraben, methyl paraben EDTA di-sodium, triethanolamine and distilled water in quantity to prepare 100gm of gel. The quantity of distilled water required for the formulation is divided into two parts. In one part the exact amount of extract was dissolved and to this calculated amount of ethanol and propylene glycol was added and in other part carbopol 940 was dissolved and to that solution propyl paraben, methyl paraben and EDTA disodium was added. Both of that solutions were mixed in beaker and triethanolamine was drop wise added in mixture to obtained gel consistency [10-14]. Master formula for batch F1 to F9 was given in table 1.

Table 1: Formulation of gel

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Carbopol-940 (gm)</th>
<th>Extract (gm)</th>
<th>Propylene glycol (ml)</th>
<th>Ethanol (ml)</th>
<th>Methyl Paraben (gm)</th>
<th>Propyl Paraben (gm)</th>
<th>EDTA (gm)</th>
<th>Distilled water upto(ml)</th>
<th>Triethanolamine (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>4</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>100</td>
<td>q.s</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>9</td>
<td>2.5</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>100</td>
<td>q.s</td>
</tr>
<tr>
<td>F3</td>
<td>2.5</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>100</td>
<td>q.s</td>
</tr>
<tr>
<td>F4</td>
<td>4</td>
<td>9</td>
<td>2.5</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>100</td>
<td>q.s</td>
</tr>
<tr>
<td>F5</td>
<td>2.5</td>
<td>9</td>
<td>2.5</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>100</td>
<td>q.s</td>
</tr>
<tr>
<td>F6</td>
<td>2.5</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>0.2</td>
<td>0.02</td>
<td>0.03</td>
<td>100</td>
<td>q.s</td>
</tr>
</tbody>
</table>
d) Evaluation of gel formulation

1. **pH**: The pH of 1% aqueous solution of the prepared gel formulations were measured by a pH METER (Lab Electronics Ltd.) \(^{[15]}\).
2. **Viscosity**: The viscosity of gel formulations were determined using Brook Field viscometer with spindle no.4 at 10 rpm \(^{[15]}\).
3. **Spread ability**: The spread ability of gel formulations were determined at 24 hours after permeation by using the spreading diameter of 1 gm of gel between two horizontal plates (20 cm× 20cm) after one min \(^{[16, 17]}\).
4. **Drug content**: About 1gm of gel was accurately weighed and transferred to 100ml volumetric flask to which about 70 ml distilled water was added. After mixing, volume was made up to 100ml with distilled water. The content was filtered through suitable filter paper. An aliquot of 1ml was pipetted out from filtrate. The extract was estimated spectrophotometrically by using Shimadzu UV-VIS Spectrophotometer- 1700 at 281 nm \(^{[18]}\).
5. **In vitro diffusion study**: The diffusion studies of the prepared gel formulations were carried out in Franz Diffusion Cell for studying the dissolution release pattern of gel through cellophane membrane. Gel sample (1gm) was taken on cellophane membrane and the diffusion studies were carried out at 37± 1°C using distilled water as dissolution medium. Five milliliters of each sample was withdrawn periodically at an interval of 1 hour for 8 hours and each sample was replaced with equal volume of dissolution medium. The samples were analyzed for drug content by using distilled water as blank \(^{[18, 20]}\).
6. **Extrudability study**: After the gel formulations were set in the container, it was filled in collapsible tubes. The extrudability of formulations were determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds \(^{[21]}\).
7. **Homogeneity**: After gels have been set in container all formulated gels were tested for homogeneity by visual inspections. They were tested for appearance and presence of any aggregates.
8. **Grittiness**: All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence obviously preparations fulfills the requirements of freedom from particulated matter and from grittiness as desired for any topical preparations \(^{[23]}\).

### III. RESULT AND DISCUSSION

a) **Phytochemical investigation of *Tridax Procumbens***

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Determination of steroids</td>
<td>Upper layer at surface appeared red in color and showed yellow to green fluorescence</td>
<td>Presence of steroids</td>
</tr>
</tbody>
</table>
b) Evaluation of gel formulation

1. **pH of gel**: pH of the gel formulations were in the range of 4.06-5.62 which lies in the normal pH range for skin and would not produce any skin irritation. pH values of F1 – F9 formulation shown in Table 3.

2. **Viscosity of gel**: The viscosity of gel formulations generally reflects its consistency. Decrease in viscosity of gel formulations showed increase in drug release. Viscosity of F1 - F9 formulations were shown in table 3.

3. **Spreadability**: spreadability of F1-F9 formulation showed in table 2. F9 gel formulation showed maximum spreadability then other gel formulations.

4. **Drug content**: drug content of formulation F1-F9 showed in table 3. The maximum drug content was found in formulation F9.

5. **In vitro diffusion study**: invitro diffusion study was carried out in diffusion cell for 8 hours, showed F9 formulation with maximum drug release as compared to other gel formulations showed in table 4.

6. **Extrudability**: the extrudability of formulation F1-F9 showed in table 3.

7. **Homogeneity**: homogeneity of formulation F1-F9 showed in table 3. The formulation F3 and F4 were found to be non-homogenous. Rest of the formulations found to be homogenous.

8. **Grittiness**: grittiness of formulation F1-F9 showed in table 3. The formulation F3 and F4 were found to be gritty but all other formulations are found to be free from grittiness.

### Table-3: Evaluation of gel formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>pH</th>
<th>Extrudability (g/m/cm²)</th>
<th>Spreadability (mm)</th>
<th>Viscosity (cps)</th>
<th>Homogeneity</th>
<th>Grittiness</th>
<th>Drug Content (%)</th>
<th>Diffusion Study (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>4.06</td>
<td>750</td>
<td>36.66</td>
<td>1308</td>
<td>Homogenous</td>
<td>NO</td>
<td>89.77</td>
<td>87.33</td>
</tr>
<tr>
<td>F2</td>
<td>5.12</td>
<td>1000</td>
<td>54.33</td>
<td>1210</td>
<td>Homogenous</td>
<td>NO</td>
<td>94.44</td>
<td>91.66</td>
</tr>
<tr>
<td>F3</td>
<td>4.83</td>
<td>800</td>
<td>36.33</td>
<td>1276</td>
<td>Aggregation</td>
<td>YES</td>
<td>92.88</td>
<td>91.22</td>
</tr>
<tr>
<td>F4</td>
<td>4.52</td>
<td>600</td>
<td>37.66</td>
<td>1368</td>
<td>Aggregation</td>
<td>YES</td>
<td>91.22</td>
<td>86.77</td>
</tr>
<tr>
<td>F5</td>
<td>4.84</td>
<td>820</td>
<td>36.66</td>
<td>1400</td>
<td>Homogenous</td>
<td>NO</td>
<td>91.66</td>
<td>87</td>
</tr>
<tr>
<td>F6</td>
<td>4.82</td>
<td>1100</td>
<td>30.66</td>
<td>1504</td>
<td>Homogenous</td>
<td>NO</td>
<td>90.44</td>
<td>84.88</td>
</tr>
</tbody>
</table>
Natural remedies are more acceptable in the belief that they are effective with lesser side effects than the synthetic ones. Herbal formulations have growing demand globally. It is a very good attempt to establish the herbal gel formulation containing extract of *Tridax Procumbens*. This study revealed that the developed herbal formulation F9 was comparatively better than other batches of formulation.

**IV. REFERENCES**


