

Wound Healing Effectivity of *Ageratum conyzoides* L. Leaf Ethanolic Extract (Purple Flower Type), *Centella asiatica* (L.) Urban Leaf Ethanolic Extract, and Astaxanthin Combination Gel Preparation in Diabetic Animal Model

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ABSTRACT

The diabetic wounds are one of the global burden diseases. In our previous study, *Ageratum conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1% combination gel preparation showed remarkable wound healing effectivity. However, this wound healing effectivity in the diabetic condition is still unknown. There were three groups used, namely negative group (placebo/gel base), positive group (oxoferin/tetrachlorodecaoxide), and test group (*Ageratum conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1%). Each group consisted of three male Wistar rats. We conducted diabetes induction with alloxan (175 mg/Kg BW i.p) and followed by an incision 1.5 cm on the back. All of these groups were given treatment once daily and monitored for 14 days. The results of the study showed the test group indicated significantly better effectivity than positive and negative groups ($p < 0.05$). The percentages of the wound healing effectivity of the test, positive and negative groups were 62.74%, 51.77%, and 41.65%, respectively. *Ageratum conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1% combination gel preparation has excellent potential to be developed as a commercial product to treat diabetic wound conditions.

INTRODUCTION

Diabetes is one of the top 10 leading causes of death worldwide (Forouzanfar *et al.*, 2016). Global incidence, prevalence, death, and disability-adjusted life-years were projected to increase in 2025 to 26.6 million, 570.9 million, 1.59 million, and 79.3 million in 2025, respectively (Lin *et al.*, 2020). Diabetic wounds or diabetic foot ulcers are a common complication in diabetic patients with the prevalence of this condition reaching 11.6% and the prevalence increased over time (Abdissa *et*

al., 2020; Lin *et al.*, 2020). It is estimated that the prevalence of this condition in Asia will reach over 5.5% (95%CI: 4.6-6.4%) (Zhang *et al.*, 2017). This condition is related to an increase in amputation incidence and mortality (Robbins *et al.*, 2008; Kim *et al.*, 2018). In a cross-sectional survey study conducted by Bondor *et al.* (2016), that involved 21,174 patients, 14.85% reported a history of foot ulcers and 3.6% reported an amputation. Moreover, the diabetic foot ulcer relates to an increase of 5-year mortality rate between 43-55% (Robbins *et al.*, 2008).

However, there are no guidelines that recommend any topical treatment of this condition (Everett *et al.*, 2018; Schaper *et al.*, 2020). In our previous study, *Ageratum conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1% combination gel preparation showed remarkable wound healing effectivity (Sukmawan *et al.*, 2021). However, the level of this wound healing effectivity in the diabetic condition is still unknown. Accordingly, we conducted a wound healing effectivity study of this combination in a diabetic animal model.

METHODS

Ethical Clearance

All the procedures of this study were conducted according to the appropriate institutional guidelines 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

Ageratum conyzoides L. leaf (purple flower) and *Centella asiatica* leaf were collected from Galunggung Mountain Area at February

2021, District of Tasikmalaya, West Java, Indonesia. The location to collected of these plants was the same as our previous wound healing study (Sukmawan *et al.*, 2021). We collected leaf samples from *A. conyzoides* purple flower type only. These plants were authenticated by the Department of Biology, Padjajaran University. Additionally, astaxanthin was purchased from Sigma Aldrich ($\geq 97\%$; HPLC).

Extract Preparations

Ageratum conyzoides L. leaf (purple flower) and *Centella asiatica* leaf were shade dried and coarsely powdered by the grinder and stored in an airtight container plus silica gel at room temperature. The dried leaf of *Ageratum conyzoides* L (1000 grams purple flower type and *Centella asiatica* (1000 gram) was extracted by maceration methods using ethanol 96% as a solvent for 24 hours and repeat three times. The extract was filtered and evaporated using a rotary evaporator at 60°C until no drops of solvent were seen, then followed by a water bath to obtain a concentrated extract (IKA RV-10). The percentage yield was reported and preserved in a refrigerator until further use.

Table 1. Gel preparation formula

Formula	(%)
<i>Ageratum conyzoides</i> L. Extract	10
<i>Centella asiatica</i> (L.) Urban Extract	5
Astaxanthin	0.1
Carbopol 934	2
Propylenglycol	2
DMDM Hydantoin	0.5
Triethanolamine	q.s
Tween 80	q.s
Aquadest	ad 15 g

Table 2. The result of phytochemical screening of *A. conyzoides* and *Centella asiatica*

Secondary Metabolites/ Yield	<i>Ageratum conyzoides</i> L.		<i>Centella asiatica</i> L. Urb	
	Simplicia	Extract	Simplicia	Extract
Flavonoid	+	+	+	+
Alkaloid	+	+	+	+
Saponin	+	+	+	+
Polyphenol	+	+	+	+
Tannin	-	-	-	-
Quinone	+	+	+	+
Steroid	+	+	+	+
Monoterpene/ Sesquiterpene	+	+	+	+
Yield		21.09%		13.69%

+: positive result.

-: negative result.

Table 3. The result of gel preparation evaluation

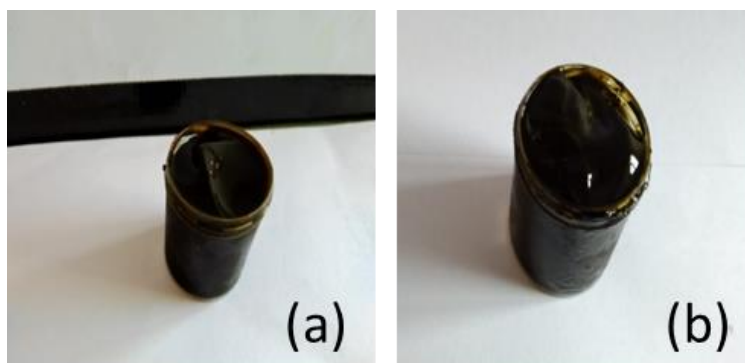
Parameters	Cycle 1	Cycle 6	Recommendation
Organoleptic	Color: Blackish Green Odor: typical Consistency: Viscous	Color: Blackish Green Odor: typical Consistency: Viscous	Unchanged
Homogeneity	Homogen	Homogen	Homogen
pH	6	6	4-6.5
Viscosity	2300 cp	2650 cp	2000-4000 cp
Spreadability	6.5 cm	5.2 cm	5-7 cm

Table 4. The results of the wound healing study

Groups	Wound Healing Effectivity (%)
Test group	62.74±0.761**
Positive group	51.77±1.303*
Negative Group	41.65±1.117

* showed significant difference compared with the negative group ($p < 0.05$).

** showed significant difference compared with negative and positive groups ($p < 0.05$).

**Figure 1.** Organoleptic evaluation between Cycle 1 (a) and Cycle 6 (b)

Phytochemical Screening

The phytochemical analysis of the secondary metabolites is according to Fransworth (1996). The results of this analysis included alkaloids, flavonoids, saponins, polyphenols, tannins, quinones, steroids, monoterpenes/sesquiterpenes.

Formulation and Evaluation of Gel Preparation

The formulation of the gel preparation was according to Sukmawan *et al.* (2021) (Table 1) with slight concentration modification of the carbopol 934 from 1% to 2%. The evaluation of this formulation included organoleptic, pH, homogeneity, viscosity, spreadability, and stability test (Sukmawan *et al.*, 2021).

Wound Healing Activity Study

There were three groups namely: negative group (placebo/gel base), positive group (oxoferin-tetrachlorodecaoxide) (PT. Pharos), and test group (*Ageratum conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1%). Each of group consisted of three male Wistar rats. We conducted diabetes induction with alloxan (175 mg/Kg BW i.p) (Sigma Aldrich 98%). The diabetic animals (blood glucose concentration > 200 mg/dL) (Accu check-performa) then conducted an incision 1.5 cm on the back. All groups were given treatment once daily and

monitored for 14 days of the wound length using a Vernier caliper.

Data Analysis

The data was presented in an average \pm standard deviation (SD). We used ANOVA and post-hoc (LSD) to calculate the difference between these groups with a 95% confidence interval (CI) and significance set as $p < 0.05$ using SPSS 16.00 (IBM Corp, Armonk, NY, USA).

RESULTS AND DISCUSSION

Phytochemical Screening and Extract Yields

The phytochemical screening study of *A. conyzoides* and *C. asiatica* in simplicia and extract

preparations showed positive for flavonoids, alkaloids, saponins, polyphenols, quinones, steroids, and monoterpenes/sesquiterpenes. However, there was a negative result for tannins. The percentage yields of the *A. conyzoides* and *C. asiatica* extract were 21.09% and 13.69%, respectively (Table 2).

Gel Preparation Evaluation

All of the evaluations of the gel preparation fulfilled the criteria of the gel preparation, including those of organoleptic, homogeneity, pH, viscosity, and spreadability (Table 3 and Figure 1) (Voight, 1994; Garg *et al.*, 2002).

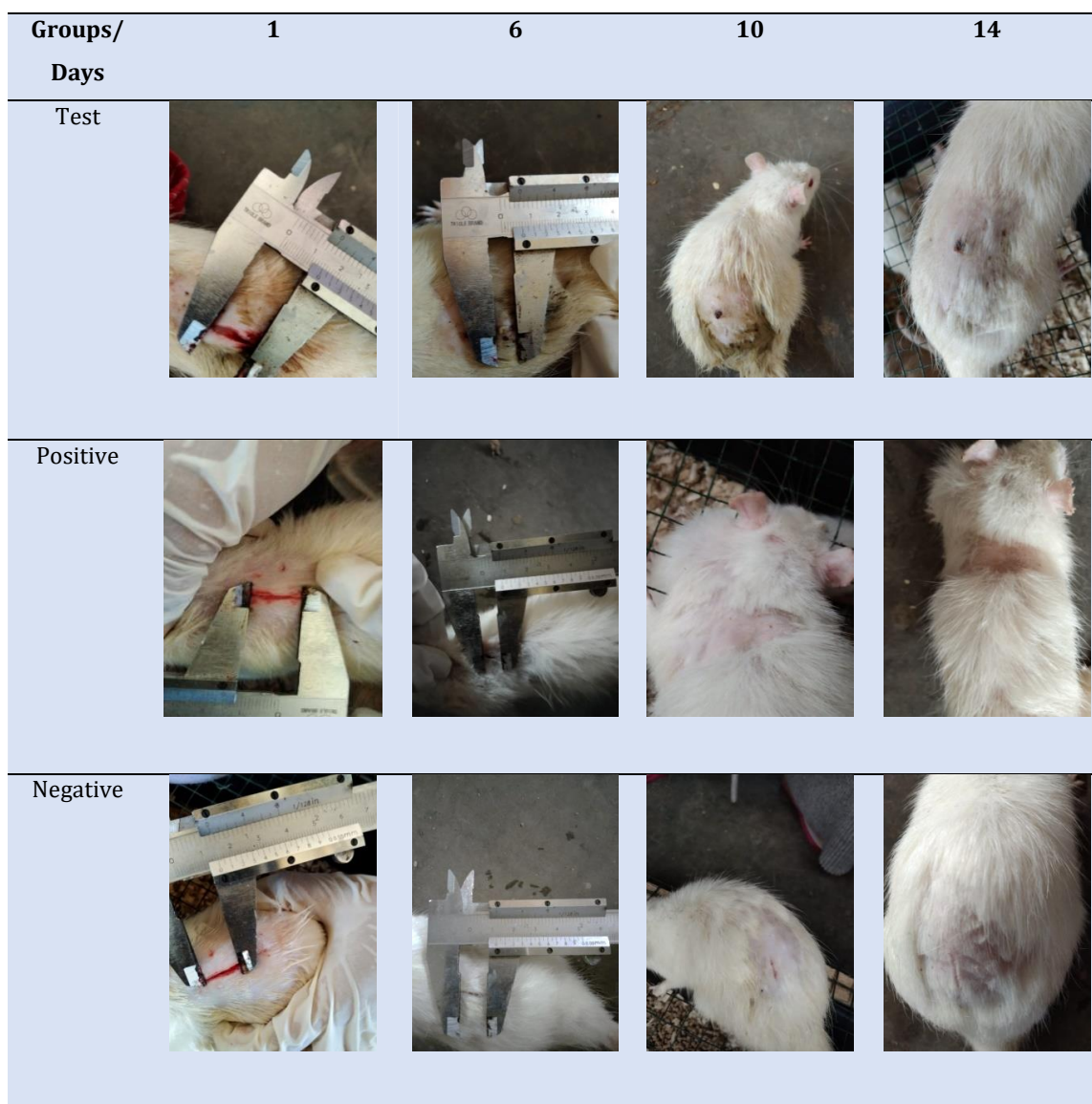


Figure 2. The result of wound healing effectivity

Wound Healing Study in Diabetic Animal Model

The results of our study of *Ageratum conyzoides* L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1% combination gel preparation showed superior efficacy than oxoferin (tetrachlorodecaoxide) and negative group ($p=0.000$; 95%CI). As known tetrachlorodecaoxide is very effective to treat an ulcer in diabetic patients (Parikh *et al.*, 2016). The percentage wound healing effectivity of the test, positive and negative groups were 62.74%, 51.77%, and 41.65%, respectively. Moreover, the average days of complete healing or the wound completely closed of the incision wound of the test, positive, and negative groups were 9.67, 10.67, and 12.67 days, respectively (Table 4 and Figure 2).

The wound healing effect of this combination may be related to many mechanisms of action. The extracts of *Ageratum conyzoides* L showed anti-inflammation, anti-oxidant, immunomodulatory activity, cell repairing, and also strengthening of the skin cells, with anti-bacterial agency (Sartika, 2010; Sukmawan and Aryanti, 2016; Safani *et al.*, 2019; Suhendy *et al.*, 2019). *Centella asiatica* showed collagen stimulation, epidermis formation, anti-oxidant, anti-inflammation, and anti-bacterial activity (Somboonwong *et al.*, 2012; Kurnianto *et al.*, 2017). Whereas, astaxanthin showed powerful anti-oxidant activity, tissue repair, and collagen formation (Meephanan *et al.*, 2017; Yun *et al.*, 2010).

CONCLUSION

Ageratum conyzoides L. leaf ethanolic extract (purple flower type) 10%, *Centella asiatica* (L.) Urban leaf ethanolic extract 5%, and astaxanthin 0.1% combination gel preparation showed powerful wound healing activity, therefore this combination has excellent potential to be developed as a commercial product to treat diabetic wound conditions.

The research limitations are that only the qualitative wound healing effectivity measurement was done, without any quantitative determinations such as cytokine level, collagen formation, the strength of the skin cells, etc. Moreover, additional research is needed, with the administration more than once daily of this combination gel in longer study times.

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