ABSTRACT

Objective: The objective of the study was to determine the wound healing activity of the combination of ethanolic extract of *Ageratum conyzoides* L. Leaf (white and purple), *Centella asiatica*, and astaxanthin gel preparation.

Materials and Methods: In-gel preparation, we had 3 different formulas of gelling agents, namely: Carbopol 934 (1%), HPMC (9%), and Na-CMC (4%). Then, we evaluated these formulas including organoleptic, pH, spreadability, and viscosity. In order to determination of wound healing activity, we had 6 groups, including negative (Placebo), positive (Bioplacenton), BP5 (*A. conyzoides* L. Leaf Ethanolic Extract (White flower type) 5%, *C. asiatica* L. Urb Leaf Ethanolic Extract 2.5%, Astaxanthin 0.05%), BU5 (*A. conyzoides* L. Leaf Ethanolic Extract (White flower type) 5%, *C. asiatica* L. Urb Leaf Ethanolic Extract 2.5%, Astaxanthin 0.05%), BU10 (*A. conyzoides* L. Leaf Ethanolic Extract (Purple flower type) 10%, *C. asiatica* L. Urb Leaf Ethanolic Extract 5%, Astaxanthin 0.1%) and BP10 (*A. conyzoides* L. Leaf Ethanolic Extract (White flower type) 10%, *C. asiatica* L. Urb Leaf Ethanolic Extract 5%, Astaxanthin 0.1%). All groups were treated by incision as long as 1.5 cm. Observation of the wound length was conducted for 14 days.

Results: Based on the evaluation test, carbopol 934 (1%) gelling agent formula was better than HPMC and Na-CMC. Meanwhile, the percentage of wound healing activity for negative, positive, BP5, BU 5, BU10, and BP10 groups were 72.51%, 69.36%, 70.14%, 81.70%, 86.84%, and 80.21%, respectively. The BU5 and BU10 showed significant activity (p<0.05) compared to positive and negative groups.

Conclusion: BU10 provided the best wound healing activity and very potential to be developed as a commercial product.

Key Words: *Ageratum conyzoides* L, *Centella asiatica*, Astaxanthin, Gel Preparation, Wound Healing.

ÖZ
Amaç: Çalışmanın amacı, *Ageratum conyzoides* Leaf (beyaz ve mor), *Centella asiatica* ve astaxanthin jel preparatının etanolik ekstraktının kombinasyonunun yara iyileştirici aktivitesini belirlemekti.

**Gereç ve Yöntemler:** Jel içi hazırlamada, 3 farklı jelleştirme ajanı formülümüz vardı: Carbopol 934 (% 1), HPMC (% 9) ve Na-CMC (% 4). Daha sonra bu formülleri organoleptik, pH, yayılabilirlik ve viskozite dahil olmak üzere değerlendirildik. Yara iyileştirme aktivitesini belirlemek için negatif (Plasebo), pozitif (Bioplacenton), BP5 (*A. conyzoides* L. Leaf Ethanolic Extract (Beyaz çiçek tipi)% 5, *C. asiatica* L. Urb Leaf Ethanolic Extract olmak üzere 6 grubumuz vardı. % 2,5, Astaxanthin% 0,05), BU5 (*A. conyzoides* L. Leaf Ethanolic Extract (Mor çiçek türü)% 5, *C. asiatica* L. Urb Leaf Ethanolic Extract% 2,5, Astaxanthin% 0,05), BU10 (*A. conyzoides* L. Leaf Ethanolic Extract (Mor çiçek türü)% 10, *C. asiatica* L. Urb Leaf Ethanolic Extract% 5, Ve Astaxanthin% 0,1) ve BP10 (*A. conyzoides* L. Leaf Ethanolic Extract (Beyaz çiçek türü)% 10, *C. asiatica* L. Urb Leaf Ethanolic % 5 ve Astaxanthin% 0,1 ekstrakte edin). Tüm gruplar 1,5 cm uzunluğunda kesi ile tedavi edildi. Yara uzunluğunun gözlenmesi 14 gün boyunca gerçekleştirilirdi.

**Bulgular:** Carbopol 934 (% 1) jelleştirici madde formülü, değerlendirme testine göre HPMC ve Na-CMC'den daha iyiydi. Negatif, pozitif, BP5, BU 5, BU10 grupları için yara iyileştirme aktivitesi yüzdesi % 72,51, % 69,36, % 70,14, % 81,70, % 86,54 ve % 80,21 BU5 ve BU 10, pozitif ve negatif gruplara kıyasla anlamlı aktivite gösterdi (p <0,05).

**Sonuç:** BU10, en iyi yara iyileştirme aktivitesini ve ticari bir ürün olarak geliştirme potansiyeli sağladı

**Anahtar kelimeler:** Ageratum conyzoides L, Centella asiatica, Astaxanthin, Jel Hazırlama, Yara İyileştirme.

**INTRODUCTION**

Wound is defined as physical, chemical or thermal injuries or insult that results in an opening or breaking in the integrity of the skin or disruption of anatomical and functional integrity of living tissues. Globally, wound prevalence reached ~8.2 million people, and Medicare cost ranged from $28.1 billion to $96.8 billion. Many wound healing products available in the market. However, at present, there is no standard topical treatment for wound healing. Bioplacenton is a topical preparation that available in the market. This product is commonly used for wound healing treatment in Indonesian society. The ingredients of this product including neomycin sulfate 0.5% and placenta extract 10%. Placenta extract showed acceleration healing of wound size, followed by decreased of TGF and increased of VEGF and CD31. *Ageratum conyzoides*, *Centella asiatica*, and astaxanthin have been shown to have wound healing activity. Ethanol extract of *A. conyzoides* showed an increase of 40% of tissue tensile strength, a decrease of 33% time of re-epithelialization, higher of collagen, and cellular infiltration. Different fraction of *C. asiatica* (Hexane, ethyl acetate, methanol, and water extract) showed tensile strength, developed epithelization, and keratinization of the wounds. Asiaticoside and madecassoside from *C. asiatica* play an important role in this wound healing activity. Astaxanthin is a powerful antioxidant, which is isolated from a lobster. Besides this antioxidant activity, astaxanthin provides wound healing activity through the decrease of iNOS and an increase of Col1A1 and bFGF. *Col1A1* provides instruction for making collagen which supports many tissues including skin, in a while, bFGF regulates many biological functions including tissue repair. However, the wound healing activity of these combinations is still unknown. Therefore, we conducted a wound healing activity study of *A. conyzoides*, *C. asiatica*, and astaxanthin combination gel preparation.
MATERIALS AND METHODS

Ethical clearance
All of the procedures were according to the guide for the care and use of laboratory animals and approved by Bakti Tunas Husada Health Science College Ethical Committee with no. 03/kepk-bth/04/20.

Plant materials and extract preparation
*A. conyzoides* Leaf and *C. asiatica* Leaf were collected from Galunggung Mountain Area, Tasikmalaya, West Java. This plant was authenticated by the School of Life Science and Technology, Institut Teknologi Bandung. In a while, astaxanthin was obtained from Sigma Aldrich. The Leaf was shade dried and coarsely powdered by the grinder and stored in an airtight container at room temperature. The dried Leaf of *A. conyzoides* L (1000 gram Purple flower type Leaf and 1000 gram White Flower type leaf) and *C. asiatica* (2000 gram) were used for maceration using ethanol 96% for 24 hours and repeat three times. The extract was filtered and concentrated using a rotary evaporator at 60°C. The percentage yield was reported and preserved at a refrigerator at 4°C until further use.

Standardization of simplicia
Standardization of simplicia including organoleptic, microscopic, and secondary metabolite analysis. The secondary metabolite phytochemical determination including alkaloid, flavonoid, polyphenol, quinone, tannin, monoterpenes-sesquiterpenes, triterpenoid, and steroid according to Fransworth.

Preformulation of gel preparation
The objective of the gel pre-formulation was to determine the best gel formula from three basis including carbopol 934 1%, Hydroxypropylmethylcellulose (HPMC) 9% and Natirum-Carboxymethylcellulose (Na-CMC) 4%. The preformulation evaluation including organoleptic, pH, homogeneity, viscosity, and spreadability.

Wound healing activity test
We have 6 groups, including negative (Placebo), positive (Bioplacenton), BP5 (*A. conyzoides* L. (White flower) 5%, *C. asiatica* L. Urb 2.5%, Astaxanthin 0.05%), BU5 (*A. conyzoides* L. (Purple flower) 5%, *C. asiatica* L. Urb 2.5%, Astaxanthin 0.05%), BU10 (*A. conyzoides* L. (Purple flower) 10%, *C. asiatica* L. Urb 5%, and Astaxanthin 0.1%) and BP10 (*A. conyzoides* L. (White flower) 10%, *C. asiatica* L. Urb 5%, and Astaxanthin 0.1%). All groups was treated by incision wound as long as 1.5 cm. To determine the wound healing capacity, we conducted once daily observation of the wound length using calipers for a 14 day.

Analysis data
The obtained data were analyzed using SPSS 16.00 with ANOVA and followed by posthoc test Least Singinificat Difference (LSD). A difference was considered significant if the p-value was less than 0.05.

RESULTS AND DISCUSSION

Standardization of Simplicia
Table 1. Organoleptic and microscopic

<table>
<thead>
<tr>
<th>Simplicia</th>
<th>Organoleptic</th>
<th>Microscopic</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Simplicia</th>
<th>Organoleptic</th>
<th>Microscopic</th>
</tr>
</thead>
</table>
**Centella asiatica** L. Urb

- **Shape:** powder
- **Colour:** green
- **Odour:** aromatic typical
- **Taste:** bitter
- **Stomata, hair cover, oxalic acid, sklerenkim, epidermis, wooden vessel**

**Ageratum conyzoides** L. leaf (white flower type)

- **Shape:** powder
- **Colour:** pale green
- **Odour:** aromatic typical
- **Taste:** bitter
- **Stomata, hair cover, secretion cells and essential oil, stomata**

**Ageratum conyzoides** L. leaf (purple flower type)

- **Shape:** powder
- **Colour:** green
- **Odour:** aromatic typical
- **Taste:** bitter
- **Stomata, hair cover, secretion cells, epidermis, wooden vessels**

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**Table 2. Non specific parameter**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Results (%)</th>
<th>Standard (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td><em>Ageratum conyzoides</em> L. leaf (white flower type)</td>
<td>5.33</td>
<td>&lt;10</td>
</tr>
<tr>
<td>b.</td>
<td><em>Ageratum conyzoides</em> L. leaf (purple flower type)</td>
<td>5.33</td>
<td>&lt;10</td>
</tr>
<tr>
<td>c.</td>
<td><em>Centella asiatica</em> L. Urb</td>
<td>6.67</td>
<td>&lt;10</td>
</tr>
<tr>
<td>2</td>
<td>Ash content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td><em>Ageratum conyzoides</em> L. leaf (white flower type)</td>
<td>11.84</td>
<td>&lt;13</td>
</tr>
<tr>
<td>b.</td>
<td><em>Ageratum conyzoides</em> L. leaf (purple flower type)</td>
<td>10.57</td>
<td>&lt;13</td>
</tr>
<tr>
<td>c.</td>
<td><em>Centella asiatica</em> L. Urb</td>
<td>10.60</td>
<td>&lt;18.05</td>
</tr>
<tr>
<td>3</td>
<td>Dry shrinkage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td><em>Ageratum conyzoides</em> L. leaf (white flower type)</td>
<td>8.7</td>
<td>&lt;10</td>
</tr>
<tr>
<td>b.</td>
<td><em>Ageratum conyzoides</em> L. leaf (purple flower type)</td>
<td>9.91</td>
<td>&lt;10</td>
</tr>
<tr>
<td>c.</td>
<td><em>Centella asiatica</em> L. Urb</td>
<td>9.68</td>
<td>&lt;11</td>
</tr>
<tr>
<td>4</td>
<td>Yields</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td><em>Ageratum conyzoides</em> L. leaf (white flower type)</td>
<td>20.16</td>
<td>&gt;7.2</td>
</tr>
<tr>
<td>b.</td>
<td><em>Ageratum conyzoides</em> L. leaf (purple flower type)</td>
<td>13.87</td>
<td>&gt;7.2</td>
</tr>
<tr>
<td>c.</td>
<td><em>Centella asiatica</em> L. Urb</td>
<td>11.74</td>
<td>&gt;7.2</td>
</tr>
</tbody>
</table>

**Table 3. Phytochemical screening**

<table>
<thead>
<tr>
<th>Secondary metabolite</th>
<th><em>Ageratum conyzoides</em> L. Simplicia Extract</th>
<th><em>Centella asiatica</em> L. Urb Simplicia Extract</th>
</tr>
</thead>
</table>
The standardization evaluation was including organoleptic, microscopic, non-specific parameters, and phytochemical screening. The results of the organoleptic, microscopic (table 1), water content, ash content, dry shrinkage, and yields (table 2) were fulfilled of the Indonesia Materia Medica Standard and Indonesia Herbal Pharmacopeia. Therefore, due to the fulfillment of the national standard criteria, these simplicia were qualified for further wound healing test activity. Phytochemical screening study showed positive for flavonoid, alkaloid, saponin, polyphenol, tannin, quinone, steroid-triterpenoid, and monoterpene-sesquiterpene, but negative for tannin (table 3).

Evaluation of the gel preparation
Table 4. Preformulation of gel preparation

<table>
<thead>
<tr>
<th>Substances</th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbopol 934</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose (HPMC)</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na-Carboxymethylcellulose (Na-CMC)</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Propylenglycol</td>
<td>15</td>
<td>2,5</td>
<td>2</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>-</td>
<td>-</td>
<td>qs</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0,15</td>
<td>0,2</td>
<td>-</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0,18</td>
<td>0,18</td>
<td>-</td>
</tr>
<tr>
<td>Tween 80</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Aquades</td>
<td>Ad 20 g</td>
<td>Ad 20 g</td>
<td>Ad 20 g</td>
</tr>
</tbody>
</table>

+ : Positive results, - : Negative results
The organoleptic evaluation showed carbopol 934 gel preparation gave the best texture and color than Na-CMC and HPMC (figure 1). Therefore, carbopol 934 bases in three concentrations (0.5%, 1%, and 1.5%) were used for further gel preparation formula evaluation (Table 5). The evaluation including stability, organoleptic, pH, viscosity, and spreadability for 3 cycles in two temperatures 20°C and 40°C (Table 6).

The results of the evaluation showed Carbopol 1% gave the best formulas and fulfilled the criteria. Therefore, Carbopol 1% (F3b) was chosen to be incorporated with the combination of *A. conyzoides* L Leaf Ethanolic Extract (white, and purple flower type), *C. asiatica* Leaf Ethanolic Extract, and Astaxanthin.
The Determination of wound healing activity

![Graph showing wound healing activity test](image)

**Figure 2.** Wound healing activity test

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Negative</th>
<th>Positive</th>
<th>BU10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
<td><img src="image" alt="Image" /></td>
</tr>
</tbody>
</table>
Wound Healing is comprised in three phases including inflammation, proliferation, and remodeling. The first phase is an inflammation condition that involves polymorphnuclear (PMN) and macrophage which last 3-5 days; the second phase marked with a new tissue formation, fibroblast, endotel and collagen formation; the third phase is maturation phase that provides tensile strength, epithelium and new tissue growth. The BU10 group showed the best wound healing activity than other groups (p<0.05) (negative, positive, BP5 and BP10), but non-superior (p>0.05) with BU5. The wound healing percentage of BU10 reach 86.54% with complete remission time in the days 8th. Meanwhile, positive groups (bioplacenton) showed no difference with negative control (p>0.05). Until now, we could not confirm this phenomenon.

The wound healing activity of BU10 may due to the secondary metabolite composition in *A. conyzoides* L (purple flower type), *C. asiatica*, and the antioxidant activity of astaxanthin. Flavonoid composition in *A. conyzoides* L such as kaempferol and quercetin showed anti-inflammation, anti-oxidant and immunosuppressive activity. Alkaloid and saponin composition of *A. conyzoides* L also has a role in wound healing activity through fibroblast initiation, anti-inflammation, cell repairing, and strength of the skin cells. Moreover, *C. asiatica* secondary metabolites such as Asiaticoside and madecassoside play important role in wound healing activity, although madecassoside is more effective than asiaticoside. Asiaticoside stimulated collagen, epidermis formation, antioxidant activity and anti-inflammation activity which resulting scars formation inhibition.

**CONCLUSION**
The combination of *A. conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *C. asiatica* L. Urb leaf ethanolic extract 5%, and Astaxanthin 0.1% showed the best wound healing activity and very potential to be developed as a commercial product. Moreover, in the
next study, we need to determine the relationship between antioxidant and wound healing activities.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
The authors declares no conflict of interest of this research.

REFERENCE
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