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Original Article

Phytochemical screening and gel stability test of ethanol extract of *Chromolaena odorata* L. from Indonesia

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Abstract

Chromolaena odorata L. is a widely used traditional herbal medicine known for its medicinal usage throughout the world. It has been used by the community for its anti-cancer, anti-bacterial, anti-inflammatory, antipyretic, anti-diabetic, and anti-diarrheal, and it also plays a role in hemostasis and wound healing. This study aims to analyze the chemical compounds of *C. odorata* leaves from Indonesia and to determine the gel stability of its ethanol extract. In this study, *C. odorata* leaves were collected from Lamkawe Village, Darul Imarah District, Aceh Besar Regency, Aceh, Indonesia. The extraction process was conducted using the maceration method. The active chemical compounds of the ethanol extract of *C. odorata* leaves were analyzed through phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis. The results of the phytochemical screening indicated it contained terpenoids, saponins, flavonoids and phenolics. The most abundant secondary compounds identified were glycidyl palmitate (17.02%), n-9-octadecenoic acid (Z) (15.68%), hexadecanoic acid and l-(+)-ascorbic acid 2,6-dihexadecanoate (12.19%) and 16-hentriacontanone. The 7.5% ethanol extract of *C. odorata* leaves showed good stability.

Keywords: *Chromolaena odorata*, gel, phytochemical, wound healing, stability

Introduction

Traditional medicine, which uses plants as ingredients, has been widely accepted and applied globally, including Indonesia. Plants produce hundreds of thousands of low molecular weight compounds, categorized by researchers into primary and secondary metabolites as well as hormones. Primary metabolites are needed for plant growth; secondary metabolites mediate plant interactions with the environment, while hormones regulate metabolic processes in organisms (Erb and Kliebenstein., 2020). Secondary metabolite compounds are not only used by plants for their growth processes but also have bioactive capabilities that help plants defend themselves against environmental challenges such as temperature, climate, pests, and plant diseases. These compounds can also be used to treat various types of diseases in humans.

Chromolaena odorata L. is one of the weed plants that can be used as medicine because it is rich in secondary metabolites. It originates from South and Central America and has spread to tropical areas of Asia, Africa, and the Pacific, including Indonesia (Budha Magar et al., 2023). *C. odorata* contains phenolics and flavonoids with potential effects against bacterial infections, diabetes, and oxidative stress (Budha Magar et al., 2023). Studies have shown that compounds in the leaves of *C. odorata* have anti-cancer (Yusuf and Fahriani, 2022), antioxidant (Michael et al., 2015), anti-diabetic, antipyretic, anti-inflammatory, and anti-diarrheal activities (Salsabila et al., 2021). It also plays a role in hemostasis and wound-healing processes (Salsabila et al., 2021) and accelerates wound closure (Pandith et al., 2013a).

The Indonesians, particularly the Acehnese, use leaves of *C. odorata* for various treatments, including wound healing. The use of traditional medicine for wound healing has long been carried out by the community because of its great potential in promoting recovery. Various parts of the plant have been traditionally used to treat wounds, burns, and skin infections (Vijayaraghavan et al., 2017a). A study revealed that several herbal medicines have real activity in treating wounds and this activity is caused by flavonoids, alkaloids, saponins and phenolic compound (Budha Magar et al., 2023). The antioxidant effects of flavonoids in *C. odorata* leaf extracts could inhibit the oxidation process and higher levels of flavonoid content can be achieved if ethanol is used as a solvent (Budha Magar et al., 2023).

Gels are pharmaceutical preparations commonly used as a topical medication. They have several advantages, such as a cooling sensation, ease of application, even spreadability, and moisturizing effects, which promote tissue growth and speed up the wound healing process (Liu et al., 2022; Budha Magar et al., 2023). The aim of this study was to determine the phytochemical content and gas chromatography-mass spectrometry (GC-MS) analysis (GC-MS) of ethanol extracts of *C. odorata* leaves collected from Lamkawe, Aceh Besar, and then formulate them into a gel for practical application and enhanced wound healing.

Methods

Sample collection and identification

The leaves of *C. odorata* were collected from Lamkawe village, Aceh Besar district, Aceh, Indonesia, located at coordinates 5°30'11"N and 95°19'51"E (Figure 1A). Plant identification was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia. The results showed that the plant was a member of the *Asteraceae* family, specifically *C. odorata*, as stated in the identification letter no. 254/UN 11.1.8.4/TA.00.03/2004. Documentation of *C. odorata* can be seen in Figure 1B.

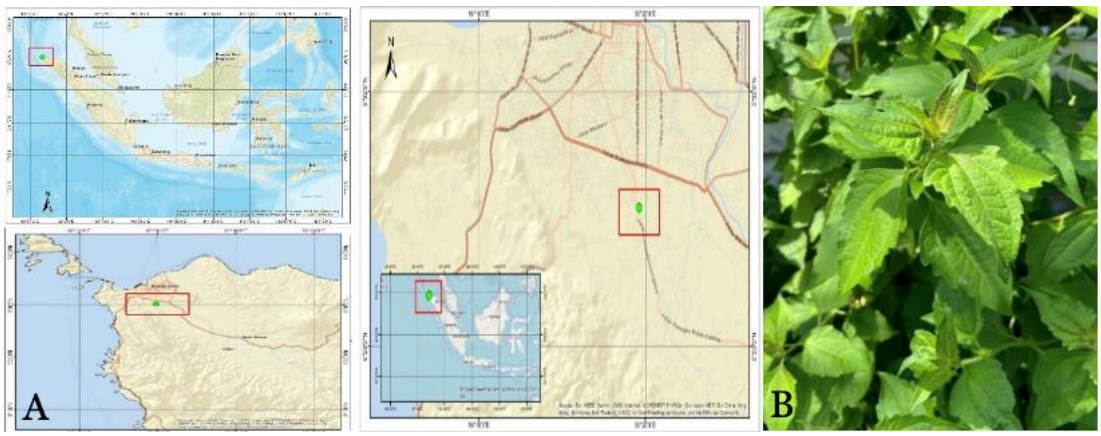

Figure 1. (A) Sampling location in Lamkawe village of Aceh Besar district, Aceh, Indonesia (B) *C. odorata* plants.

Extraction of *Chromolaena odorata*

The leaves were cleaned, dried in an open room without direct sunlight, and then a dry weight of 1.40 kg was obtained. Subsequently, the dried leaves were ground using a blender to obtain 1.30 kg of powder. The simplicia leaves of *C. odorata* were placed into a macerator and soaked in a 70% ethanol solvent at a ratio of 1:3, then left for 24 hours with stirring four times every 24 hours. After 24 hours, the mixture was then filtered using filter paper to obtain the filtrate. The residue was soaked again in a new 70% ethanol solvent, and this entire process was carried out 2 x 24 hours, using a total of 26 liters of ethanol. The filtrates obtained were dried using a *vacuum rotary evaporator* at a temperature of 50°C to obtain a thick extract. The resulting extract was further evaporated in an oven at 50°C. The yield was 6.36%.

Screening of qualitative phytochemical compounds

The qualitative phytochemical screening of *C. odorata* leaves included tests for alkaloids, steroids, triterpenoids, saponins, flavonoids, phenolics, and tannins. Alkaloids were tested using Mayer's, Wagner's, and Dragendroff's reagents. Steroids and terpenoids were examined with the Liebermann-Burchard reagent, where a red color indicated terpenoids and a blue-green color indicated steroids. Saponins were examined using the shuffling method. The foam formed would be stable for approximately 30 minutes. Flavonoids were examined by dissolving 0.50 g of an extract with Mg powder and 0.50 M HCl; phenolics were examined using 1% FeCl3, while tannins were tested using gelatine plus H_2SO_4.

Screening of quantitative phytochemical compounds

The GC-MS analysis of the ethanol extract of *C. odorata* leaves was carried out using the Thermo Scientific ISQ 7000 Single Quadrupole GC-MS instrument. A total of 5 µL of filtered extract was injected into the GC-MS system using helium (He) as the carrier gas through a column under constant pressure at a flow rate of 1.2 mL/minute and a split ratio of 8:1 psi. The injector temperature was set at 250°C, the detector temperature was at 230°C, and the operating temperatures were 280°C and 140°C. The components were detected using a mass detector, and their mass spectrum fragmentation patterns were compared with the spectrometer database of the National Institute of Standards and Technology Mass Spectral Database (NIST-MS).

Preparation and stability test of gel

The gel formulation follows the method described by Masyudi et al. (2022), using a 0.5% Carbopol base. This modification results in variations in concentration formulation, as presented in Table 1.

Table 1. *Chromolaena odorata* leaf ethanol extract gel formulation

Formulation	Negative control	Concentration of *C. odorata* gel		
		5%	7.5%	10%
Ethanol extract of *C. odorata* leaves	0.0	5	7.5	10
Carbopol 940	0.5	0.5	0.5	0.5
Triethanolamine (TEA)	1.0	1.0	1.0	1.0
Propylene glycol	15	15	15	15
Methylparaben	0.2	0.2	0.2	0.2
Aquadest	100	100	100	100

The preparation of the gel extract began with the making of the gel base. First, 0.50 g of Carbopol 940 was weighed, followed by the addition of 1 g of TEA and 15 g of propylene glycol. The mixture was then stirred well. Next, 0.2 g of methylparaben was added and stirred until the mixture became homogeneous. Once the base gel was formed, the *C. odorata* leaf extract was added (5 g (K1 group), 7.5 g (K2 group) or 10 g (K3 group)). Distilled water was then added to make the total volume 100 mL, and the mixture was stirred until homogeneous.

The gel stability test was carried out using the Cycling test method (Mudhofar MN, et al., 2024). The Cycling test is an accelerated stability testing method that subjected the gel to certain time intervals and temperatures with the aim of accelerating the changes that usually occur at normal temperatures. In this study, the Cycling test involved placing the gel preparation at 4°C for 24 hours, then moving it to a place with a temperature of 40°C for 24 hours. This cycle was performed six times.

The gel stability test included assessments of organoleptic, pH, sticking power, spreadability, and viscosity. pH measurement was carried out using a pH meter. Adhesion strength was tested by placing two glass objects together and calculating the time it took for them to separate using a stopwatch. The spreadability test was carried out by placing 0.50 g of the gel on a glass plate over graph paper and then covering it with another glass plate under different loads (no load, load 50 g, load 100 g, and load 150 g).

Gel viscosity was measured using a viscometer Cone and Plate with spindle 7 (Lamy Rheology, French). Measurement was made by placing the sample in the middle of the board, positioned right under the cone. The cone then was rotated at various speeds and the sample was shifted in a transparent room. The spindle was allowed to move freely within the hydrogel, and the results were recorded. The viscosity test was carried out for 10 minutes at a speed of 100 rpm.

Statistical analysis

The results were presented as mean±standard deviation (SD). Comparisons between the groups were performed using a one-way ANOVA followed by the least significant difference post hoc test with SPSS 22.0 (IBM Corp., NY, USA). The $p<0.05$ was considered to be statistically significant.

Results

Stability test of gel

The organoleptic test results indicated that the gel had a semi-solid form, black color, and the characteristic odor of the extract, which did not change before and after the cycling test. The test results also found that the gel preparation was homogeneous, with no coarse grains found in the preparation either before or after the Cycling test.

The pH results showed the values before and after the Cycling test within normal pH (NC=6.80–6.80, K1=6.60–6.70, K2=6.30–6.70, K3=6.20–7.20). The adhesion test showed the time before and after the Cycling test were: NC=4.58–4.90, K1=4.36–4.75, K2=6.30–6.70, and K3=6.20–7.20 seconds. Spreadability test results for no load, load 50 g, load 100 g, and load 150 g before and after the cycling test were: NC=5.00–2.60, 5.70–3.10, 5.85–3.45, 6.10–6.80; K1=6.25–6.75, 6.357–6.35, 6.9–7.45, and 6.95–6.70; K2=5.60–5.25, 6.20–5.30, 6.50–6.00, and 6.95–6.70; and K3=4.30–5.05, 5.10–6.00, 5.40–6.55, and 6.00–7.20 cm. Viscosity test results before and after the Cycling test were: NC=3962–5718, K1=1838–1761, K2=2143–2045, and K3=1086–1687 cP.

Qualitative phytochemical analysis

Qualitative phytochemical results did not show the alkaloid content of Mayer's reagent, Dragendroff's reagent, or Wagner's reagent. The Liebermann-Burchard test showed the presence of terpenoids but not steroids. The flavonoid test produced a brick-red color, indicating the presence of flavonoids. The shuffling method resulted in stable foam, indicating the saponin content. A red color formed when Mg and 0.50 M HCl were added, indicating the phenolic content. The absence of a white precipitate when examining gelatine with H_2SO_4 showed that there was no tannin content.

Quantitative phytochemical analysis

Figure 2 shows the chromatogram, and Table 2 shows all chemical compounds of the ethanol extract of *C. odorata*. A total of 33 putative compound peaks were identified, and the most abundant compound was glycidyl palmitate at 17.02%. Glycidyl palmitate has the molecular formula of $C_{19}H_{36}O_3$, which is a fatty acid with a hydroxyl group. This compound was detected at 39.12 minutes with a molecular weight of 312.5 g/mol. The second dominant compound was 9-octadecenoic acid (Z)-, with the formula of $C_{18}H_{34}O_2$, detected at 42.02 minutes with a molecular weight of 282.5 g/mol and the amount was 15.68%.

The n-hexadecanoic acid compound with the formula group $C_{16}H_{32}O_2$ was detected at 42.02 minutes at 12.19%, with a molecular weight of 256.4 g/mol, ranking third and belonging to the fatty acid group. Another compound detected at 42.02 minutes was l-(+)-ascorbic acid 2,6-dihexadecanoate. l-(+)-Ascorbic acid 2,6-dihexadecanoate, with the formula group $C_{38}H_{68}O_8$ and a molecular weight of 652.9 g/mol, is a derivative of vitamin C. GC-MS results at 52.92 minutes showed the compound of 16-hentriacontanone, with the formula group $C_{38}H_{68}$ and a molecular weight of 436.8 g/mol.

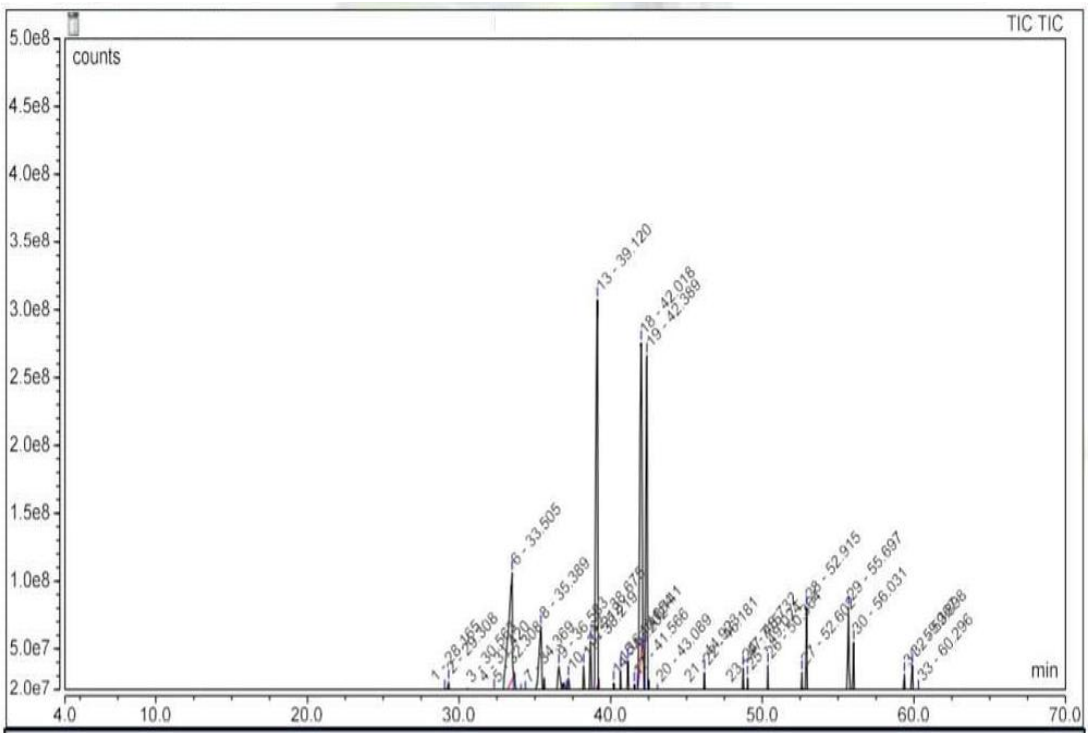

Figure 2. The GC-MS chromatogram of the ethanol extract of *Chromolaena odorata* L.

Table 2. All chemical compounds of the ethanol extract of *Chromolaena odorata* L.

Retention time (minutes)	Compound	Relative area (%)
28.17	Tetradecanoic acid, pentadecanoic acid, tridecanoic acid	2.04
29.31	Tetradecanoic acid, tridecanoic acid, pentadecanoic acid	2.17
30.56	Tetradecanoic acid, pentadecanoic acid, tridecanoic acid	0.57
31.42	Pentadecanoic acid, tetradecanoic acid, octadecanoic acid	0.50
32.31	Hexadecanoic acid, methyl ester, pentadecanoic acid, 14-methyl-, methyl ester	0.32
33.51	n-Hexadecanoic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, pentadecanoic acid	12.19
34.37	n-Hexadecanoic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, pentadecanoic acid	0.75
35.39	Myristic acid glycidyl ester, glycidyl palmitate, pentadecanoic acid	6.08
36.58	cis-13-octadecenoic acid, trans-13-octadecenoic acid, cis-vaccenic acid	2.28
37.21	Octadecanoic acid, 15-hydroxypentadecanoic acid, glycidyl palmitoleate	0.64
38.22	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester, glycerol 1-palmitate, glycidyl palmitate	1.13
38.67	Glycidyl palmitoleate, glycidyl (z)-9-heptadecenoate, 9-octadecenoic acid (z)-, oxiranylmethyl ester	2.15
39.12	Glycidyl palmitate, myristic acid glycidyl ester, palmitic anhydride	17.02
40.20	Glycidyl palmitate, glycidyl (z)-9-heptadecenoate	0.92
40.65	Glycidyl palmitate, Myristic acid glycidyl ester, docosanoic anhydride	0.90
41.14	1-Cis-vaccenoylglycerol, oleic acid, 3-hydroxypropyl ester, 2,3-dihydroxypropyl elaidate	1.80
41.57	Octadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester, Stearic anhydride	0.91
42.02	9-Octadecenoic acid (Z)-, oxiranylmethyl ester, glycidyl (z)-9-heptadecenoate, glycidyl palmitoleate	15.68
42.39	Glycidyl palmitate, glycidol stearate, myristic acid glycidyl ester	11.48
43.09	Myristic acid, 9-hexadecenyl ester, (Z)-, diethyl n-hexadecylmalonate, 1,3-O-Benzylidene glyceryl-2-myristate	0.35
44.92	Glycidyl palmitoleate, glycidyl (z)-9-heptadecenoate, 9,12,15-Octadecatrienoic acid, 2- (acetyloxy)-1-[(acetyloxy) methyl] ethyl ester, (Z,Z,Z)-	0.32
46.18	Decyl sulfide, diethyl n-hexadecylmalonate, 5-ethyl(dimethyl)silyloxytridecane	1.04

Retention time (minutes)	Compound	Relative area (%)
47.72	Nonacosan-14-one, 14-heptacosanone, Z-5-methyl-6-heneicosen-11-one	0.24
48.73	1-Heptatriacotanol, Diethyl n-hexadecylmalonate, 9-octadecenoic acid (Z)-, hexyl ester	1.10
49.02	Diethyl n-hexadecylmalonate, silane, dimethyl(dodec-2-enyloxy) heptyloxy-, 3-dimethyl(octyl)silyloxytridecane	0.78
50.36	Nonacosan-14-one, 14-heptacosanone, 16-hentriacontanone	0.93
52.60	Z-5-methyl-6-heneicosen-11-one, e-10,13,13-trimethyl-11-tetradecen-1-ol acetate, trans-9-octadecenoic acid, pentyl ester	1.32
52.92	Nonacosan-14-one, 16-hentriacontanone, 14-heptacosanone	2.29
55.70	Z-5-Methyl-6-heneicosen-11-one, -pentatriacontene, e-10,13,13-trimethyl-11-tetradecen-1-ol acetate	4.33
56.03	16-Hentriacontanone, 18-Pentatriacontanone, nonacosan-14-one	2.11
59.37	trans-9-octadecenoic acid, pentyl ester, 12-Methyl-E,E-2,13-octadecadien-1-ol, trans-13-octadecenoic acid	2.09
59.90	trans-9-octadecenoic acid, pentyl ester, 17-pentatriacontene, 2-undecanol oleate	2.73
60.30	18-Pentatriacontanone, octadecanoic acid, 2-propenyl ester, octadecanoic acid, ethynyl ester	0.83

Discussion

This study identified the content of terpenoids, saponins, flavonoids, and phenolics from phytochemical tests but did not find alkaloids, steroids, or tannins. A previous study found that the phytochemical content of methanol extract of *C. odorata* leaves contains alkaloids, flavonoids, tannins, saponins, terpenoids, anthraquinones, cardiac glycosides and carbohydrates (Vijayaraghavan et al., 2017b). Another study conducted in Ie Suum, Aceh Besar, showed that the extract contained phytochemicals in the form of flavonoids, phenolics, alkaloids, terpenoids, saponins, steroids, and tannins (Santi and Candra, 2023).

Terpenoid compounds have various biological activities, including anticancer, antimicrobial, anti-inflammatory, antioxidant, and anti-allergic (Masyita et al. 2022). The saponin content found in plants could have anti-inflammatory, antioxidant, anti-apoptosis, and anti-coagulation activities (Trinh et al., 2022). A study reported the phytochemical content in the form of flavonoids and phenolics found in *C. odorata* have potential effects against bacterial infections, diabetes, and oxidative stress in humans (Budha Magar et al., 2023). Another study also proved that phenolics and flavonoids are good sources of natural antioxidants obtained from plants (Tyagi and Agarwal, 2017).

Flavonoid is one of the most interesting and promising chemical constituents of natural products for treating skin problems. The presence of hydroxyl groups in its chemical structure is very important for its antibacterial, antifibrotic, antioxidant, and anti-inflammatory effects due to its high degree of hydroxylation (Trinh et al., 2022). Flavonoids also have anti-inflammatory, angiogenesis, re-epithelialization, and antioxidant effects, which play a very important role in wound healing (Zulkefli et al., 2023).

Flavonoids can be classified into various classes, such as flavones, flavonols, flavanols, flavanones, isoflavones, anthocyanins, and chalcones (Zulkefli et al., 2023). New flavanones isolated from the methanol extract of *C. odorata* leaves are odoratenin, isosacuretin, and subscadenin, which are described as 5,3'-dihydroxy-7,6'-dimethoxyflavanone. Odoratenin has the potential to be a source of antioxidants (Putri and Fatmawati, 2019). The compounds contained in *C. odorata* can act independently or synergistically to mediate different biological properties (Olawale et al., 2022).

Vijayaraghavan et al. identified the phytochemical content of *C. odorata* leaves from India and found differences in phytochemical content using ethanol and water extracts (Vijayaraghavan et al., 2017b). Phytochemical screening of the water extract

in their study identified that the extract contained saponins and alkaloids, while the ethanol extract was rich in terpenoids and glycosides but did not contain alkaloids. This indicated that different solvents could produce different phytochemical contents (Vijayaraghavan et al., 2017b). Additionally, there are several factors that influence the secondary metabolites of a plant, such as growing location, rainfall, humidity, temperature, and soil quality (Ance et al., 2018)

Our GC-MS indicated that the most abundant putative compounds were glycidyl palmitate, 9-octadecenoic acid (Z), n-hexadecanoic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate and 16-hentriacontanone. The compound n-9-octadecenoic acid (Z) has larvicidal activity and is widely used in lotions and oil preparations (Ajanaku Christiana et al., 2019; Rahman Lefta H, 2022). Ascorbic acid (vitamin C) has antioxidant activity and is widely used as a supplement in the pharmaceutical, cosmetic, food, beverage, and animal feed industries (Wang et al., 2018). Hentriacontane is a long-chain alkane compound with anti-inflammatory, antitumor, and antimicrobial activity. A study found that hentriacontane could suppress pro-inflammatory cytokines (TNF-a, IL-6, MCP-1 and IL-1b) and anti-inflammatory cytokines (IL-10) (Khajuria et al., 2017). Apart from that, the hentriacontane compound also has a regulatory effect on NF-κB (Khajuria et al., 2017).

The results of the gel stability test showed that the negative control, 5 and 7.5 g of *C. odorata* groups, had a pH that corresponded to the skin's pH range of 4 to 7. The adhesion test showed that all groups had an adhesion of more than 1 second. The 5 g *C. odorata* group has a good semi-solid spreadability in the range of 5 to 7 cm. The negative control and 7.5 g of *C. odorata* group viscosity tests were in the range of 2000–50,000 cP, which results in better drug contact with the skin. This evaluation is in accordance with a previous study which demonstrated that the gel stability test of the ethanol extract of *C. odorata* leaves was proven to be stable after 30 days (Zamram et al., 2022).

The group with 7.5% of *C. odorata* leaves gel met topical drug standards by fulfilling the requirements for organoleptic, homogeneity, adhesion, spreadability, and viscosity tests. The ethanol extract of *C. odorata* leaves gel that met the stability test reflects the combination of ingredients used in the formulation, which did not cause changes during the study. These results suggested that the gel base with Carbopol is the right choice as delivering the active ingredient in the ethanol extract of *C. odorata* leaves, making it suitable for application as a therapy for wounds or other skin diseases.

Conclusion

The most abundant identified putative compounds of ethanol extract of *C. odorata* leaves were glycidyl palmitate, 9-octadecenoic acid (Z), n-hexadecanoic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate and 16-hentriacontanone. The gel stability test of the ethanol extract of *C. odorata* leaves met topical drug standards based on organoleptic, homogeneity, adhesion, spreadability, and viscosity tests.

Authors' contributions

Study Concept and Design: PH, AY, and GG; Acquisition of Data: PH, AY, and GG; Analysis and Interpretation of Data: PH, AY, and GG; Drafting of the Manuscript: PH, AY, and GG; Critical Revision of the Manuscript for Important Intellectual Content: PH, AY, and GG; Statistical Analysis: PH, AY, and GG; Study Supervision: PH, AY, and GG.

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Physiology and Histology Laboratory of Veterinary Medicine Faculty, Universitas Syiah Kuala, Indonesia.

Ethical Approval

Not required.

Conflict of interests

The authors declare no conflict of interest regarding this study.

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