



RESEARCH ARTICLE

Bioactive phytoconstituents and hemostatic and angiogenetic activities of *Chromolaena odorata* L. leaf extract gel on an animal epistaxis model

[version 1; peer review: 2 approved with reservations]

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Abstract

Introduction: Epistaxis occurs in approximately 60% of the general population globally and herbal medicine for its treatment including *Chromolaena odorata* L. could be one of the alternatives. The aims of this study were: (a) to determine the putative compounds and the bioactivities of *C. odorata* leaf extract; and (b) to assess their hemostatic and angiogenesis properties in an animal epistaxis model.

Methods: The putative compounds of *C. odorata* extract were determined using gas chromatography and mass spectroscopy (GC-MS) and the bioactivities were determined using the Molinspiration Cheminformatics 2018 software. The hemostatic and angiogenesis activities were assessed using an epistaxis model of male rabbits (*Oryctolagus cuniculus*), on which three concentrations of *C. odorata* were tested. The gel was applied twice a day until 21 days. The hemostatic activity was evaluated by measuring the clotting time and the angiogenesis activity was evaluated by measuring the serum blood electrolyte, serum CD34 and CD68 levels and the histopathology of fibroblast and new blood vessels.

Results: Eight putative compounds with activities that increased immune responses and angiogenesis by having antithrombotic, antioxidant and anti-inflammatory effects were identified. Those compounds had a range of bioactivity to G-protein coupled receptor ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. Our data suggested that *C. odorata* extract had an effect on the levels of sodium, potassium and chloride. There was no significant difference in the mean levels of CD34 and CD68 among treatment and control groups, $p=0.443$ and $p>0.050$, respectively. The extract had no significant effect in inducing the growth of fibroblasts. Our data indicated that *C.*

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odorata extract induced angiogenesis significantly ($p=0.018$).

Conclusions: The *C. odorata* extract gel consisted of the compounds that contribute in antithrombotic, antioxidant and anti-inflammatory activities and these compounds increased the angiogenesis during wound healing in the epistaxis model.

Keywords

Epistaxis, *Chromolaena odorata*, antithrombotic, antioxidant, angiogenesis

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Introduction

Epistaxis (nasal bleeding) is a symptom of several diseases such as nose trauma and cardiovascular diseases. It has been reported in 60% of the general population, most commonly in individuals younger than 10 or older than 50.¹ Neovascularization (organic formation of blood vessels) and angiogenesis (growth of blood vessels from the pre-existing vasculature) are core mechanisms in epistaxis healing by involving cluster of differentiation 34 (CD34) as a marker of hematopoietic progenitor cells, assisted by CD68, which is able to clear cellular waste and facilitate the attraction and activation of macrophages.²

Herbal medicine for epistaxis management has been intensively studied recently since it could be safer. *Chromolaena odorata* L. (*C. odorata*) is a prairie weed found in almost all parts of Indonesia and other tropical countries. The leaves contain compounds such as flavonoids, saponins, tannins and terpenoids.^{3,4} All of which are important in the coagulation underlying wound healing, due to their antioxidant activities that inhibit oxidative damage.⁴ A previous study reported that the 70% ethanolic extract of *C. odorata* was highly effective for stopping bleeding in Wistar rats⁵ and its compounds, like stigmasterol and scutellarin tetramethyl ether, have hemostatic⁶ and anti-inflammatory properties.⁷ Another study also found that *C. odorata* leaves in a cream formulation stimulated granulation tissue formation and wound re-epithelialization by increasing fibroblasts and skin cell proliferation, which have been clinically and histologically proven.⁸

Epistaxis can be treated with a variety of topical vasoconstrictor agents such as in a gel formulation. Gel is less greasy and easily washed off, so it leaves the treated wound with no unpleasant smell.⁹ This study aimed to assess the hemostatic and angiogenesis effects of *C. odorata* leaf extract gel in an animal epistaxis model.

Methods

Study setting

An experimental study was conducted to assess the hemostatic and angiogenesis activities of *C. odorata* leaf extract gel in an animal epistaxis model. A total of 30 healthy male rabbits (*Oryctolagus cuniculus*) were divided into five groups (one group for each negative and positive control and three groups for different concentrations of *C. odorata* extract). After epistaxis was created in all animals, the *C. odorata* extract gel was applied twice a day and the animals were followed until 21 days. The hemostatic activity was evaluated by measuring the clotting time required after the first gel was applied. The angiogenesis activity was evaluated by measuring *C. odorata* extract's effect on serum inflammatory cell profiles, serum CD34 and CD68 expression and the histopathology of angiogenesis and fibroblast on days 7, 14 and 21 post-epistaxis.

C. odorata extract

The leaves of *C. odorata* were collected from Darussalam, Banda Aceh, Indonesia and herbarium identification was conducted at the Department of Biology in the Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia. The extraction procedure was conducted at the Pharmacology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. In brief, 5 kg of leaves were cleaned with distilled water, sliced into pieces, and dried in the shade for 72hrs. The dried leaves were mechanically powdered with a blender. A total of 1,500 g of powder was divided into 10 bottles of dark macerator, 70% ethanol was added and they were left for 18hrs at room temperature. The mixture was separated by filtration using flannel twice, using the 70% ethanol. The filtrate was concentrated with a rotary evaporator (Buchi R300, Wellington, New Zealand) until a pure extract was obtained.¹⁰

Compounds identification of *C. odorata* extract

The putative compounds were determined using quantitative analysis with gas chromatography and mass spectroscopy (GC-MS) (QP2010 SE, Shimadzu, Kyoto, Japan), controlled with LabSolutions DB/CS software (Shimadzu, Kyoto, Japan). It was started by methylating *C. odorata* extract in which 15 ml of methanol was added. The mixture was then left for 2hrs at room temperature and evaporated until dried. The sample obtained was then added with 10 ml of methanol containing 2 ml of HCl, heated for 1.5hrs and it was separated using column chromatography with silica gel for the stationary phase and hexane-chloroform (9:1) for the mobile phase. The injector temperature was 250°C. The oven temperature was set at 140°C for 10 mins and increased by 7°C/min to 250°C for 10 mins.

Bioactivity test of *C. odorata* extract

The bioactivity score of *C. odorata* extract was calculated using web-based software (Molinspiration Cheminformatics 2018 on the Web, Bratislava, Slovak Republic). The result was based on the compounds activity in response to G-protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. The bioactivity was assessed using a score scale: greater than 0.00 indicated significant biological activity, between -0.50 and 0.00 indicated moderate activity and less than -0.50 indicated inactivity.¹¹

Preparation of gel formulation

Four gel formulations were each made using different concentrations of *C. odorata* extract. The ingredients are presented in Table 1. A total of 5, 10 and 15 g of *C. odorata* extract was used to formulate the gel-II (5% w/w, called *Co*_{5%} group), gel-III (10% w/w, called *Co*_{10%} group) and gel-IV (15% w/w, called *Co*_{15%} group), respectively, and then dissolved in a heated water bath, at approximately 45°C. To create a homogenous gel, glycerin, propylene glycol and water were added to the mixture while continuously stirring with sodium carboxymethyl cellulose (Na-CMC). The gel was kept overnight at 10–15°C in a dark room. The final weight was adjusted with aquadest to yield a total weight of 100 g.

Gel evaluation

Evaluation of gel preparations included organoleptic qualities, pH, homogeneity, viscosity and spreadability. An organoleptic test was conducted by assessing the gel visually including the shape, color and odor of each gel preparation. The pH of gel was determined using a universal pH indicator stick. To assess the homogeneity, the gel was applied to a piece of glass to check whether it showed any coarse grains. A digital Rion viscometer (Thermo Scientific, Brookfield, WI, US) was used to assess gel viscosity. The spreadability, the rate (in seconds) at which two slides separate from gel placed between them, was measured using a 7.5 cm glass slide and the specified weight tied to the upper plate was 20 g.

Animal preparation

A total of 30 healthy male rabbits (*Oryctolagus cuniculus*) were used in this study. The animals were maintained separately in sterile cages with 12hr light/dark cycles at 22°C ± 4°C. Food and water were provided *ad libitum*. The animal adaptation was carried out for one week prior to study. Ill and underweight rabbits were excluded.

Epistaxis model

All rabbits were sedated with 5 mg/kg intramuscular xylocaine hydrochloride (23.32 mg/mL) and 50 mg/kg ketamine chloride (Parker Davis and Company, Detroit, Michigan, US). A standard mucosal wound was made with a 3mm surgical punch to the rabbit nasal septum. The surgical punch was applied to the nasal mucosa over the right and left front of the septum using slight pressure and was rotated 90° clockwise, followed by 90° counterclockwise to assemble a full-thickness mucosal injury without damaging the cartilage of the nose septum. A total of 0.2 ml of the gel was applied soon after the surgical punch and repeated twice a day. All procedures were conducted by a registered veterinarian with animal care certification.

Homeostasis assessment

To measure the homeostasis effect of *C. odorata* extract, the time required for the blood to stop after the first gel was applied was measured. The times were expressed in minutes.

Blood electrolyte analysis

To measure the blood electrolytes, blood samples were collected on days 7, 14 and 21. Electrolytes in serum samples were examined using an electrolyte analyzer with the ion-selective electrode method (Guangzhou Happpycare Electronics, Guangdong, China). Briefly, the suction needle of the electrolyte analyzer was inserted into the serum cup and left to collect the serum for ± 2 seconds. The results of the serum electrolyte levels were recorded as per the manufacturer's protocol (Guangzhou Happpycare Electronics, Guangdong, China).

Measurement of CD34 and CD68 expression

The titers of serum CD34 and CD68 were measured using Rabbit Cluster of Differentiation 34 (CD34) and CD68 ELISA Kits from ELK Biotechnology (ELK Biotechnology, Wuhan, China). Venous blood samples were collected from the

Table 1. Composition of gel formulation.

Ingredients	Group			
	C _{Negative}	Co _{5%}	Co _{10%}	Co _{15%}
Ethanol extract of <i>C. odorata</i> (w/w)	0	5	10	15
Sodium carboxymethyl cellulose (Na-CMC)	5	5	5	5
Glycerin	10	10	10	10
Propylene glycol	5	5	5	5
Aquadest	q.s.p.	q.s.p.	q.s.p.	q.s.p.

q.s.p.: quantity sufficient to prepare 100 grams of gel.

lateral ear vein on days 7, 14 and 21, and serum was prepared by centrifuging the blood at 4000 rpm for 3 mins. A total of 10 μ L of serum was used to measure the level of CD34 and CD68 following the manufacture's protocol (ELK Biotechnology, Wuhan, China). The sensitivity limit of both kits is 0.065 ng/mL while the detection range is between 0.16–10 ng/mL.

Measurement of angiogenesis

To provide the histopathology of angiogenesis, the animals were sacrificed using chloroform at day 21. The nasal septal mucosa was preserved using formaldehyde, and a tissue dehydration process was conducted using serial concentrations of alcohol based on standard protocol.¹² The histopathology slices were prepared as thin as 4 μ m each based on the protocol.¹² Angiogenesis was measured by counting the number of fibroblasts and the new blood vessels through histopathology examination. They were assessed and counted using a BX51M light microscope (Olympus, Tokyo, Japan) with 400x magnification. Each observation was repeated in 5 fields of view.

Statistical analysis

Bleeding time, the concentration of serum electrolytes, inflammatory cell number, titers of CD34 and CD68 were compared between groups using one-way analysis of variance (Anova) or Kruskal-Wallis analysis as appropriated. All analyses were conducted using SPSS software ver. 24 (SPSS Inc., Chicago, IL, USA) (RRID:SCR_019096).

Ethical approval

The protocol for this study and its use of rabbits was approved by the Research Ethics Committee at the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh (no. 127/KEPH/XII/2021). To minimize the distress to the animals, the appropriate housing with *ad libitum* feeding was provided during the study while to minimize the pain, anesthesia was used before the rabbits were sacrificed. The procedures were conducted by a veterinarian with animal care certification.

Results

The putative compounds of *C. odorata*

The putative compounds found in the ethanol extract of *C. odorata* were antithrombotic, antioxidant and anti-inflammatory, indicated as nos. 9, 12, 19, 20, 21, 27, 33, and 35 in Table 2. The eight compounds have effects that increase immune responses and angiogenesis. Figure 1 depicts the quantity and the tendency of peak values of related compounds.

Bioactivity test of *C. odorata* compound

The bioactivity scores for each putative compound from *C. odorata* extract are presented in Table 3. Compound 19 (neomenthol) was the most inactive while compound 35 (octacosanol) was the most active against all six molecule groups.

Among the eight antithrombotic, antioxidant and anti-inflammatory compounds, our findings enabled the following observations:

- a. GPCR ligand: compounds 9, 12, 21, 27, 33 and 35 were highly active (> 0), compound 20 was moderately active (≤ 0) and compound 19 was inactive (< -0.50).
- b. Ion channel modulator: compounds 12, 20, 21, 27 and 35 were highly active (> 0), and compounds 9, 19 and 33 were moderately active (≤ 0).
- c. Kinase inhibitor: compound 35 was highly active (> 0), compounds 9, 20, 21, 27, and 33 were moderately active (≤ 0) and compounds 12 and 19 were inactive (< -0.50).
- d. Nuclear receptor ligand: compounds 12, 21, 27, 33, 35 were highly active (> 0), compounds 9 and 20 was moderately active (≤ 0) and compound 19 was inactive (< -0.50).
- e. Protease inhibitor: compounds 21, 33, and 35 were highly active (> 0), compound 9, 12, 20, and 27 were moderately active (≤ 0) and compound 19 was inactive (< -0.50).
- f. Enzyme inhibitor: compounds 9, 12, 20, 21, 27, 33, and 35 were highly active (≥ 0) and only compound 19 was moderately active.

Table 2. GCMS analysis showing the putative compounds of *C. odorata* leaf ethanol extract.

Peak #	Retention time	Area %	Compounds
1	13.416	0.31	β -Copaen-4 α -ol
2	15.839	0.31	δ -Cadinene
3	16.263	0.32	α -Calacorene
4	17.019	1.52	(+)-Spathulenol
5	17.153	3.01	(-)-Caryophyllene oxide
6	17.531	0.65	(E)-Solanone
7	17.634	1.66	(-)-Caryophyllene oxide
8	18.781	0.72	Longifolenaldehyde
9	18.959	0.58	Longiverbenon (Vulgaron B)
10	19.039	0.63	2,5-Furandione, 3-(dodecenyl) dihydro-
11	20.810	0.61	Ledol
12	21.565	3.23	Phytol, acetate
13	22.157	0.32	Platambin
14	22.288	0.83	2-Hexadecen-1-ol, (CAS) Phytol
15	22.944	0.32	1-Cyclohexene-1-butanol, α ,2,6,6-tetr
16	23.020	0.84	Hexadecanoic acid, methyl ester (CAS) Me
17	23.888	5.39	l-(+)-Ascorbic acid 2,6-dihexadecanoate
18	25.829	0.73	9,12,15-Octadecatrienoic acid, methyl ester
19	26.047	5.25	neo-Menthol
20	26.664	4.02	Pentadecadien-1-ol
21	26.865	1.02	Octadecanoic acid
22	28.539	0.60	n-Tetracosanol-1
23	33.720	0.63	Stigmasta-5,22-dien-3-ol, (3 β ,22E)- (C
24	33.852	1.43	1-Heptacosanol
25	34.112	2.47	5-Hydroxy-4',7-dimethoxyflavanone
26	34.968	1.36	9-Octadecenamide, (Z)-(CAS) Oleoami
27	35.501	22.96	Squalene
28	35.601	2.80	Stigmast-5-en-3-ol, (3 β ,24S)-(CAS) Cl
29	36.318	6.08	1-Hexacosanol
30	37.025	8.35	Phenol, (3S)-7-O-Methoxymethylvestitol
31	37.894	11.79	4-Acetyl-3-hydroxy-2,6-dimethoxytoluene
32	38.203	1.92	4H-1-Benzopyran-4-one, 5-hydroxy-2-(4-h
33	38.780	2.26	β -Ocopherol
34	39.131	4.47	Silane, dimethyl(3-methylphenoxy)ethoxy-
35	39.435	0.64	Octacosanol
Total		100.00	

Homeostasis in the epistaxis model

In epistaxis rabbits, each concentration of *C. odorata* ethanol extract gel had a different ability to stop the bleeding (Table 4). The one-way Anova revealed that there was no significant difference in bleeding time between the groups ($p=0.928$).

Blood electrolyte levels in epistaxis wound healing

The levels of the blood electrolyte of rabbits in the treatment and control groups on days 7, 14 and 21 are presented in Table 5. In rabbits, the normal range values of the electrolytes are sodium (130-155 mmol/L), potassium (4.0-6.5 mmol/L).

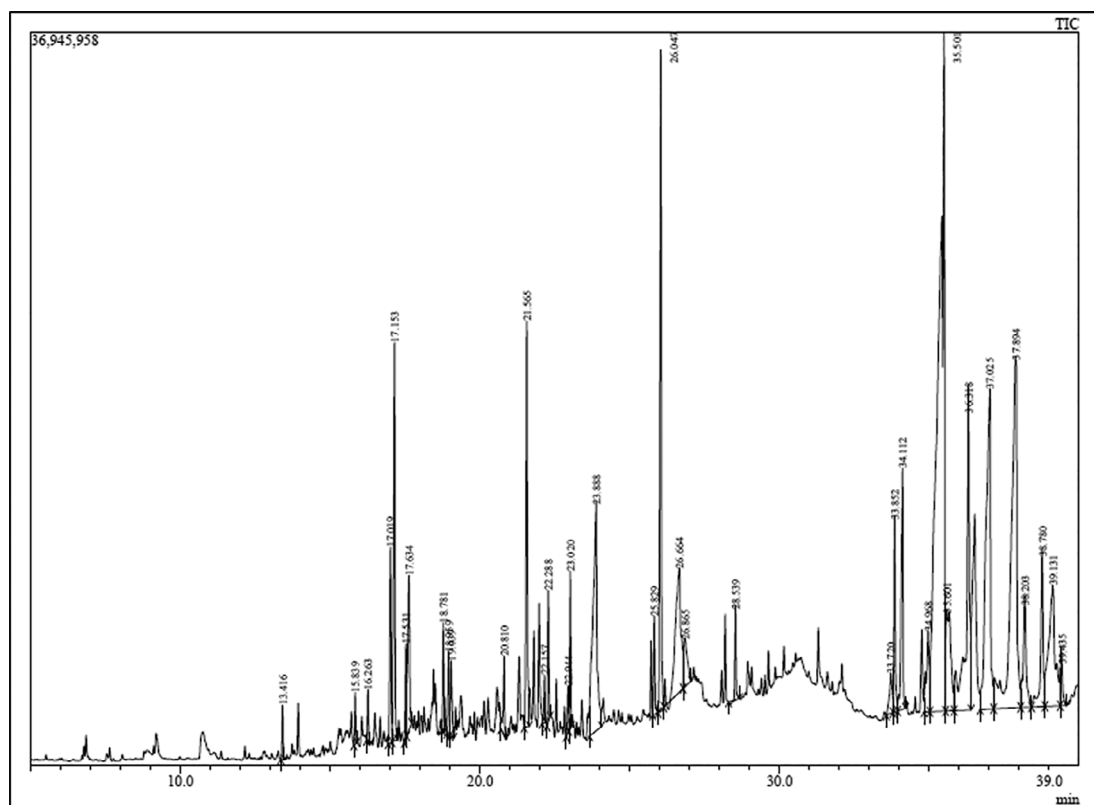


Figure 1. GC-MS pattern of chemical compounds obtained from *C. odorata*.

dL) and chloride (92-120 mmol/L).¹³ Almost all the animals from all groups had normal electrolyte levels on days 7, 14 and 21 post-epistaxis. Our data suggested that there were no significantly different levels of Na, K and Cl among groups in each time series (Table 5).

CD34 and CD68 levels

After 7 days of treatment, CD34 concentrations were relatively higher in the positive control and the treatment groups compared to the negative control group and this was maintained until day 21. At days 7, 14 and 21 the highest CD34 concentration was demonstrated in *C. odorata* 5%, *C. odorata* 15%, and positive control, respectively (Table 6). Negative control had the lowest CD34 level at each time point. However, our data suggested that there was no significant difference in the mean levels of CD34 among groups on days 7, 14 and 21 with $p=0.072$, $p=0.093$ and $p=0.093$, respectively. The CD34 levels also showed no significant difference between days 7, 14 and 21 ($p=0.443$).

In comparison to negative controls, *C. odorata* 10% and 15% groups had consistent higher CD68 levels compared to the negative control group at all time points (i.e., days 7, 14 and 21) (Table 7). On day 14, the 15% *C. odorata* was able to induce CD68 expression more effectively. However, there was no significant difference in the mean levels of CD68 among groups on days 7, 14 and 21 with $p=0.075$, $p=0.078$ and $p=0.188$, respectively. There were no significant CD68 levels between days 7, 14 and 21 ($p>0.050$).

Fibroblast and angiogenesis profile in epistaxis healing

At 7 days post-epistaxis, animals in the *C. odorata* 15% and 10% groups had a higher percentage of fibroblasts compared to the *C. odorata* 5% group or positive control. At 14 days of recovery, *Co*_{10%} group had a better ability to induce an increase in fibroblast cells, while positive control was the best on day 21 after recovery (Figure 2). One-way Anova analysis revealed that the percentage of the fibroblasts was no different between days 7, 14 and 21 ($p=0.765$).

Our data suggested that the highest concentration of *C. odorata* (15%) demonstrated the ability to initiate angiogenesis, which was greater than the *Co*_{10%} and *Co*_{5%} groups. In all groups, the longer the recovery time, the higher percentage of angiogenesis. In general, the positive control group played a lesser role in angiogenesis than the treatment groups (Figure 3). There were no significant differences in angiogenesis between time series (7, 14 and 21 days of receiving

Table 3. The IUPAC name and bioactivity score of volatile putative compounds isolated from *C. odorata* leaf ethanol extract.

No.	Chemical compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	β -Copaen-4 α -ol	-0.19	0.26	-0.57	0.45	-0.17	0.27
2	δ -Cadinene	-0.58	-0.02	-0.75	0.00	-0.68	0.19
3	α -Calacorene	0.01	0.15	-0.74	0.24	-0.36	0.14
4	(+)-Spathulenol	-0.42	-0.28	-0.68	0.28	-0.36	0.06
5	(-)-Caryophyllene oxide	-0.08	0.14	-0.86	0.62	0.00	0.57
6	(E)-Solaneone	-0.67	-0.27	-0.37	-0.11	-0.70	-0.01
7	(-)-Caryophyllene oxide	-0.29	0.05	-0.75	0.04	-0.24	0.60
8	Longifolenaldehyde	-0.41	-0.10	-1.05	0.14	-0.53	0.16
9	Longiverbenon (Vulgaron B)	0.01	-0.10	-0.42	-0.06	-0.06	0.15
10	2,5-Furandione, 3-(dodecenyldihydro-	-0.50	-0.29	-0.82	-0.22	-0.48	-0.13
11	Ledol	-0.01	0.07	-0.44	0.24	-0.1	0.23
12	Phytol, acetate	0.07	0.19	-0.68	0.62	-0.20	0.48
13	Platambin	-0.01	0.27	-0.21	0.12	-0.09	0.30
14	2-Hexadecen-1-ol,(CAS) Phytol	-0.11	-0.05	-0.34	-0.09	-0.13	0.04
15	1-Cyclohexene-1-butanal, α ,2,6,6-tetr	-	-	-	-	-	-
16	Hexadecanoic acid, methyl ester (CAS) Me	-	-	-	-	-	-
17	l-(+)-Ascorbic acid 2,6-dihexadecanoate	-0.23	-0.85	-0.67	-0.61	-0.05	-0.31
18	9,12,15-Octadecatrienoic acid, methyl ester	0.19	0.12	-0.22	0.17	0.04	0.26
19	neo-Menthol	-0.76	-0.30	-1.36	-0.60	-0.67	-0.22
20	Pentadecadien-1-ol	-0.17	0.06	-0.45	-0.17	-0.26	0.03
21	Octadecanoic acid	0.11	0.05	-0.20	0.17	0.06	0.20
22	n-Tetracosanol-1	0.08	0.02	0.01	0.12	0.10	0.10
23	Stigmasta-5,22-dien-3-ol, (3 β ,22E)- (C	0.12	-0.08	-0.48	0.74	-0.02	0.53
24	1-Heptacosanol	0.07	0.02	0.01	0.11	0.09	0.09
25	5-Hydroxy-4',7-dimethoxyflavanone	0.02	-0.27	-0.24	0.36	-0.09	0.13
26	9-Octadecenamide, (Z)-(CAS) Oleoami	0.04	-0.05	-0.12	-0.03	0.07	0.12

Table 3. *Continued*

No.	Chemical compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
27	Squalene	0.04	0.01	-0.10	0.19	-0.03	0.16
28	Stigmast-5-en-3-ol, (3 β ,24S)-(CAS) Cl	0.14	0.04	-0.51	0.73	0.07	0.51
29	1-Hexacosanol	0.07	0.02	0.01	0.10	0.08	0.08
30	Phenol, (3S)-7-O-Methoxymethylvestitol	-	-	-	-	-	-
31	4-Acetyl-3-hydroxy-2,6-dimethoxytoluene	-0.60	-0.47	-0.93	-0.57	-0.95	-0.35
32	4H-1-Benzopyran-4-one, 5-hydroxy-2-(4-h	-	-	-	-	-	-
33	β -Ocopherol	0.21	-0.01	-0.20	0.35	0.21	0.14
34	Silane, dimethyl(3-methylphenoxy)ethoxy-	0.66	0.22	0.46	-0.14	-0.47	0.15
35	Octacosanol	0.07	0.02	0.01	0.10	0.08	0.08

Table 4. The effect of *C. odorata* extract on bleeding time in the animal epistaxis model.

Group	Mean bleeding time (minutes)						Mean
	1 st Rabbit	2 nd Rabbit	3 rd Rabbit	4 th Rabbit	5 th Rabbit	6 th Rabbit	
C _{015%}	0.52	1.13	0.40	0.58	1.11	0.55	0.71
C _{010%}	0.39	0.43	1.22	1.00	0.31	0.15	0.58
C _{05%}	0.49	0.44	0.56	0.55	0.25	0.48	0.46
C _{Positive}	0.48	0.41	0.4	0.35	0.49	0.27	0.40
C _{Negative}	1.27	1.21	1.07	1.32	1.11	1.12	1.18
p-value							0.928

C: control group

Table 5. The effect of *C. odorata* extract on serum electrolyte of epistaxis model rabbit.

Group	Day 7 (n=10)			Day 14 (n=10)			Day 21 (n=10)		
	Concentration (mmol/L)			Concentration (mmol/L)			Concentration (mmol/L)		
	Na	K	Cl	Na	K	Cl	Na	K	Cl
C _{015%}	140	7.5	103	142	4.3	103	140	7.5	103
C _{010%}	132	6.4	95	140	4.9	106	136	3.9	98
C _{05%}	137	6.3	101	149	4.0	110	140	4.1	103
C _{Positive}	123	6.4	89	136	4.5	101	133	4.1	98
C _{Negative}	144	7.1	104	134	4.2	101	135	4.0	104
p-value	0.072	0.070	0.068	0.067	0.072	0.094	0.091	0.070	0.099

Na: natrium (sodium), K: kalium (potassium), Cl: chloride

Table 6. The effect of *C. odorata* extract on serum CD34 level in the animal epistaxis model.

Group	Day 7 (n=10)		Day 14 (n=10)		Day 21 (n=10)	
	OD Mean $\pm$ SDV	Level (ng/mL)	OD Mean $\pm$ SDV	Level (ng/mL)	OD Mean $\pm$ SDV	Level (ng/mL)
C _{015%}	0.11 $\pm$ 0.01	10.72	0.15 $\pm$ 0.01	11.55	0.14 $\pm$ 0.03	11.26
C _{010%}	0.12 $\pm$ 0.01	10.95	0.12 $\pm$ 0.03	10.86	0.12 $\pm$ 0.04	10.86
C _{05%}	0.13 $\pm$ 0.01	11.10	0.11 $\pm$ 0.03	10.76	0.14 $\pm$ 0.00	11.34
C _{Positive}	0.04 $\pm$ 0.01	9.34	0.13 $\pm$ 0.02	11.06	0.15 $\pm$ 0.00	11.44
C _{Negative}	0.13 $\pm$ 0.00	8.093	0.06 $\pm$ 0.06	9.75	0.09 $\pm$ 0.02	7.378
p-value		0.072		0.093		0.093

C: control group, SD: standard deviation value, OD: optical density

C. odorata extract gel) ($p=0.478$). However, the Kruskal-Wallis test suggested that there was a significant difference in percentage of angiogenesis between concentrations of *C. odorata* ($p=0.018$). The profile of fibroblasts and new blood vessels in epistaxis at day 7 from all groups is presented in [Figure 4](#).

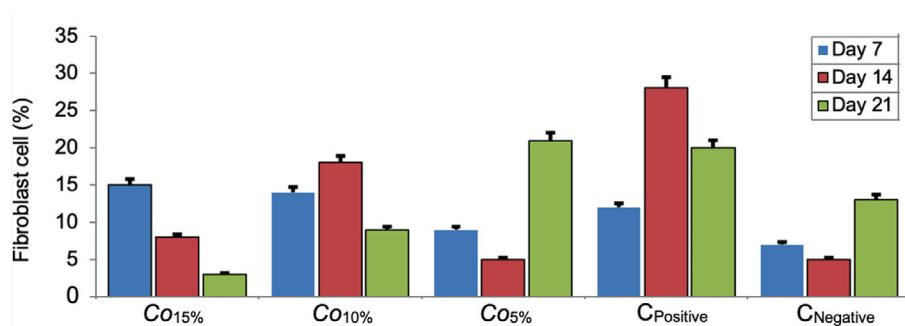
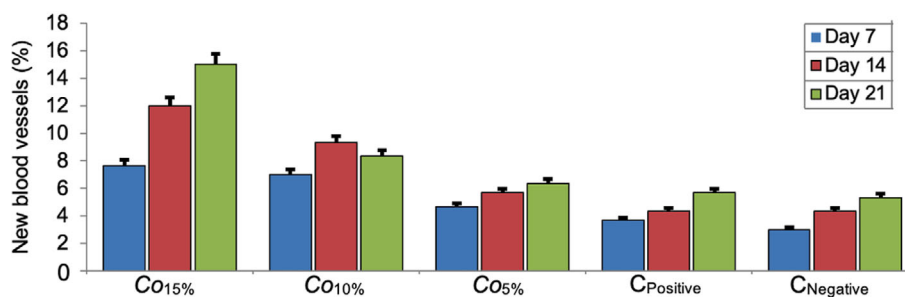
Discussion

Our data indicated that *C. odorata* leaf extract gel contained antioxidant, anti-inflammatory and antithrombotic compounds that might increase the homeostatic and angiogenesis properties observed in this study. These putative compounds are longiverbenone (vulgaron B) (0.58%), phytol, acetate (3.23%), neo-menthol (5.25%), pentadecadien-1-ol (4.02%), octadecanoic acid (1.02%), squalene (22.96%), beta-tocopherol (2.26%) and octacosanol (0.64%). The synergism between *C. odorata* compounds and blood clotting factors in extrinsic and intrinsic pathways could accelerate the blood clotting, including the conversion of prothrombin to thrombin. Thrombin is an enzyme converting fibrinogen

Table 7. The effect of *C. odorata* extract on serum CD68 level in the animal epistaxis model.

Group	Day 7 (n=10)		Day 14 (n=10)		Day 21 (n=10)	
	OD Mean $\pm$ SDV	Level (ng/mL)	OD Mean $\pm$ SDV	Level (ng/mL)	OD Mean $\pm$ SDV	Level (ng/mL)
C _{015%}	0.067 $\pm$ 0.028	0.51	0.141 $\pm$ 0.112	1.20	0.184 $\pm$ 0.090	1.60
C _{010%}	0.075 $\pm$ 0.024	0.59	0.077 $\pm$ 0.032	0.60	0.086 $\pm$ 0.057	0.69
C _{05%}	0.087 $\pm$ 0.166	0.69	0.013 $\pm$ 0.075	0.01	0.122 $\pm$ 0.021	1.03
C _{Positive}	0.086 $\pm$ 0.006	0.68	0.031 $\pm$ 0.033	0.17	0.106 $\pm$ 0.047	0.88
C _{Negative}	0.012 $\pm$ 0.194	0.00	0.017 $\pm$ 0.037	0.04	0.022 $\pm$ 0.052	0.09
p-value		0.075		0.078		0.188

C: control group, SD: standard deviation value, OD: optical density

**Figure 2.** Fibroblast percentage in epistaxis healing process over time after being treated with different concentrations of *C. odorata* L leaf gel extract. Bar errors indicates percentage of error.**Figure 3.** Angiogenesis, represented by the percentage of new blood vessels, in epistaxis healing process over time after being treated with different concentrations of *C. odorata* L leaf gel extract. Bar errors indicates percentage of error.

into fibrin that covers the wound and subsequently blood clotting.¹⁴ A number of these compounds are also involved in the wound healing phases, including tissue proliferation and remodeling.¹⁵

Our study also assessed the bioactivities of *C. odorata* compounds as GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. The biological activities of those six molecules might contribute in increasing the angiogenesis during epistaxis recovery. Through the ligand mechanism of GPCR, the activation of proteolytic enzymes involved in wound healing is possible.¹⁶ These enzymes degrade necrotic cells originating from injured cells, regulate the cell maturation and multiplication, synthesize collagens and regulate perivascular fibrin.¹⁷

The ion channel owned by the active compounds of *C. odorata* could bind to the intracellular domain of the sodium channel with a voltage that blocks the entry of sodium into the cell to prevent depolarization. Depolarization is responsible for initiating a response or signals of pain conduction in peripheral nerves that act on wound healing.¹⁸ Modulator channel

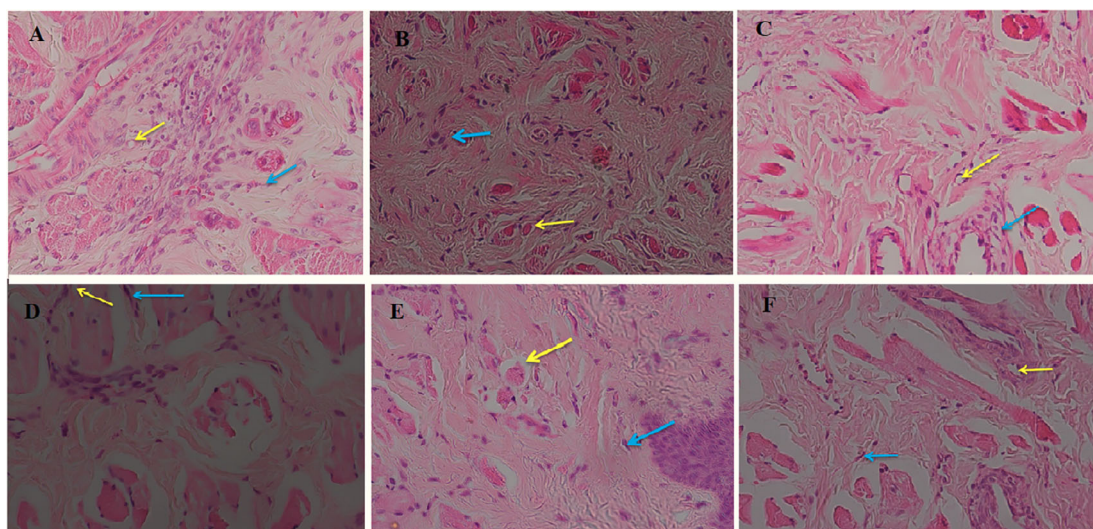


Figure 4. The profile of fibroblasts and new blood vessels in epistaxis after being treated with ethanol extract gel of *C. odorata* at day 7 with 400-time magnifications. (A) Negative control, (B) Positive control, (C) *C. odorata* 5%, (D) *C. odorata* 10%, (E and F) *C. odorata* 15%. Yellow arrow (new blood vessels), blue arrow (fibroblast).

ions open pores in cell membranes, causing the generation of electric currents. Activation of the receptor will open ion channels so that the charge across the plasma membrane will be distributed effectively. This increased activation will help the cells to proliferate in the process of tissue repair.¹⁹

In terms of wound healing, enzyme inhibitors interfere with the response of inflammatory cells such as neutrophils. In contrast, the presence of active compounds from *C. odorata* provides an inflammatory cell response to release pyruvate kinase M2 (PKM2), an enzyme that acts in the last step of the glycolysis pathway and helps wound healing by promoting angiogenesis at the wound site.^{20,21}

Some compounds from *C. odorata* exhibit high bioactivity such as octacosanol, which means that this compound might have an impact on expression of the genes involved in wound healing.²² This process occurs through the mechanism of ligand interaction with the receptor. Binding ligands activate nuclear receptors including lipophilic substances such as endogenous hormones, vitamin A, vitamin D and xenobiotic hormones. Since the expression of a large number of genes is regulated by nuclear receptors, ligands that activate these receptors can have important effects on any cell of an organism undergoing repair or other response to the environment.²³

C. odorata compounds also have bioactivity against protease inhibitors. In the wound healing process, proteases and their inhibitors contribute to a balance between degradation and deposition of the extracellular matrix (ECM), which is important for the timely and coordinated healing of wounds. However, when the balance is disturbed, the wound develops chronicity, characterized by an increase of proteases and a decrease of protease inhibitors.²⁴ Excessive proteases will degrade ECM, interfering with wound healing.²⁵ This mechanism allows wound healing to also occur in epistaxis with *C. odorata* binding to excess proteases. In the inflammatory phase, proteases remove the damaged ECM, followed by a proliferation phase where proteases help increase the degradation of capillary basement membrane for angiogenesis, assisting migration and cell release. In the remodeling phase, various cells are mobilized to the injured location to reconstruct the tissue, regulated by the ECM.²⁶

C. odorata increased the expression of CD34 and CD68 level but had no significant effect compared to control groups. CD34 is a marker of increased fibroblast cells, which are critical in angiogenesis during wound healing. They break down fibrin clots, build new collagen and ECM structures to support other cells needed for efficient wound healing.^{27–29} The increase in CD68, which is expressed by monocytes and macrophages, is in line with a decrease in inflammatory cells and stimulation of fibroblast activity which has an impact on angiogenesis.^{30,31} Nevertheless, slight fibroblast cell proliferation still occurred in the wound area in the present study. In addition, our study clearly demonstrated that the number of new blood vessels was significantly frequent at wounds from animals given the highest concentration of *C. odorata* (15%), followed by the 10% and 5% concentrations. This suggests that *C. odorata* extract could induce the angiogenesis, the process that is crucial during wound healing.

This study has some limitations that need to be discussed. The number of animals used in this study was very small and a study with bigger sample size is required. The present study assessed the level of CD34 and CD68 from the blood and such a source might not be the best indicator. Further study assessing the expression of CD34 and CD68 directly from the wound histopathology using immunohistochemistry is important. It is recommended for further studies be undertaken such as docking analysis³² to predict further interactions between the putative compounds of the *C. odorata* extract and critical molecules in the angiogenesis pathway.

Conclusions

We identified eight putative compounds with antithrombotic, antioxidant and anti-inflammatory activities from *C. odorata* extract: longiverbenon (Vulgaron B), phytol (acetate), neo-Menthol, pentadecadien-1-ol, octadecanoic acid, squalene, beta.-tocopherol and octacosanol. These putative compounds have a range of bioactivities as GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. Those putative compounds with their bioactivities might contribute in inducing the angiogenesis during the wound healing processes in epistaxis observed in the present study.

Data availability

Underlying data

Figshare: Bioactive phytoconstituents and hemostatic and angiogenetic activities of *Chromolaena odorata* L. leaf extract gel on animal epistaxis model. <https://doi.org/10.6084/m9.figshare.20676942>.³³

The project contains the following underlying data:

- Master Table.xlsx (raw data of gas chromatography–mass spectrometry (GC-MS)).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Acknowledgements

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References

1. Tunkel DE AS, Payne SC, Ishman SL, *et al.*: **Clinical practice guideline: nosebleed (Epistaxis).** *Otolaryngol. Head Neck Surg.* 2020; **162**: S1–S38.
[Publisher Full Text](#)
2. Salim MN, Masyita D, Harris A, *et al.*: **Anti-inflammatory activity of *Jatropha curcas* Linn. latex in cream formulation on CD68 expression in mice skin wound.** *Vet World.* 2018; **11**(2): 99–103.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Akinmoladun AC, Akinloye O: **Effect of *Chromolaena odorata* on hypercholesterolemia-related metabolic imbalances.** *Proceedings of The Akure-Humboldt Kellogg/3rd SAAT Annual Conference on Medicinal Plants.* 2007; 287–90.
4. Phan TT, Hughes MA, Cherry GW: **Effects of an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin) on the proliferation of human keratinocytes and on their migration in an in vitro model of reepithelialization.** *Wound Repair Regen.* 2001; **9**(4): 305–313.
[PubMed Abstract](#) | [Publisher Full Text](#)
5. Pandith HTS, Wongkrajang Y, Gritsanapan W: **In vivo and in vitro hemostatic activity of *Chromolaena odorata* leaf extract.** *Pharm. Biol.* 2012; **50**(9): 1073–1077.
[PubMed Abstract](#) | [Publisher Full Text](#)
6. Triratana TSR, Naengchomnong W: **Effect of *Eupatorium odoratum* on blood coagulation.** *J. Med. Assoc. Thai.* 1991; **74**(5): 283–287.
[PubMed Abstract](#)
7. Pandith HZX, Thongpraditchote S, Wongkrajang Y, *et al.*: **Effect of Siam weed extract and its bioactive component scutellarein tetramethyl ether on anti-inflammatory activity through NF-KB pathway.** *J. Ethnopharmacol.* 2013; **147**(2): 434–441.
[PubMed Abstract](#) | [Publisher Full Text](#)
8. Revi Yenti RA, Afriani L: **Formulasi krim ekstrak etanol daun kirinyuh (*Eupatorium odoratum* L.) untuk penyembuhan luka.** *PharmaMedika.* 2011; **3**(1): 227–230.
9. Herdiana Y: **Formulasi gel uudesilenil fenilalanin dalam aktivitas sebagai pencerah kulit.** Universitas Padjajaran; 2007.
10. Aiyalu R, Govindarajan A, Ramasamy A: **Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model.** *Braz. J. Pharm. Sci.* 2016; **52**(3): 493–507.
[Publisher Full Text](#)
11. Husain A, Ahmad A, Khan SA, *et al.*: **Synthesis, molecular properties, toxicity and biological evaluation of some new substituted imidazolidine derivatives in search of potent anti-inflammatory agents.** *Saudi. Pharm. J.* 2016; **24**(1): 104–114.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
12. Anderson G, Bancroft J: **Tissue processing and microtomy.** Bancroft J, editor. Edinburgh Churchill Livingstone; 2022.
13. Jones RT: **Normal values for some biochemical constituents in rabbits.** *Lab. Anim.* 1975 Apr; **9**(2): 143–147.
[PubMed Abstract](#) | [Publisher Full Text](#)
14. Weisel JW, Litvinov RI: **Fibrin formation, structure and properties. sub-cellular biochemistry. Fibrous Proteins: Structures and Mechanisms.** Cham: Springer; 2017; 405–56.
15. Guo S, Di Pietro LA: **Factors affecting wound healing.** *Journal of dental research.* *J. Dent. Res.* 2010; **89**(3): 219–229.
[Publisher Full Text](#)
16. Fu J, Lee T, Qi X: **The identification of high-affinity G protein-coupled receptor ligands from large combinatorial libraries using multicolor quantum dot-labeled cell-based screening.** *Future Med. Chem.* 2014; **6**(7): 809–823.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

17. Sinclair RD, Ryan TJ: **Proteolytic enzymes in wound healing: the role of enzymatic debridement.** *Australas. J. Dermatol.* 1994; **35**(1): 35–41.
[PubMed Abstract](#) | [Publisher Full Text](#)
18. Ciotu CI, Tsantoulas C, Meents J, *et al.*: **Noncanonical Ion Channel Behaviour in Pain.** *Int. J. Mol. Sci.* 2019; **20**(18): 4572.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Waszkielewicz AM, Gunia A, Szkaradek N, *et al.*: **Ion channels as drug targets in central nervous system disorders.** *Curr. Med. Chem.* 2013; **20**(10): 1241–1285.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Zhang Y, Li L, Liu Y, *et al.*: **PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis.** *Wound Repair Regen.* 2016; **24**(2): 328–336.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Hanh TTH, Hang DTT, Van Minh C, *et al.*: **Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*.** *Asian Pac. J. Trop. Med.* 2011; **4**(10): 760–763.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Sonoda J, Pei L, Evans RM: **Nuclear receptors: decoding metabolic disease.** *FEBS Lett.* 2008; **582**(1): 2–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Sladek FM: **What are nuclear receptor ligands?** *Mol. Cell. Endocrinol.* 2011; **334**(1–2): 3–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
24. McCarty SM, Percival SL: **Proteases and Delayed Wound Healing.** *Advances in Wound Care (New Rochelle).* 2013; **2**(8): 438–447.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Rushton I: **Understanding the role of proteases and pH in wound healing.** *Nurs. Stand.* 2007; **21**(32): 68–74.
[Publisher Full Text](#)
26. Serena TE: **Development of a novel technique to collect proteases from chronic wounds.** *Advances Wound Care (New Rochelle).* 2014; **3**(12): 729–732.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Goichberg P: **Current understanding of the pathways involved in adult stem and progenitor cell migration for tissue homeostasis and repair.** *Stem Cell Rev. Rep.* 2016; **12**(4): 421–437.
[Publisher Full Text](#)
28. Mir MA, Hamdani SS, Sheikh BA, *et al.*: **Recent advances in metabolites from medicinal plants in cancer prevention and treatment.** *Curr. Immunol. Rev.* 2019; **15**(2): 185–201.
[Publisher Full Text](#)
29. Dhar R, Kimseng R, Chokchaisiri R, *et al.*: **2',4-Dihydroxy-3',4',6'-trimethoxychalcone from *Chromolaena odorata* possesses anti-inflammatory effects via inhibition of NF- κ B and p38 MAPK in lipopolysaccharide-activated RAW 264.7 macrophages.** *Immunopharmacol. Immunotoxicol.* 2018; **40**(1): 43–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
30. Gurevich DB, Severn CE, Twomey C, *et al.*: **Live imaging of wound angiogenesis reveals macrophage orchestrated vessel sprouting and regression.** *EMBO J.* 2018; **37**(13): e97786.
[Publisher Full Text](#)
31. Chistiakov DA, Killingsworth MC, Myasoedova VA, *et al.*: **CD68/ macrophage: not just a histochemical marker.** *Lab. Investig.* 2017; **97**: 4–13.
[Publisher Full Text](#)
32. Purnama A, Mardina V, Puspita K, *et al.*: **Molecular docking of two cytotoxic compounds from *Calotropis gigantea* leaves against therapeutic molecular target of pancreatic cancer.** *Narra J.* 2021; **1**(2): e37.
[Publisher Full Text](#)
33. Husni TRT: **Underlying data for 'Bioactive phytoconstituents and hemostatic and angiogenetic activities of *Chromolaena odorata* L. leaf extract gel on an animal epistaxis model.'** *Figshare.* 2022.
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Version 1

Reviewer Report 05 March 2024

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Nur 'Ainun Mokhtar 

UiTM Kampus Bertam, Pulau Pinang, Malaysia

1. Introduction

- Introduction provides sufficient information. The problem statement or research question should also be included in this section.

2. Methods.

- Compound identification: the method needs citation.
- Blood electrolyte analysis: kindly state whether the samples were collected after treatment/induction/etc (when referring to 7, 14 and 21 days).
- In general, please include citation/reference to the methods presented/chosen in this study.

3. Results

- **CD34 & CD68 Level:** the data is best represented graphically. If possible, could you add in/supplement graph, as part of the data presented for CD34 & CD68 levels?

4. Discussion

- It was claimed that the gels formulation contains antioxidant, anti-inflammatory, and antithrombotic compounds but the author did not specify/suggested which compounds attributed to these pharmacological activities. Include citation/reference to support this claim.
- Proteases plays important roles in various wound healing stages and there are many types of proteases involved during this process. For example, matrix-metalloproteases (MMPs)¹. The discussion should highlight the specific MMPs that needs to be inhibited during wound healing at distinguished stages.
- Blood electrolytes level was not discussed.

5. Conclusion

- Conclusions are too brief and partially adequate. It is recommended that authors report all results, significant or not. Include the significance of this research output and future directions in the abstract or main body conclusion.

References

1. Kandhwal M, Behl T, Singh S, Sharma N, et al.: Role of matrix metalloproteinase in wound healing. *Am J Transl Res*. 2022; **14** (7): 4391-4405 [PubMed Abstract](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Natural products, wound healing, Pharmaceuticals, Bioinformatics, Network Pharmacology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 25 May 2023

<https://doi.org/10.5256/f1000research.138691.r171646>

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Mashani Mohamad

Faculty of Pharmacy, Universiti Teknologi Malaysia, Puncak Alam Campus, Selangor, Malaysia

This research article reports on an investigation of the phytochemistry of *Chromolaena Odorata* and effectiveness of its gel formulation on rabbit epistaxis model. The body of content of this article is well structured. However, there are a few concerns and comments based on the given review questions. These are listed below:

1. Presentation of the research work was clearly presented but the accuracy in the methodology, results and discussions need some clarifications. This will be addressed in separate points further below. Majority of the literature cited (about 60%) are from 2010-2020 and there is a reference that dated back in 1975.
2. The study design is appropriately described with technically achievable outcomes.
3. The details of method and analysis is partly sufficient, where some parts of the method need improvement as below:
 - There are no citation/references for compound identification, gel formulation, gel evaluation, epistaxis model and measurement of angiogenesis (only tissue processing reference was cited).
 - Time intervals between two gel applications were not stated.
 - Positive control for epistaxis model was not stated.
 - The term 'homeostasis assessment' – is this supposed to mean hemostasis assessment?
 - The measurement of angiogenesis method needs to be more detailed in terms of calculation of the measurement: is it qualitative or quantitative? Who and how many observers were included in the observation?
4. The statistical analysis and interpretation is partly appropriate. The bleeding time data (Table 4) is presented without standard error. Significant value was not indicated in Fig 3.
5. Conclusions drawn from the results are partly adequate. It is suggested that the authors mention all the results obtained, either it is significant or not. The significance of this research output and future directions can be included in the abstract or main body conclusion.
6. Other than the review questions given, below are the additional comments and questions to the authors:
 - The abstract is well written. However, there is a contradictory statement in result (electrolytes levels were not affected due to the data being not significant). The exact percentage of *C. odorata* that give the best effect was not stated. Conclusion can be improved by including a summary of overall results and future benefit of this study.
 - Introduction is brief but informative. The problem statement/research question should be presented at the end of this part, before the research objective.
7. Comment on the presented results are as follows:
 - It was stated that the putative compounds were antithrombotic, antioxidant and anti-inflammatory, but it is not specified which 8 compounds are related to these properties.
 - Gel evaluation results are important to ensure applicability but were not presented.
 - It was mentioned that animals were sacrificed at day 21. However, the histology is presented at day 7. Was a separate group is allocated for each duration (7, 14 & 21 days)?

- Representative histology images are presented without scale bar and no brief description on morphology to be observed. Figure B, D and F is darker compared to the other 3. Suggest standardizing the brightness and contrast for all images.
 - The arrow shape and size are inconsistent.
 - The fibroblast in figure A is questionable as it is not pointed at blue staining, just white. Fibroblast and new blood vessel in D is at the very edge of image and is not clear. Figure E new blood vessel is too wide and seen as artifact.
8. Comment on the discussions are as follows:
- Discussions in para 1 can be strengthened by providing the exact reaction in the process of suggested mechanism/pathway where the compounds are associated. Which exact compound that contributes to the clotting process? A figure showing these association will be much better.
 - Ion channel is essential to promote activity of wound healing, not preventing it¹. This contradicts paragraph 3 discussion.
 - Octacosanol is an antioxidant and considered as anti-angiogenesis². This contradicts paragraph 5 discussion.

References

1. Lee K, Seo PJ: Wound-Induced Systemic Responses and Their Coordination by Electrical Signals. *Front Plant Sci.* 2022; **13**: 880680 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Wang T, Liu YY, Wang X, Yang N, et al.: Protective effects of octacosanol on 6-hydroxydopamine-induced Parkinsonism in rats via regulation of ProNGF and NGF signaling. *Acta Pharmacol Sin.* 2010; **31** (7): 765-74 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phytochemistry, wound healing, histology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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