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# Bioactive Compounds Analysis and Antioxidant Evaluation of *Chromolaena odorata* (L) for Development of Anti-Inflammatory Herbal Cream

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## Abstract

**Introduction:** *Chromolaena odorata* (L.), commonly known as Siam weed (Khmer name: Tontrean khet) that is widely used traditional medicine in Cambodia to treat wound, burn, and skin infections due to its diverse pharmacological properties. Despite its traditional use, scientific validation for its development into a standardized topical formulation in Cambodia remains limited.

**Objective:** This study aimed to evaluated the secondary metabolites and antioxidant activities of *C. odorata* leaves extraction and developed into a stable anti-inflammatory herbal cream. **Method:** Mature leaves were collected from Siem Reap province in Cambodia and extracted using 70% of ethanol. Crude extraction was analyzed for TPC, TFC, TCC, and TAIC including antioxidant capacity using FRAP and DPPG assays. An oil-in-water (o/w) cream was formulated with 1% extract and subjected to physicochemical and accelerated stability testing (8 weeks at 20 °C and 40 °C).

**Results:** Extraction yield was 7.65%. Bioactive analysis revealed TPC of 80.46 ± 7.52 mg GAE/g CE, TFC pf 27.21 ± 2.20 mg QE/g of CE, TCC of 0.75 ± 0.01 mg EE/g of CE, and TAIC of 4.55 ± 0.01 mg AE/g of CE. Significant antioxidant activities with FRAP assay of 1.12 ± 0.22 and DPPH $IC_{50}$ of 2.03 ± 0.288 µg/mL. A strong correlation was observed between TPC and TFC (r = 0.955) and between TCC and DPPH scavenging (r = 0.946). The Formulated cream was smooth, homogeneous, and physical stable with pH of 5.06 which was compatible with human skin.

**Conclusion:** *C. odorata* is rich source of bioactive phenolic and tannin with potent antioxidant properties. Successful

formulation of a stable, skin-compatible herbal cream validated its potential as a natural therapeutic product for topical applications.

## Introduction

Cambodia is a country with rich and diverse biodiversity with abundant natural resources that support livelihood and traditional healthcare knowledge and practice [1]. A wide variety of herbal species that are used to prevent and treat diseases with local knowledge that has been passed down through generation. Among all herbal species, *Chromolaena odorata* (L.) or Siam weed (Khmer name: Tontrean khet) is the herb that belongs to Asteraceae Family, and it is known as an invasive weed native to tropical American, African, Asia, as well as Australia where it has become the major agriculture weed species [2]. Although widely known for its invasive behavior, parts of this plant are used as traditional medicines to treat wound, burn, or skin infections with demonstrates multiple pharmacological properties such as anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant activities [3]. *C. odorata* contains rich bioactive compounds such as phenolic, flavonoid, and tannin which are recognized for their potential anti-inflammatory, antioxidant, and therapeutic properties to identify effective non-antibiotic natural compounds for preventing and treating illness [4].

Traditionally, the fresh leaves of *C. odorata* have been used as a natural skin remedy by locals in rural areas in Cambodia such as crushing or grinding and applying it directly on an open wound or cut, to accelerate wound healing, stop minor bleeding, and relieve insect bite and reduce inflammation [5]. Moreover, *C. odorata* is rich in bioactive compounds such as flavonoids, terpenoids, tannins, and essential oils, which contribute beneficial effects on skin including anti-inflammatory, anti-microbial, and wound healing properties [6].

Due to these effects on skin conditions, *C. odorata* is a good option for scientists' research and development of the natural or combination of natural-synthetic into topical products, such as cream [7]. Its plant extract can be formulated into an anti-inflammatory cream, which offers multiple therapeutic effects. Nevertheless, the several evaluation tests were conducted to assess the quality and stability of *C. odorata* compound for the formulation through pH testing, stability testing, and determining bioactive compounds and antioxidant activities [8].

Additionally, this study aims to evaluate the secondary metabolites compounds and antioxidant activities of *C. odorata* for development of the into herbal cream formulation as the natural product, in order to validate therapeutic effectiveness and ensure long-term safety on the skin usage.

**Keyword:** Bioactive compounds, herbal cream, *Chromolaena odorata*, anti-inflammatory; wound healing

## Method

### Materials and Reagents

Folin-Ciocalteu reagents, aluminum chloride, ferric chloride, ferrous sulphate, sodium hydroxide, sodium carbonate, acetic acid, sulfuric acid, hydrochloric acid, and ethanol (analytical grade) were purchased from Merck KGaA (Darmstadt, Germany). Gallic acid was brought from Acros Organics BV (Geel, Belgium) while Quercetin was got from HiMedia Laboratories Pvt.Ltd. (Mumbai, India). 2,2-Diphenyl-1-picrylydrazyl was purchased from Tokyo Chemical Industry Co,Ltd (Tokyo, Japan) 2,4,6-Tri(2-pyrideyl)-s-triazine (TPTZ) was bought from Thermo Fisher Scientific (Fair Lawn, NJ, US). Vanillin was obtained from Acros Orgnic, Janssen Pharmaceuticalaan 2a (Geel Belgium). All other reagents were acquired from standard commercial suppliers.

### Plant collection and authentication

*C. odorata* leaves were obtained in Pouk commune, Pouk district, Siemreap province, Cambodia, and authenticated by botanists.

### Plant Extraction

Mature leaves were cleaned and air-dried under sunlight for 2-3 weeks at atmospheric temperature (approximately 37-38 $^{o}$C). The dried leaves were grounds into coarse powder. About 200 g of powered was extracted with 70% ethanol via maceration technique for a week, with intermittent shaking [9]. The extraction was further filtered through Whatman No. 1 filter paper and concentrated crude under reduced pressure using a rotary evaporator. Crude extraction was stored in a refrigerated at 4 degrees Celsius ($^{o}$C) until use [10].

### Total Phenolic Content (TPC)

The crude extraction was dissolved with 1 mg per mL of ethanol. TPC was determined using the Folin-Ciocalteu method [11]. Gallic acid was used as the standard and dissolved in ethanol to prepare a series of concentrations ranging from 20 to 60 µL/mL. For the assay, 15 µL of the extraction was mixed with 120 µL of Folin-Ciocalteu in the 96 well-microplate and incubated for 5 min at room temperature in the dark, covered with aluminum foil. Then sodium carbonate ($Na_2CO_3$, pH7.5) solution was added following by incubation for 90 min at room temperature.

The absorbance of the blue complex was measured at 725 nm using a microplate spectrophotometer (Thermo Scientific$^{TM}$ Multiskan$^{TM}$ FC, Boston, MA, USA). TPC was

calculate from gallic acid calibration curve (y = 0.0265x + 0.0382, $R^2$ = 0.9904), with blank, standards, and sample analyzed in five replicates. The results were reported as milligrams of gallic acid equivalent (GAE) per gram of crude extraction (CE).

**Total Flavonoid Content (TFC)**

TFC was determined by using Aluminum Chloride ($AlCl_3$) colorimetric method [12]. Quercetin was dissolved in ethanol and prepared at different concentrations from 20 to 60 µL/mL to generate the standard calibration curve. For sample analysis, crude extraction was dissolved in ethanol (1mg/mL), and 100 µL of crude extraction was added to the 96 well-microplates following by the addition of 2% of $AlCl_3$. The reaction mixtures were incubated for 60 min in the dark at temperature.

The measurement was finalized with five replications of blank, standard, and sample. The absorbance was measured at the wavelength of 400 nm in a microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA). TFC was calculated from the quercetin standard curve (y = 0.0461x + 0.0019, $R^2$ = 0.9946). The results were reported as milligrams of quercetin equivalent (QE) per gram of crude extraction.

**Total Tannin Content (TCC)**

TCC was determined by using the 4% of vanillin reagent in the 96 well microplate [13]. Epicatechin was prepared at different concentrations from 20 to 100 µL/mL. Then crude extraction was dissolved in ethanol (1 mg/mL) and transferred 50 µL to the 96-microplate and mixed with 150 µL of vanillin and 75 µL of Hydrochloride acid (HCL) to incubate in the dark at room temperature for 15 min.

The absorbance measurement was detected with five replications at 500 nm in a microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA). TCC was calculated from epicatechin standard curve (y = 0.111x - 0.0759, $R^2$ = 0.9982). The results were reported as milligram of epicatechin equivalent (EE) per gram of crude extraction.

**Total Alkaloid Content (TAIC)**

TAIC was performed using citrate phosphate buffer (pH 4.7) [12]. Atropine was used as the standard at different concentration (0.01 to 0.1 mg/mL). Crude extraction 10 mg was dissolved in 1,000 µL of 2N HCL as the sample. Then 1,000 µL of sample was added with 5 mL of citrate phosphate and 5 mL of bromocresol green with 5 mL of chloroform in test tubes and mixed solution by shaking. The solution was incubated in the dark at room temperature for 30 min. Chloroform layer showed below the layer of sample and standard mixture which was analysis at 420 nm using the UV spectrometry (GENESYSTM 10S UV-Visible Spectrophotometer, Thermo Fisher Scientific, Madison, WI, USA).

The experiment was conducted in five replications. TAIC was estimated from atropine standard curve (y = 0.1153x + 0.0146, $R^2$ = 0.9995). The results were described as milligram of atropine equivalent (AE) per gram of crude extraction.

**Reducing Antioxidant Power based on FRAP Assay**

FRAP assay method was analyzed the reduction by mixing of acetate buffer (pH 3.6), ferric chloride (FeCl3), and 2,4,6-Tri(2-pyridyl)-s-trianzine (TPTZ) solution in a 10:1:1 ration [14]. Crude extraction 1 mg was dissolved in ethanol and transferred to the 96-microplate in 1:1 ration with FRAP reagent for five replication and was incubated for 30 min at room temperature.

The experiment was measured at 593 nm with a microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA). Standard curves of ferrous sulfate ($FeSO_4$) with various concentration (50, 100, 150, 200, and 250 nM) was liner (y = 0.0022x + 0.0113, $R^2$ = 0.9958), and Gallic acid was a positive control (20, 50, 100, 150, and 200 µg/mL) was linear (y = 0.0053x - 0.0028, $R^2$ = 0.9974).

**DPPH Radical Scavenging Assay**

The free radical scavenging activity was evaluated using 2,2-Diphenyl-1-picrylhydrayl or DPPH assay to show the reaction when color changed from deep violet to light yellow [15]. Crude extraction was dissolved in 1 mg in ethanol and diluted in different concentration (100, 250, 500, 750, and 1,000 µg/mL). Then 100 µL of extraction was mixed with 100 µL of DPPH solution in ratio of 1:1 in the 96 well-microplates. The reaction was incubated for 30 min in the dark at room temperature.

DPPH radical was measured at 515 nm with a microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA). Gallic acid with different concentrations (100, 250, 500, 750, and 1,000 µg/mL) was presented as the standard curve (y = 0.2275x + 82.824, $R^2$ = 0.9973). The experiment was carried out for five replications.

DPPH radical scavenging capacity was described as percentages of DPPG radical inhibition at 50% ($IC_{50}$). The inhibitory activity was followed by the formula [16]:

$$\text{Inhibition (\%)} = \frac{Abs\ control - Abs\ sample}{Abs\ control} * 100$$

Where Abs control was absorbance of DPPH radical and ethanol; Abs sample was absorbance of DPPH radical and *C. odorata* extraction.

## Formulation and Physicochemical Evaluation

The development of anti-inflammatory Cream was followed from pervious article **[3]**.

### Formulation of Anti-Inflammatory Cream

The oil and water phases were placed in separate beakers and heated to 70 °C. The oil phase was added to the water phase while stirring continuously until an oil-in-water solution was formed. The cream was generated when the consistency was satisfactory, and the look was opaque. 1% of *C. odorata* leaf extract was combined with the base, along with methylparaben, a preservative, and peppermint oil, which provides scent and antioxidant properties.

#### *pH of the Cream*

A standard buffer solution was used to calibrate the pH meter. About 1 g of cream was diluted with 9 ml of distilled water, and the pH was determined.

#### *Homogeneity*

The uniformity of the formulation was assessed by touch and appearance.

#### *Appearance*

The cream's appearance was evaluated based on its color, transparency, roughness, and grain qualities.

#### *After Feel*

Smoothness, slippery consistency, and the amount of residue remaining after applying a set amount of cream were evaluated.

#### *Type of Smear*

The kind of film or smear that developed on the skin after the cream was applied was examined.

#### *Removal*

The ease of removal of the cream was tested by draining the treated area with tap water.

#### *Accelerated Stability Testing*

Cream was separated into two parts and stability tested at 20 °C ± 1 °C and 40 °C ± 1 °C in an incubator with 75% relative humidity. The parameters were monitored weekly for 8 weeks.

#### *Data Analysis*

Results were exhibited as mean ± standard deviation (SD) of five replications (n = 5). Correlation coefficient analysis was performed using Statistical Package for the Social Science (SPSS) Version 20, SPSS Inc, Armonk, NY, USA. A p-value < 0.05 was considered statistically significant.

## Results

### Secondary Metabolic Compounds

The extraction yield (%) of *C. odorata* dried leaves using ethanol 70% was 7.65% (w/w). Quantitative bioactive compounds exhibited TPC were 80.46 ± 7.52 mg GAE/g CE that represented the most abundant class of secondary metabolite while TFC was 27.21 ± 2.20 mg QE/g of CE, suggesting that flavonoid contributed essentially to overall phenolic composition. Respectively, TCC was low at 0.75 ± 0.01 mg EE/g of CE, and TAIC found 4.66 ± 0.01 mg AE/g of CE. The low standard deviation value proved good reproducibility and consistency of analytical procedures (n = 5). After all, phenolic compound was explained as the predominant bioactive constituents in ethanol extraction.

### Antioxidant Activities

FRAP assay of *C. odorata* extraction exhibited the reducing power was 1.06 ± 0.08 millimoles (mM) GAE/g of CE that indicated its ability to reduce ferric ($Fe^{3+}$) to ferrous ion ($Fe^{2+}$), and antioxidant activity was determined by DPPH assay reported an $IC_{50}$ of 2.03 ± 0.14 µg/mL that suggested *C. odorata* possessed considerable antioxidant activity (Table 2).

**Table 1.** Secondary metabolic compounds and antioxidant activities in dry leaves extraction of *C. odorata*.

| Secondary Metabolic Compounds/Antioxidant Activities | Results (mean ± SD) |
|---|---|
| TPC (mg GAE/g of CE) | 80.46 ± 7.52 |
| TFC (mg QE/g of CE) | 27.21 ± 2.20 |
| TTC (mg EE/g of CE) | 0.75 ± 0.01 |
| TAIC (mg AE/g of CE) | 4.66 ± 0.01 |
| FRAP (mM GAE/g of CE) | 1.06 ± 0.08 |

| | |
|---|---|
| DPPH $IC_{50}$ (µg/mL) | 2.03 ± 0.14 |

**Formulation and Stability**

The composition of formulated anti-inflammatory cream was showed in Table 2. The oil-in-water (o/w) emulsion combination was developed successfully by using Vaseline, cetyl alcohol, and liquid paraffin as oil phase while water, glycerin, and triethanolamine composed water phase. Vitamin E was blended as the antioxidant, and methylparaben was presented as a preservative to ensure microbiological stability. Crude extraction was mixed 1% (w/w) into the emulsion base.

**Table 2.** Formulation and composition of antibacterial and anti-inflammatory cream

| Components | Amount (g) |
|---|---|
| **Phase A (oil phase)** | |
| Vaseline | 20.56 |
| Cetyl alcohol | 7.64 |
| Liquid paraffin | 4 |
| Vitamin E | 1 |
| **Phase B (water phase)** | |
| Water | 140 (ml) |
| Glycerin | 9.5 |
| Triethanolamine | 1.92 |
| Methylparaban | 1.92 |

Extraction of *C. Odorata* leaves resulted in a stable greenish cream concentration with a slightly herbal aroma (figure 1). During formulation, the extract was added into the emulsion base without difficulty and produced a cream with smooth texture and homogeneity consistency. The final product shows a light peppermint fragrance, good spread ability on the skin, and rapid absorption without leaving a greasy residue.

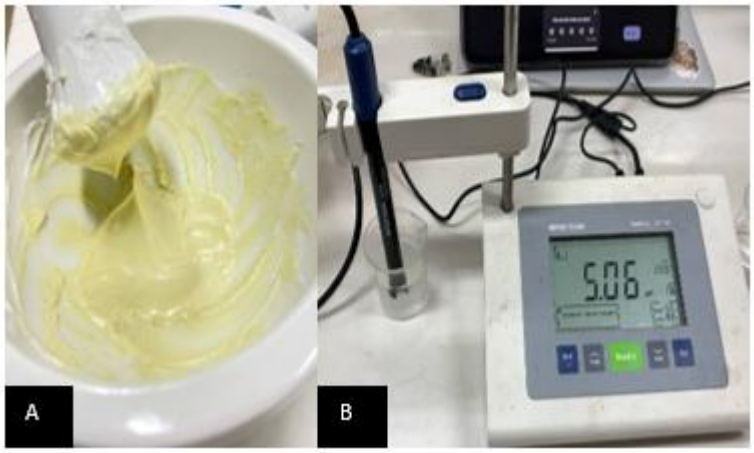


Figure 1. (A) Formulated cream. (B) pH 5.06 of cream.

Stability evaluation showed that the cream remained physically stable when stored at temperatures between 20 °C and 40 °C with 75% relative humidity, with no visible phase separation or change in color throughout the testing period. The pH of the cream formulation was found to be 5.06 (Figure 1), which falls within the acceptable range for topical cream skin application. Preliminary users show that the *C. Odorata* cream provided a soothing sensation when applied on the skin, with noticeable reduction in skin redness and overall improvement in skin comfort.

**Table 3.** Correlation coefficient (r) indicated the strength and direction of relationship between secondary metabolites and antioxidant activities (FRAP and DPPH). Value close to +1 indicated a strong positive correlation, value close to -1 indicated a strong negative correlation, and value near 0 suggested a weak or no linear relationship.

| Method | TPC | TFC | TCC | TAIC | FRAP | DPPH |
|---|---|---|---|---|---|---|
| **TPC** | 1 | 0.955* (P = 0.012) | 0.635 (P = 0.250) | -0.142 (P = 0.820) | 0.588 (P = 0.297) | 0.415 (P = 0.487) |
| **TFC** | 0.955* (P = 0.012) | 1 | 0.638 (P = 0.247) | -0.393 (P = 0.512) | 0.376 (P = 0.533) | 0.464 (P = 0.431) |
| **TCC** | 0.635 (P = 0.250) | 0.638 (P = 0.247) | 1 | 0.186 (P = 0.764) | -0.007 (P = 0.990) | 0.946* (P = 0.015) |
| **TAIC** | -0.142 (P = 0.820) | -0.393 (P = 0.512) | 0.186 (P = 0.764) | 1 | 0.251 (P = 0.684) | 0.153 (P = 0.806) |
| **FRAP** | 0.588 (P = 0.297) | 0.375 (P = 0.533) | -0.007 (P = 0.990) | 0.251 (P = 0.684) | 1 | -0.194 (P = 0.754) |
| **DPPH** | 0.415 (P = 0.487) | 0.464 (P = 0.431) | 0.946* (P = 0.015) | 0.153 (P = 0.806) | -0.194 (P = 0.754) | 1 |

*Statistically significant is noted as $P < 0.05$.

**Correlation Coefficient (r) between Secondary Metabolites and Antioxidant Activities**

Table 3 presented the significant positive correlation observed between TPC and TFC ($r = 0.955$, $P < 0.05$) suggested that flavonoids constitute a major portion of TPC that was presented in *C. odorata* since flavonoid is a subclass of phenolic compounds. In Addition, DPPH and TCC ($r = 0.946$, $P < 0.015$) suggested that condensed tannin plays a key role in radical scavenging activity. The outcome confirmed that phenolic compounds including flavonoids and tannins were major contributors to antioxidant potential of *C. odorata*.

## Discussion

*C. odorata* leaves were extracted using ethanol to obtain the crude extraction, which was experimentally evaluated to generate scientific evidence supporting the development of the herbal cream in Cambodia. Ethanol is a well-known solvent to extract plant compounds and is considered safer for human health, requiring fewer toxicological and pharmacokinetic evaluation compare with methanol [17]. Although crude extraction using ethanol may produce lower quantities of compounds than methanol extraction, the resulting herbal cream formulation still provides effective compounds to ensure safety for human application as the natural product.

According to previous study, *C. odorata* leaves that were extracted with 70% of ethanol too that yield extraction was 15.83%, TPC was 113.15 mg GAE/g, and DPPH was 223.33 ± 9.20 µg/mL [18]. Extensive research has been conducted on the bioactive compounds and antioxidant activities of *C.odorata*. High value of TPC (1373.75 mg GAE/g extract), TFC (301.09 mg QE/g extract), TCC (1373.75 mg TAE/g extract), and DPPH (13.04 mg/mL) were reported comparing differing form the findings of this study [19]. In Indonesia, TFC value of *C. odorata* found was 125.459 ± 0.163 mg/g that differed from results of this research [20]. Moreover, another article found TPC was 182.26 ± 1.99 mg GAE/g, TFC was 128.57 ± 7.63 mg QE/g, DPPH was 32.81 ± 5.26 µg/mL [21]. Furthermore, FRAP assay results were reported in previous studies showed considerable antioxidant activity with ferric-reducing capacities of 90.16 ± 0.76 % and 271.25 µg/mL [22][23]. The relationship between TPC and antioxidant activities that were discussed in many studies above suggesting phenolic compound play the main role in ferric-reducing and radical-scavenging mechanisms of *C. odorata.*

Frap assay assesses antioxidants 'electron-reducing ability by converting $Fe^{3+}$ to $Fe^{2+}$ while DPPH assay measures their capacity to neutralize free radicals via hydrogen condition [24][25]. Both methods are important because oxidative stress contributes to inflammation by generating reactive oxygen species (ROS) that activate inflammatory mediators [26].

Alkaloid could modulate inflammatory pathways by inhibiting pro-inflammatory mediators such as nitric oxide (NO), prostaglandins, and cytokines which helped reduce redness, swelling, and irritation in tropical application [27][28]. As reported in an earlier publication, TAIC of *C.*

*odorata* was expressed in percentages value of 8.256% and 5.938% [29]. Due to the limited availability of published data on TAIC of *C. odorata*, the obtained TAIC value was compared with studies investigating alkaloid in other medicinal plants with established wound-healing activity. Previous study on *Vernonia amygdalina* demonstrated that TAIC was 9.95 ± 0.41 mg AE/g dry material and *Solanum torvum* was 6.32 ± 2.3 mg/g in mature fruit, results were higher than *C. odorata* extraction (4.66 ± 0.01 mg AE/g CE) [30].

Bioactive compounds of *C. odorata*, particularly flavonoids and phenolic compounds exhibit anti-inflammatory activity by inhibiting medicator such as nitric oxidant (NO), cyclooxygenase (COX), and pro-inflammatory cytokines [31][32]. Following this mechanism, the extraction reduces oxidative stress and modulate inflammatory pathways with supporting their therapeutic potentials in wound healing and management of skin inflammation [33].

The strong positive correlation between TPC and TFC ($r = 0.955$, $P < 0.05$) showed that flavonoid was a major subclass of phenolic compound. Phenolic compound was wieldy recognized for antioxidant activity due to their ability to donate hydrogen atoms or electrons to stabilize free radicals through resonance mechanisms [34][35]. This relationship was supported by a study that showed phenolic and flavonoid contents correlate strongly with antioxidant activity in plant extract that indicated flavonoid contribute substantially to phenolic and associated radical-scavenging potential [36]. The strong correlation between DPPH and tannin content ($r = 0.946$, $P < 0.015$) revealed tannin was a key contributor to radical scavenging capacity which aligned with recognized ability of tannin to chelate metal ions and directly scavenge relative species [37]. The strong correlation between phenolic compounds and antioxidant activity indicates these compounds are likely the major contributors to the observed radial scavenging capacity [38].

The physical characteristic of *C. odorata* cream developed in this study, included smooth texture, homogeneity, good stability, rapid absorption, and pleasant scent. The similar finding has been reported on formulation of *C. odorata* extraction into the antibacterial cream which observed acceptable organoleptic properties, stable pH, and good physical stability over 28 days that indicated the extraction could be effectively incorporated into tropical cream system with desirable formulation attributes [39]. Additionally, another study reported the gel formulations contained *C. odorata* demonstrated favorable dispersion and acceptable organoleptic after accelerated stability testing, supporting the notion that phytochemical-rich extraction from this plant-maintained stability in semi-solid delivery systems [40].

The pH values of 5.06 presented in cream within the physiologically acceptable range for skin application for minimizing irritation and maintaining skin barrier integrity with stability assessments in *C. odorata* cream that also had pH values compatible with skin use [39]. Moreover, previous research highlights their bioactive potential of formulation cream has been shown to possess antioxidant and anti-inflammatory activity that indicated therapeutic benefits beyond cosmetic properties [41]. These finding suggest that bioactive compounds in *C. odorata* plays a synergistic role in enhancing antioxidant potential.

However, formulated cream confirmed acceptable physicochemical stability, the further examination is essential for large-scale production. Factors such as long-term stability, microbial contamination control, and consistency of active compound concentration must be carefully monitored including emulsification processes and preservation system will be required to maintain formulation quality during commercial scale-up and storage [42][43].

As the results of limitations, only ethanol extraction was conducted while other extraction solvents may yield different phytochemical profile firstly. Second, qualification of alkaloid and other bioactive was using spectrophotometry method, which may be less precise than advance chromatographic methods such as HPLC or LC-MS. Third, cream formulation was analyzed only for stability and physicochemical properties while biological efficacy and safety were not tested in vivo or in clinical setting. Further research will include detailed bioactive characterization, evaluation of antioxidant, anti-inflammatory, and antibacterial activities using cellular model, as well as clinical studies to confirm safety and therapeutic effectiveness of formulation to humans.

## Conclusion

The outcome of this study presented bioactive compounds which contributed to notable antioxidant activities of *C. odorata*. The potential benefit of leaves extraction supported therapeutic profile for anti-inflammatory activity was formulated to be skin irritation application. The herbal cream was accepted with physicochemical properties and stability that its suitability for further pharmacological and clinical evaluation. Overall, *C. odorata* leaves extraction showed the promising natural candidate for development of safe and effective topical product for skin care and inflammation management. On the other hand, future study will investigate additional experiments to guarantee its safety of formulation to human.

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