Research Paper

Formulation and Evaluation of Polyherbal Gel for Wound Healing

Nikunjana A. Patel*, Megha Patel, Rakesh P. Patel

* Department of Pharmacognosy, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat vidyanagar, Kherva, Gujarat, India
E-Mail: nikunjapatel@rediffmail.com

Abstract

Terminalia arjuna, Centella asiatica and Curcuma longa are reported to possess wound healing, anti-inflammatory, antioxidant and anti-bacterial activities. The carbopol 934 gel formulations containing different concentrations of extract of the above mentioned herbs were formulated and their wound healing activity was studied on experimentally induced open wounds in albino rats. The individual herbs were evaluated for their standard specification according to the Herbal Pharmacopoeia of India. Extracts were obtained by established procedures. HPTLC analysis for the extracts was carried out for identification of some known active constituents present in these herbs. Formulations containing 1% and 2% herbal extracts were prepared and applied topically three times a day to open wounds for 24 days post-operatively and compared with base control. The treated wounds showed a faster rate of wound contraction compared with controls. The wound contraction studies revealed that the wound contractions increase with an increase in the herbal extract concentration.

Keywords: Terminalia arjuna, Centella asiatica, Curcuma Longa, Wound Healing, Polyherbal Formulation

1. Introduction

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases (Biswa et al, 2004). Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhea, scabies, venereal disease, ulcers, snake bite, etc (Mukherjee et al, 2000). More than 80% of the world’s population still depends upon traditional medicines for various skin diseases (Babu et al, 2002). Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process (Purna et al, 2000). A large number of plants are used by folklore traditions in India for treatment of cuts, wounds and burns.

The drugs selected for this work were Terminalia arjuna, Centella asiatica and Curcuma longa. These three important herbs are reported to have significant anti-bacterial, immunomodulatory and anti-inflammatory activities (Phan et al, 2001; Rahman et al, 2004 and Liu et al, 2008) which are complementary to wound healing process. The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness and paucity of reported adverse reaction, prompted us formulate a polyherbal topical preparation and assess its wound healing ability. The combination is used in order to enhance the wound healing activity.

2. Materials and methods

2.1. Materials

The plants were selected on the basis of their antimicrobial activities and their medicinal uses reported in the literatures. The herbs (Terminalia arjuna, Centella asiatica and Curcuma longa) were purchased from plant drug supplier Sanjivani Aushadhalay, Bhavnagar, Gujarat, India. The marker compound curcumin, from Sigma
eldritch, Bombay, India while asiaticoside and arjunic
acid were purchased from Spic Pharma Ltd., Chennai,
India. All other chemicals were of analytical grade and
used without further purification.

2.2. Monographic Analysis of Herbs

The individual herbs were evaluated with regard to their
standard specifications according to the Herbal
Pharmacopoeia of India (1996). The tests carried out were
loss on drying, extractive values and ash values.

2.3. Preparation of Herbal Extract

The dried powder of Centella asiatica leaves, Curcuma
longa rhizomes and Terminalia arjuna bark were
extracted with ethanol by maceration for 72 hours.
The extract obtained was concentrated in rotary evaporator
at a temperature not exceeding 60° C and stored in
desiccators.

2.4. Identification of Active Constituents in the Herbal
Extract

A HPTLC system equipped with linomat V sample
applicator and Camag TLC scanner III, using Camag Win
CATS software was used to identification of some known
active constituents in all the three extracts according to
procedures described in literature. (Singh et al, 2005;
Mobile phases for curcuminoid, asiaticoside and arjunic
acid were chloroform: ethanol: gl. acetic acid (95:5:1),
chloroform: Gl. Acetic acid:methanol:water (60:32:12:8),
toluene: ethyl acetate: formic acid : methanol (6:3:0:1:1.0)
respectively. TLC for curcuminoid was detected at 425 nm
without derivatization while for asiaticoside and arjunolic
acid TLC were detected at 550 nm and 598 nm
respectively after derivatization with anisaldehyde
sulphuric acid reagent.

2.5. Preparation of Topical Formulations

The topical gel was prepared by soaking the carbopol 934
in water for 24 h and their compositions are given in Table
1. The herbal extracts were incorporated into prepared gel
base in two different concentrations. (Sunilkumar et al,

2.6. Antimicrobial Activity

The anti-microbial activity of each formulation was
assessed by cup plate method. The zone of inhibition was
measured in nutrient agar medium, employing Bacillus
subtilis, Staphylococcus aureus, Escherichia coli and
Pseudomonas aeruginosa as test organisms (Perumal et al,

2.7. Wound Healing Studies

An excision wound model was used for studying wound
healing activity. Wistar albino rats of either sexes
weighing 150-180 gm were randomly divided into 4
groups of six animals each. The back of the each animal
was shaved and prepared after washing with spirit. An area
of about 4 cm² was defined with a marker on the shaven
back of the animals. The circular marked area was excised
with its full thickness using a surgical sterile blade and
scissors under ether anesthesia. The formulations G1 and
G2 were applied to the wounded rats of the respective
groups, three times a day. The wound contractions were
measured as the percentage of wound reduction in the
wound area for every four days. (Sunilkumar et al, 1998
and Charde et al, 2003). The reduction in the wound size
was calculated by the formula:

\[ \text{Wound contraction} \% = \frac{\Delta A}{B} \times 100 \]  

\( \Delta A = \text{Difference in the area of the wound in mm}^2 \text{ between the initial}
\text{and on a particular post-operative day} \)

\( B = \text{Area of the wound in mm}^2 \text{ immediately after the wound excision.} \)

2.8. Statistical Analysis

The results were analyzed by one-way ANOVA and a P
value less than 0.01 was considered significant.

3. Results and Discussion

3.1. Monographic Analysis of Herbs

Table 2 shows the results of monographic analysis of the
herbs, performed according to the Indian Herbal
Pharmacopoeia and WHO guideline for quality control of herbal raw materials. It was found that the moisture content of all the extract were less than 10%. The extractive values and ash values of all the extracts were within the pharmacopoeias limit. It indicates the good quality of raw materials.

3.2. Preparation of Herbal Extract

The extract prepared had different color and odor according to raw materials from which they are extracted.

3.3. Identification of Active Constituents in the Herbal Extracts

HPTLC analysis of the herbal extracts was performed and the chromatograms of active constituents and extracts are shown in Figure 1. It was found that the $R_f$ values of the active constituents in the chromatogram of extract were match with chromatogram of active constituents. It indicates the presence of active constituent in extracts. The heights of peaks also indicate the presence of active constituents in significant amount in extract.

3.4. Preparation of Topical Formulations

The gel base without herbal extract was transparent and had good viscosity. The color was changed to brown after adding the extract and viscosity also slightly decreased due to addition of extract.

3.5. Anti-Microbial Activity of the Formulations

The results of the antimicrobial activity of formulations are illustrated in Table 3. The gel base without the herbal extracts did not show any zone of inhibition while in case of formulation G1 G2, the zone of inhibitions were found to increase on increasing the concentration of extracts both the formulations were shown the better activity against *Bacillus subtilis* and *Staphylococcus aureus* than *Escherichia coli* and *Pseudomonas aeruginosa*. Hence the results of this study confirm that the herbs possess anti-

Table 2. Monographic Analysis of Herbs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C. longa (%w/w)</th>
<th>C. asiatica (%w/w)</th>
<th>T. arjuna (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obtained Value</td>
<td>Pharmacopoeial Limit</td>
<td>Obtained Value</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>7.98±0.52</td>
<td>NMT 12</td>
<td>7.66±0.38</td>
</tr>
<tr>
<td>Alcohol Soluble Extractive</td>
<td>10.15±2.22</td>
<td>NLT 8</td>
<td>10.37±0.33</td>
</tr>
<tr>
<td>Water Soluble Extractive</td>
<td>13.29±0.25</td>
<td>NLT 9</td>
<td>15.38±0.39</td>
</tr>
<tr>
<td>Total Ash</td>
<td>7.07±0.062</td>
<td>NMT 1</td>
<td>22.32±0.32</td>
</tr>
<tr>
<td>Acid Insoluble Ash</td>
<td>0.51±0.028</td>
<td>NMT 1</td>
<td>6.19±0.23</td>
</tr>
</tbody>
</table>

3.3. Identification of Active Constituents in the Herbal Extracts

HPTLC analysis of the herbal extracts was performed and the chromatograms of active constituents and extracts are shown in Figure 1. It was found that the $R_f$ values of the active constituents in the chromatogram of extract were match with chromatogram of active constituents. It indicates the presence of active constituent in extracts. The heights of peaks also indicate the presence of active constituents in significant amount in extract.

Table 3. Antimicrobial Effect of Formulations

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Zone of inhibition (mm)</th>
<th>G1*</th>
<th>G2*</th>
<th>Nitrofurazone Cream</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td></td>
<td>13±0.2</td>
<td>19±0.7</td>
<td>22±0.9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td>14±0.4</td>
<td>16±0.6</td>
<td>20±0.8</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td>12±0.2</td>
<td>14±0.5</td>
<td>20±0.6</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td>12±0.3</td>
<td>15±0.3</td>
<td>27±0.8</td>
</tr>
</tbody>
</table>

G1*: Gel formulation with 1% extract  
G2*: Gel formulation with 2% extract
Table 4. The Effect of Formulations on Excision Wound Healing in Rats

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Wound Contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple Gel (Control)</td>
</tr>
<tr>
<td>4</td>
<td>11.28 ± 0.095</td>
</tr>
<tr>
<td>8</td>
<td>27.38 ± 0.142</td>
</tr>
<tr>
<td>12</td>
<td>46.17 ± 0.213</td>
</tr>
<tr>
<td>16</td>
<td>61.4 ± 0.172</td>
</tr>
<tr>
<td>20</td>
<td>74.82 ± 0.095</td>
</tr>
<tr>
<td>24</td>
<td>88.08 ± 0.196</td>
</tr>
</tbody>
</table>

*Significant difference between treatment groups and the control group (P ≤ 0.05)
G1<sup>+</sup>: Gel formulation with 1% extract; G2<sup>+</sup>: Gel formulation with 2% extract

Figure 1. HPTLC Chromatograms of A) C. longa Extract, B) Curcuminoids, C) C. asiatica Extract, D) Asiaticoside, E) T. arjuna Extract, & F) Arjunolic Acid
On the basis of the result obtained in the present investigation, it is possible to conclude that the gel of the extracts of above plants has significant wound healing activity. Several phytoconstituents present in plants like asiaticoside, curcuminoids and triterpenoids are known to promote wound healing process due to their anti-oxidant and anti-microbial properties. The study reveals that the better activity of polyherbal formulation may be due to the synergistic action of the plants constituents present in the formulation.

Figure 2. Wound Healing Activity of Gel Formulation G2 On A) Zero Post Wounding Day, B) Fifth Post Wounding Day, C) Tenth Post Wounding Day, D) On The Day Of Complete Healing

Figure 3. Effect of Different Gel Formulations and Standard on Wound Healing
4. Conclusion

From the above observations, it can be concluded that all the monographic parameters of the selected herbs were within in the Pharmacopoeial limit indicates the good quality of raw materials. HPTLC studies of the extracts showed the presence of selected active constituents in significant amount. Their $R_f$ values were similar to standard $R_f$ values. The microbiological studies indicated that the formulations possess anti-microbial activity against tested organisms. The wound contraction studies revealed that the wound contraction increases on increasing the concentration of herbal extract. The study also reveals that the better activity of polyherbal formulation may be due to the synergistic action of the plants constituents present in the formulation. Thus, the prepared topical gels possess a multifaceted approach in healing the wound contraction.

References

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