Journal of Vocational Health Studies

https://e-journal.unair.ac.id/JVHS

ANTIOXIDANT POTENTIAL OF TEMULAWAK (CURCUMA XANTHORRHIZA) EXTRACT GEL AS A CANDIDATE FOR WOUND HEALING

POTENSI ANTIOKSIDAN GEL EKSTRAK TEMULAWAK (CURCUMA XANTHORRHIZA) SEBAGAI KANDIDAT PENYEMBUH LUKA

Original Research Article
Penelitian

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A B S T R A C T

Background: *The complete treatment of chronic wounds remains a significant unmet medical need. To expedite the healing of chronic wounds, numerous studies have begun to utilize gels, including hydrogels or sol-gels, which incorporate a combination of antioxidant properties. Curcuma xanthorrhiza is known to contain polyphenolic compounds, which include flavonoids acting as an antioxidant.* ***Purpose:*** *Examine the potential of C. xanthorrhiza extract (CXE) gel as a candidate for wound-healing by measuring its antioxidant activity.* ***Method:*** *Three CXE gel formulas were prepared from different concentrations of CMC-Na (3, 4, and 5%). Each formula consisted of 5% CXE, 15% Propylene Glycol, 10% Glycerin, 0.25% Methylparaben, and distilled water. The quality of the CXE gel was tested through homogeneity, spreadability, pH, and viscosity tests. The antioxidant activity was measured by 2,2-Diphenyl-1-picrylhydrazil (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-reducing activity assay in a gel formula that was close the standards. The measurement results were then used in the calculation of antioxidant activity to determine the IC_{50} value.* ***Result:*** *Formulas 1, 2, and 3 meet the requirements by yielding a homogeneous gel with a spreadability of 5.37 – 5.93 cm, a pH level of 5.87 – 6.10, and a viscosity of 8.800 – 9296.67 cps. The highest DPPH and ABTS percentages were 34.04% and 5.28%, respectively. The IC_{50} values of CXE gel in DPPH and ABTS were 1973.38 µg/mL and 700.65 µg/mL, respectively.* ***Conclusion:*** *The CXE 1, 2, and 3 gel formula meets the requirements and has the potential to be used as a wound healing therapy through its antioxidant properties.*

A B S T R A K

Latar belakang: Perawatan lengkap kronis masih menjadi kebutuhan medis yang belum terpenuhi. Dalam mempercepat penyembuhan luka kronis, banyak penelitian yang mulai menggunakan gel (hidrogel atau sol-gel) dengan kombinasi sifat antioksidan. *Curcuma xanthorrhiza* diketahui mengandung senyawa polifenol yang termasuk dalam senyawa flavonoid yang berperan sebagai antioksidan. **Tujuan:** Menguji potensi gel *C. xanthorrhiza extract* (CXE) sebagai kandidat penyembuhan luka melalui pengukuran aktivitas antioksidan. **Metode:** Tiga formula gel CXE dibuat dari konsentrasi CMC-Na yang berbeda (3, 4 dan 5%). Setiap formula diberi CXE dengan konsentrasi 5%, propilen glikol dengan konsentrasi 15%, Gliserin dengan konsentrasi 10%, Metilparaben dengan konsentrasi 0,25%, dan aquadest. Kualitas gel CXE diuji melalui uji homogenitas, daya sebar, pH, dan viskositas. Aktivitas antioksidan diukur melalui uji 2,2-Diphenyl-1-picrylhydrazil (DPPH) dan 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-*reducing* pada formula gel yang mendekati standar. Hasil pengukuran kemudian digunakan dalam perhitungan aktivitas antioksidan untuk penetapan nilai IC_{50}. **Hasil:** Formula 1, 2, dan 3 memenuhi syarat sebagai gel yaitu homogen, daya sebar 5,37 - 5,93 cm, pH 5,87 - 6,10, dan viskositas 8,800 - 9296,67 cps. Aktivitas tertinggi penangkapan radikal bebas DPPH dan ABTS yaitu masing-masing 34.04% dan 5.28%. Nilai IC_{50} gel CXE pada DPPH sebesar 1973.38 µg/mL dan pada ABTS sebesar 700.65 µg/mL. **Kesimpulan:** Formula gel CXE 1, 2, dan 3 memenuhi syarat dan berpotensi untuk digunakan sebagai terapi penyembuhan luka melalui sifat antioksidannya.

A R T I C L E I N F O

Received 09 January 2023
Revised 26 January 2023
Accepted 09 October 2023
Available online 05 March 2024

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Keywords:
Antioxidant; Curcuma xanthorrhiza; Gel; Wound healing

Kata kunci:
Antioksidan; *Curcuma xanthorrhiza*; Gel; Penyembuhan luka

Journal of Vocational Health Studies p-ISSN: 2580–7161; e-ISSN: 2580–717x
DOI: 10.20473/jvhs.V7.I3.2024.166-174

INTRODUCTION

Skin damage resulting from physical, thermal, and physiological trauma can render the surrounding area unstable and potentially disrupt normal bodily function (Xu *et al*., 2020). Biologically, wound healing can occur naturally, but it is quite complicated (Hakkarainen *et al*., 2016). Minor damage to the skin possesses a high capacity for cell-generation. However, in potentially severe wounds, the wound-healing process will take longer due to inflammatory, infectious, and hypertrophic responses (Hadisi *et al*., 2018). Inhibition of wound-healing and infection is caused by excess biofluid around the wound (Shi *et al*., 2018), which is known as a chronic wound (Helary *et al*., 2015), even some wounds may never fully recover due to underlying physiological disorders (such as diabetes) (Xu *et al*., 2020). There are four phases of the wound healing process (the hemostatic, the inflammatory, the proliferative, the maturation, and the remodeling phase). When an injury occurs, platelets begin to clump together to form a thrombus in the damaged vessel and stop bleeding temporarily. Then, as the inflammatory process begins, various immune cells are attracted to the injured cells. These cells will release pro-inflammatory cytokines and inflammatory cells (neutrophils), and produce *Reactive Oxygen Species* (ROS) (Kurahashi and Fujii, 2015). ROS levels promote angiogenesis and cell migration during normal wound healing, whereas high ROS levels can interfere with or even threaten wound healing, especially in chronic wounds (Shi *et al*., 2018; Koo *et al*., 2019). Therefore, it is necessary to maintain the redox balance in cells to produce effective antioxidants. This serves to prevent aberrant cell development and inconsistent immunological responses (Xu *et al*., 2020). Redox balance, or redox homeostasis, is defined as the balance between ROS levels and antioxidant compounds that play a role in scavenging ROS (Kurahashi dan Fujii, 2015). Several studies have reported the antioxidant content of several plants or herbs, one of them being the rhizome-type plant (Tonin *et al*., 2021; Mansouri *et al*., 2021; Rosidi, 2020). One example of a plant that has healing and antioxidant properties is *Curcuma longa L.* from the *Zingiberaceae* plant family (Süntar *et al*., 2012). In this research, *C. xanthorrhiza* was used as a source of antioxidants in a gel produced for wound healing. Most of the potential pharmacological activity of CX is believed to come from various bioactive compounds and phytochemicals, one of which is flavonoids (Rohman *et al*., 2020), which are included in antioxidant compounds that have a phenolic structure. These compounds interact through the mechanism of scavenging reactive oxygen species and reducing radical species to non-radical species (Rohman *et al*., 2020). The dosage form for wound dressing is gel preparation. The gel form can regarded to an ideal preparation for wound dressing due to their excellent permeability and biocompatibility characteristics, effectively regulating humidity around the wound, creating a supportive, moist environment for wound repair (Ghomi *et al*., 2019). Several studies have reported the effects of antioxidant gels (both hydrogel and so-gel) that are beneficial in wound healing (Lee *et al*., 2012; O'Connor *et al*., 2020; Gunes *et al*., 2017; Ahmad *et al*., 2021). According to Nguyen *et al*. (2013), antioxidant hydrogels can reduce oxidative stress, enhance the wound microenvironment, and eventually promote speedy wound regeneration by removing excess ROS from chronic wounds (Nguyen *et al*., 2013; Xu *et al*., 2020).

In this research, measurement of the antioxidant activity of gel of *C. xanthorrhiza Extract* (CXE) was carried out through the DPPH (2.2-Diphenyl-1-picrylhydrazil) and ABTS (2.2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) methods. Antioxidant content has been proven to have the capacity to speed up wound healing for the treatment of chronic wounds (Thangavel *et al*., 2017). Consequently, the function of antioxidant properties could be a viable option for accelerating chronic wound repair (Mulholland *et al*., 2017). This study was conducted to evaluate the stability of *C. xanthorrhiza Extract Gel* (CXEG) and its potential in wound healing through its antioxidant properties.

MATERIAL AND METHOD

Curcuma xanthorrhiza extract (CXE) and gel preparation

The CXE or curcuma herbal dry extract is produced by PT Borobudur, a Natural Herbal Industry certified CPOTB/GMP, located in Semarang, Central Java, Indonesia. The extraction process was carried out according to the company's standard procedure, using 50% ethanol as a solvent with an additional excipient of maltodextrin (based on the CoA dry extract that was issued by PT Borobudur). The resulting extract was, then, tested for its physical characteristics through organoleptic assays, while microbiological assays were used to determine the total plate number and the number of yeasts and bacteria. The CXE was produced with a granular form of granule and was dark brown (No. Batch: 063PS01.6). CXE was used as an active ingredient in the manufacture of gels. The extract gel formula can be seen in Table 1, the gel is manufactured by CV Zweena Adi Nugraha in Sukoharjo, Central Java, using a formula developed by Sayuti (2015).

The quality of CXE gel

Homogeneity test

The homogeneity test was carried out using two transparent glass objects under the light. One of the slides was smeared with the gel preparation evenly, then the other glass slide was placed on the slide that had been smeared with gel until its position would coincide. A homogeneous gel preparation will appear to be free of coarse particles or agglomerated particles (Afianti and Murrukmihadi, 2015; Sayuti, 2015; Senja and Amelia, 2018; Anggun *et al*., 2020).

Table 1. CXE gel formulation

Material	Concentration (% w/w)		
	FI	FII	FIII
Curcuma xanthorrhiza extract (CXE)	5	5	5
Carboxymethylcellulose sodium (CMC-Na)	3	4	5
Propylene glycol	15	15	15
Glycerin	10	10	10
Methylparaben	0.25	0.25	0.25
Aquadest	100	100	100

*FI: Formula I; FII: Formula II; FIII: Formula III

Spreadability test

One of the requirements for gel formulations to meet the ideal qualities is that they should possess good spreadability when applied to the skin (Khan *et al*., 2013). The spreadability test was carried out by placing 0.5 grams of gel in the middle of a round glass scale. Another round glass weighing 150 grams is placed on top of the gel and left for one minute. The spreading diameter is recorded (Sayuti, 2015).

pH test

The pH test was performed using a calibrated pH meter with standard neutral and acidic pH buffer solutions (pH = 7.00 and 4.00). The electrodes were, then, washed using distilled water and dried. The gel preparation's pH was continuously monitored until a constant pH value was achieved. The measurements of pH were done in triplicate and average values were calculated (Khan *et al*., 2013).

Viscosity test

The viscosity test was carried out using a viscometer (Brookfield) by immersing the viscometer spindle (at 50 rpm speed) in the gel that had been placed in a beaker glass. The viscosity value of the gel preparation can be seen until the number shown is stable (Anggun *et al*., 2020).

DPPH scavenging activity assay

The DPPH method is a straightforward method for estimating antioxidants' ability to scavenge free radicals. It is based on the decrease in DPPH• solution absorbance caused by the inactivation of DPPH• radicals by the antioxidants present in the sample. The amount of 50 µL of CXE gel (Formula 2) in various concentrations (1000; 500; 250; 125; 62.5; 31.25; 15.625 µg/mL) was added into a 96-well microplate, then continued by adding 200 µL of DPPH 0.077 mmol/L (Sigma Aldrich D9132, USA) solution (in methanol). Into the control well, 250 µL DPPH was added and 250 µL sample solvent was added to the well blank. After that, the mixture was incubated for 30 minutes at 25°C (in a dark environment). Then, a microplate reader was used to measure the absorbance at 517 nm (*Multiskan^{TM} GO Microplate Spectrophotometer, Thermo Scientific, USA*) (Ginting *et al*., 2020). In this method, there is a reduction due to the DPPH solution by hydrogen donors from antioxidant compounds to form non-radical compounds (Sohn *et al*., 2003). Discoloration will occur if the extract contains active antioxidant compounds (Widowati *et al*., 2021a; Widowati *et al*., 2021b). The radical scavenging activity was calculated according to Equation 1 and the IC_{50} of DPPH activity was determined.

$$\% \text{ scavenging activity} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \times 100 \quad \ldots (1)$$

ABTS-reducing activity assay

The ABTS assay, on the other hand, is based on a decrease in the absorbance of the radical ABTS+• solution due to its inactivation by antioxidants (Christodouleas *et al*., 2015). The antioxidant activity of CXE gel (Formula 2) was determined using a free radical assay with ABTS•+ diammonium salt (Sigma Aldrich A1888-2G, USA) (Widowati *et al*., 2018). The ABTS•+ solution was made by reacting ABTS (14 mM) and $K_2S_2O_8$ (4.9 mM ; 1:1) (Merck, EM105091) for 16 hours (in a dark environment) at 25°C, then diluting with PBS (5.5 mM, pH 7.4) until the absorbance at 745 nm was 0.70 ± 0.02. In brief, 2 µL of *C. xanthorrhiza* extract gel (50; 25; 12.5; 6.25; 3.125; 1.56; 0.78 µg/mL) was put into a 96-well plate, then 198 µL of freshly prepared ABTS•+ solution was added to a 96-well plate. Into the control well, 200 µL ABTS was added, and 200 µL sample solvent was added to the well blank. The mixture was incubated at 30°C for 6 min. At 745 nm, the absorbance was measured. The percentage of reduction activity is calculated according to Equation 2 and the IC_{50} value was also calculated.

$$\% \text{ ABTS reduction activity} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \times 100 \quad \ldots (2)$$

Statistical analysis

The SPSS software Version 22.0 was used to analyze the data with Tukey HSD and the results were presented in the form of a histogram (mean ± SD) using the *GraphPad Prism Version* 8.0.1 software. *p-values* of 0.05 or less were regarded as significant.

RESULT

Quality test of CXE gel

Homogeneity test

The results of the homogeneity test on the CXE gel can be seen in Table 2. The homogeneity test is one of the most important tests in formulating preparations as though gels. This test was carried out to ensure that the formula's ingredients had been mixed properly. Based on the test results (Table 2), all replicates of the three gel formulas showed homogeneous results, meaning that there were no coarse particles or clumping particles in the gel preparations.

Spreadability test

The results of the spreadability test on gel preparations can be seen in Table 2. The spreadability test on gels was carried out as an indicator of the ability of gel preparations to spread at the application site when applied to the skin. Based on the test results (Table 2), they show that the spreadability values at FI, FII, and FIII are 5.37, 5.93, and 5.63 cm, respectively. This spreadability test value was suitable for the standard range for semifluid gel types.

pH test

In gel preparations, the pH test is used as a parameter of the physicochemical properties of a substance. This property is related to the effectiveness and stability of the active substance in gel preparations, as well as the safety of the product when applied to the skin. Based on the results of measuring the pH of the gel preparations, it shows that the pH values for FI, FII, and FIII were 6.10, 5.87, and 5.93 respectively. This pH value still falls within the standard range for gels with a pH between 4.5 and 6.5, so a qualified gel preparation formula is required.

Viscosity test

A viscosity test on gel preparations is carried out to determine the thickness of the gel preparations that are made; this value represents the amount of resistance of a fluid to flow. Based on the results of measuring the viscosity of the gel preparations, it showed that the pH values for FI, FII, and FIII were 9296.67, 8800.00, and 9136.67 respectively. This viscosity value is the classified standard range for gels with a viscosity <10.000 cps.

DPPH scavenging activity assay

The percentage of DPPH scavenging activity of *C. xanthorrhiza* extract gel can be seen in Figure 1. According to the findings, the free radical scavenging activity increased along with the sample concentration used, but at concentrations of (I), (II), (III), and (IV), it did not show a significant value.

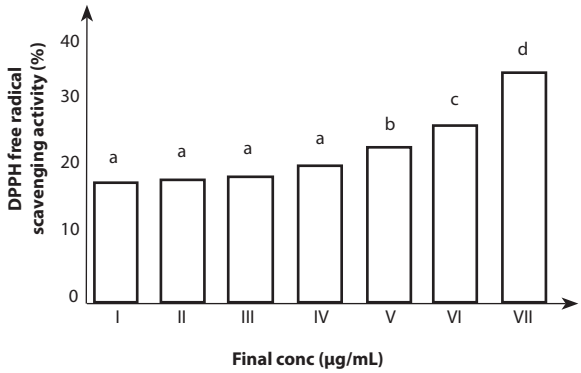

Figure 1. The effect of various concentrations of CXE gel on the free radical scavenging activity of DPPH

Based on Figure 1, data are presented as mean ± standard deviation, for each treatment, the assay was performed in triplicate. The different superscript (a, b, c, d) marks significant differences among various

Table 2. The results of quality test of CXE gel

Test	Result			Standart	Inference
	FI	FII	FIII		
Homogeneity	Homogenous	Homogenous	Homogenous	The powder is homogeneous, with no clumping and particles that spread evenly	All gels qualify
Spreadability	5.37 ± 0.47	5.93 ± 0.73	5.63 ± 0.42	3 - 5 cm for semi-stiff, 5 - 7 cm for semifluid	All gels qualify
pH	6.10 ± 0.26	5.87 ± 0.21	5.93 ± 0.28	Skin pH (4.5 to 6.5)	All gels qualify
Viscosity	9296.67 ± 495.71	8800.00 ± 876.07	9136.67 ± 527.86	Maximum value: 10000 cps	All gels qualify

*FI: Formula I; FII: Formula II; FIII: Formula III

Table 3. IC_{50} value of CXE gel toward DPPH free radical scavenging and ABTS-reducing activity

Assays	Sample	Equation	R2	IC_{50} value (µg/mL)
DPPH	CXE gel	y = 0.0163x + 18.081	0.99	1973.38 ± 219.93
ABTS	CXE gel	y = 0.0709x + 1.7969	0.99	700.65 ± 142.14

concentrations of CXE gel (*p-value* < 0.05, Tukey's HSD test). Roman numeral I - VII represents the final concentration of CXE gel. I: 15.625 µg/mL; II: 31.25 µg/mL; III: 62.5 µg/mL; IV: 125 µg/mL; V: 250 µg/mL: VI: 500 µg/mL; VII: 1000 µg/mL.

It can be seen at the highest concentration of the samples, concentrations of (VII) exhibited the highest free radical DPPH scavenging activity with a significant value (34.04 ± 2.40 µg/mL), followed by concentrations of (VII), (V), and (VI), and the lowest activity at concentrations (I) with the value of 17.82 ± 0.10 µg/mL. The Inhibitory Concentration (IC_{50}) value of *C. xanthorrhiza* extract gel toward DPPH free radical scavenging activity can be seen in Table 3.

ABTS-reducing activity assay

The percentage of ABTS-reducing activity of CXE gel can be seen in Figure 2. Among the variation of sample concentrations, it can be seen that the CXE gel at the highest concentration of (VII) has a large ABTS reducing activity significantly (5.28 ± 0.94 µg/mL). However, the value of ABTS-reducing activity was comparable to the concentration of the CXE gel. The ABTS-reducing activity was significantly elevated in a dose-dependent manner.

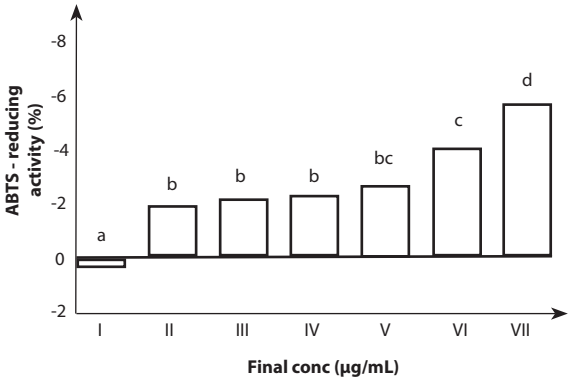

Figure 2. The effect of various concentrations of CXE gel on the ABTS-reducing activity.

Based on Figure 2, data are presented as mean ± standard deviation, for each treatment, the assay was performed in triplicate. The different superscript (a, b, bc, c, d) marks significant differences among various concentrations of *Curcuma xanthoriza* CXE gel (*p-value* < 0.05, Tukey's HSD test). Roman numeral I - VII represents the final concentration of CXE gel. I: 0.78125 µg/mL; II: 1.5625 µg/mL; III: 3.125 µg/mL; IV: 6.25 µg/mL; V: 12.5 µg/mL: VI: 250 µg/mL; VII: 50 µg/mL.

The IC_{50} value of CXE gel extract in reducing ABTS free radicals can be seen in Table 3. The results showed that the IC_{50} values of CXE gel were 1973.38 µg/mL in the DPPH free radical scavenging activity and 700.65 µg/mL for ABTS-reducing activity.

DISCUSSION

Chronic wounds that have a protracted inflammatory response produce an excessive amount of ROS that surpasses the antioxidant capacity of the cells and prevents the wound from moving from the inflammatory to the proliferative phase (Deng *et al*., 2019; Malone-Povolny *et al*., 2019) The reaction to normal tissue injury is a sequence of intricate processes, but a prompt and systematic functional recovery process (Mayet *et al*., 2014). These intricate processes can be divided into four categories: hemostasis, inflammation, proliferation, and maturation (Xu *et al*., 2020). The wound-healing process occurs dynamically and is governed by a complex biological process that is classified according to the nature of the wound (chronic or acute). Gharibi *et al*. (2015) mentioned that wound dressings must be able to protect the wound from external pressure and maintain its dimensional stability during the healing process. In addition, wound dressings must be able to provide and maintain moist environmental conditions around the wound that are caused under these conditions, then the acceleration of the healing process can occur (Gharibi *et al*., 2015).

Numerous studies demonstrate that biocompatible multifunctional hydrogels are the ideal option for biomedical and pharmaceutical applications because they can provide multistage and multifunctional combination therapies (Fuchs *et al*., 2020). The combination of antioxidant activity with hydrogel has resulted in a new revolution in the treatment of chronic wounds, based on the quick advancement of antioxidant research and the considerable hazards of ROS in chronic wound repair. In this study, we tried to make a gel preparation using CXE as the active ingredient. There are three gel formulas made with different concentrations of the CMC-Na composition. The different compositions of CMC-Na did not affect the results of the homogeneity and pH tests (Table 2), which looked homogeneous and had a pH value that was within the skin pH range (4.5 - 6.5) (Sayuti, 2015). Even other studies suggest that a pH value close to a neutral pH (6.6) is preferable because it poses a lower risk of skin irritation (Khan *et al*., 2013). Meanwhile, in the spreadability test, the first formula shows a lower spreadability value. The low dispersion value means that the CXE gel will easily spread and absorb with little friction. That is, this formulation can maintain good wet contact time when applied to the skin (Khan et al., 2013). Furthermore, the viscosity values for the three gel formulas align well with the standard preparation values (with a value of <10000 cps). Other studies indicate that the viscosity of the gel preparation ranges from 2000 - 4000 cps, with some mentioning values around 5000 - 6000 cps (Senja and Amelia, 2018).

In this study, in addition to serving as an active ingredient in the gel, *C. xanthorrhiza* extract was also used as an antioxidant agent. *C. xanthorrhiza* (family *Zingiberaceae*), better known as Javanese turmeric or temulawak, is a plant that is widely used as traditional herbal medicine in Indonesia (Ramdani *et al*., 2016; Rohman *et al*., 2019). The main substance in CX is starch, at 48.18 – 59.64% (Rohman *et al*., 2019). *C. xanthorrhiza* is also known to have active compounds identified as curcuminoids, terpenoids, and other phenolic compounds (Zhang *et al*., 2014; Rahmat *et al*., 2021; Kesumayadi *et al*., 2021). Rohman *et al*. (2019) also stated that CX has a volatile oil content of 3 - 12% (phellandrene, tumerol, camphor, borneol, sineol, and xanthorrhizol), sesquiterpenes, flavonoid (epicatechin, myricetin, quercetin, apigenin, catechin, kaemferol, naringenin, and luteolin) and curcuminoids (Rohman *et al*., 2019).

Plant polyphenols have a phenolic hydroxyl structure, and the ortho-phenolic hydroxyl group is rapidly oxidized to a quinone structure. Polyphenols have significant antioxidant and free radical scavenging properties due to the environment's consumption of oxygen and the strong potential to capture free radicals such as ROS. Other research indicate that the most abundant metabolite in *C. xanthorrhiza* is xanthorrhizol (a terpenoid) (Mary *et al*., 2012; Rahmat *et al*., 2021), which is known to have free radical scavenging activity. Several studies revealed the natural potential of antioxidant activity in *C. xanthorrhiza* through various methods, including DPPH, superoxide anion, *Ferric Reducing Antioxidant Power* (FRAP), ABTS, and metal bonding activity (Devaraj *et al*., 2014; Rosidi *et al*., 2016; Dosoky and Setzer, 2018; Sukweenadhi *et al*., 2020; Widyastuti *et al*., 2021).

The reagent DPPH can be used to examine a compound's capacity to scavenge free radicals. When used in the DPPH assay, the extracts were capable of converting the stable radical DPPH into yellow (DPPH-H) (Kumar *et al*., 2014; Widowati *et al*., 2015b). The DPPH method was used by Rosidi *et al*. (2016) to evaluate the antioxidant activity of CXE. Based on the research, the CXE extract under study showed quite strong antioxidant activity, with an IC_{50} value of 87.01 ppm (Rosidi *et al*., 2016). Another study stated that the IC_{50} value of CXE through the DPPH assay was 64.27 ppm (Kesumayadi *et al*., 2021). Sahoo *et al*. (2021) reported that the IC_{50} value of the leaf essential oil in the CX is 55.57 ± 0.02 µg/mL. Besides that, Sukweenadhi *et al*. (2020) reported that CXE has an IC_{50} value is 538 ± 12.8 ppm (Sukweenadhi *et al*., 2020). While in this study, the IC_{50} value of CXE gel in DPPH free radical scavenging activity was 1973.38 µg/mL, this value is classified as weak (tends to be inactive) according to the references.

In addition, the relative capacity of an antioxidant to scavenge the produced ABTS is measured by the ABTS-reducing activity assay. A potent oxidizing agent ($K_2S_2O_8$) reacts with the ABTS salt to produce the ABTS. Antioxidants play a role in hydrogen donors, which help reduce the color of the ABTS solution and are then measured through the absorption spectrum at a wavelength of 745 nm (Widowati, *et al*., 2015a). In the measurement of the antioxidant activity by the ABTS method, the IC_{50} CXE gel value was 700.65 g/mL. Another study claimed that the IC_{50} of CX with the ABTS method is 82 ± 3.06 ppm (Sukweenadhi *et al*., 2020). In the study by Sahoo *et al*. (2021), it was reported that the IC_{50} value of CX leaf essential oil was 24.42 g/mL (Sahoo *et al*., 2021). It can be claimed that the antioxidant activity of the extracted gel in the current study is included in the weak category. In the present study, the weak antioxidant activity in gel preparations could be attributed to the fact that the concentration of the extract added to the formulation was only 5% (w/w). Hasanah *et al*. (2017) reported that the antioxidant activity of gel preparations could be affected by the concentration of the extract added to the formula and the length of time it was stored (Hasanah *et al*., 2017).

A more orderly transition between the inflammatory, proliferative, and remodeling stages of wound healing is made possible by the antioxidant dressing, which modifies the inflammatory phase of wound healing by reducing excessive cell activation (Comino-Sanz *et al*., 2021). Antioxidants have the role of scavenging free radicals in biological cells that have a detrimental effect on living things. By eliminating the byproducts of inflammation, antioxidants lessen these negative effects on wounds. They prevent oxidative damage to protease inhibitors by reducing the excess proteases and ROS that are typically created by neutrophil buildup in the damaged location (Süntar *et al*., 2012). Instead of limiting damage and changing cell membranes, an easier protection mechanism is the direct interaction of a compound (antioxidant) in the extract with hydrogen peroxide (Süntar *et al*., 2012). Wound healing has been demonstrated to be facilitated by substances with high radical-scavenging capacity.

CONCLUSION

The characteristics of the CXE gel preparation are homogeneous, and from the overall quality gel test, it meets the requirements. The greatest concentration of CXE gel has an antioxidant activity value even if the IC_{50} value falls into the weak category. CXE gel still has the potential to be a wound healer if the concentration of CXE in the composition of gel manufacture is raised. Further, in-vivo studies are needed on the use of CXE gel as well as on optimizing the concentration of the extract in the gel's composition to demonstrate this.

ACKNOWLEDGMENTS

The authors are grateful to Arethai Medikai Utama, Bandung, West Java, Indonesia, which supplied the laboratory facilities and research methodology for this study. The author states that there is no conflict of interest with the parties involved in this research.

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