

Chlorogenic acid content, essential oil compositions, and *in vitro* antioxidant activities of *Chromolaena odorata* leaves

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ABSTRACT

Chromolaena odorata (L.) R. M. King and H. Rob. is a Thai medicinal plant used for the treatment of wounds, rashes, diabetes, and insect repellent. The leaves of *C. odorata* were collected from 10 different sources throughout Thailand. The chemical constituents of essential oils were hydro-distilled from the leaves and were analyzed by gas chromatography-mass spectrometry. Chlorogenic acid contents were determined by thin-layer chromatography (TLC) - densitometry with winCATS software and TLC image analysis with ImageJ software. The TLC plate was developed in the mobile phase that consisted of ethyl acetate:water:formic acid (17:3:2). Antioxidant activities were examined by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging and β -carotene bleaching assays. *C. odorata* essential oil has shown the major components of pregeijerene, dauca-5, 8-diene, (E)-caryophyllene, β -pinene, and α -pinene. The chlorogenic acid content of *C. odorata* leaves was determined by TLC-densitometry and TLC image analysis. Results have shown that TLC-densitometry and TLC image analysis method were not statistically significantly different. DPPH radical scavenging and β -carotene bleaching assays of ethanolic extract of *C. odorata* leaves showed its antioxidant potential.

Key words: Antioxidant activity, chlorogenic acid, *Chromolaena odorata*, essential oil, quantitative analysis

INTRODUCTION

Chromolaena odorata (L.) R. M. King and H. Rob. (syn. *Eupatorium odoratum* L.) was known in common name as Siam Weed, Christmas Bush, or Common Floss Flower which is a species in the family Asteraceae. This plant is widely distributed in Asia, Africa, and the Pacific. It may reach 1 m or more as a standing shrub. The three-nerved

leaves are deltoid to ovate-lanceolate with a dentate margin. The leaves are aromatic when crushed. The inflorescences are corymbs of white, lavender, or pink.^[1,2] In herbal medicine, leaf extracts with salt are used as a gargle for sore throats and colds. It is also used to scent aromatic baths.^[3] Extracts of *C. odorata* have been shown to inhibit *Neisseria gonorrhoeae* that causes gonorrhea *in vitro*^[4] and to accelerate blood clotting.^[5] In Thailand, the plant is used for the treatment of wounds, rashes, diabetes, and insect repellent. From the literature, it was found that the leaf extracts of *C. odorata* are more beneficial than the other parts. The chemical constituents of which were isolated from this plant may be responsible for its

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pharmacological activities. *C. odorata* has been shown its pharmacological activities such as anti-inflammatory, antimicrobial, blood coagulating, insecticidal and antioxidant activities.^[6] Polyphenolic extract exhibited a slight antioxidant effect.^[7] Chlorogenic acid is a natural chemical compound which is known as an antioxidant. These polyphenols were found in a wide distribution of plants. Structurally, chlorogenic acid is a combination of a caffeic acid moiety bound to quinic acid [Figure 1].^[8] Thereby, the aims of this study are to analyze the chemical constituents of essential oil by gas chromatography-mass spectrometry (GC-MS) and to investigate the content of chlorogenic acid. ImageJ free software was used to compare with thin-layer chromatography (TLC) densitometry while antioxidant activities testing by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and β -carotene bleaching assay for evidence-based efficacy of this crude drug.

MATERIALS AND METHODS

Crude extract preparation

The leaves of *C. odorata* were collected from 10 different sources throughout Thailand and authenticated by Assoc. Prof. Dr. Nijsiri Ruangrunsi, Chulalongkorn University. Voucher specimens were deposited at the College of Public Health Sciences, Chulalongkorn University, Thailand. Each authentic sample was dried in hot air oven at 45°C and ground to powders.

Preparation of standard solutions

The standard chlorogenic acid was purchased from Sigma-Aldrich, USA. The stock solution of chlorogenic acid was prepared in 95% ethanol and diluted to obtain the series of standard solution (0.05, 0.15, 0.25, 0.35, and 0.45 mg/ml). These solutions were stored in a refrigerator at 4°C.

Preparation of ethanol extracts of *Chromolaena odorata*

The powders of *C. odorata* (5.0 g) were extracted with ethanol by Soxhlet apparatus. The extract was filtered and

evaporated *in vacuo* to dryness. The yield was recorded. The extract was appropriately dissolved in 95% ethanol to obtain the concentration of 20 mg/ml.

Thin-layer chromatography-densitometry

Three microliters of 10 ethanol extracts and standard solutions were applied on the TLC plate (Silica gel 60 GF254) by Linomat 5 applicator (Camag, Switzerland). The plate was developed in a TLC chamber that consisted of ethyl acetate:water:formic acid (17:3:2) as mobile phase. The plate was scanned under wavelength at 330 nm using TLC scanner 3 (Camag, Switzerland) with winCATS software (Camag, Switzerland). Chlorogenic acid was quantitated by peak area. The test was done in triplicate.

Thin-layer chromatography image analysis by ImageJ software

TLC plate was photographed under ultraviolet (UV) light at 254 nm by a digital camera and saved as tiff files. Chromatographic peak and peak area was obtained using ImageJ free software (Department of Health and Human Services, National Institutes of Health (NIH) in the United State). The test was done in triplicate.

Method validation

TLC quantitation of chlorogenic acid in *C. odorata* leaves was validated. Calibration range, accuracy, repeatability, intermediate precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness were performed according to the International Conference on Harmonisation guideline.^[9]

Data analysis

The chlorogenic acid contents between TLC image analysis and TLC-densitometry were compared by paired *t*-test statistical analysis.

Extraction of essential oils

The fresh leaves of *C. odorata* were cut into small pieces and coarsely crushed. The essential oil was extracted by hydro-distillation with Clevenger apparatus.^[10]

Gas chromatography/mass spectrometry analysis

The analysis was performed using a Finnigan trace GC ultra with DSQ Quadrupole detector. BPX5 fuse silica column (30 m × 0.25 mm, 0.25 μ m film thicknesses) was used as stationary phase. The oven temperature started from 60°C to 240°C with a constant rate of 3°C/min. The carrier gas was helium with the flow rate of 1 ml/min. One microliter of the oil (1:100 in high-performance liquid chromatography grade methanol) was injected by Finnigan Autoinjector AI3000 with a split ratio of 100:1. MS was performed by EI positive mode at 70 electron volts. The chemical constituents were identified by matching mass spectra and retention time indices with Adams Essential Oils Mass Spectral library and

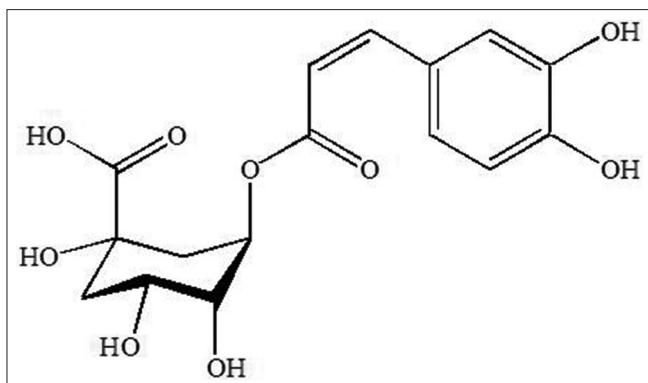


Figure 1: Structure of chlorogenic acid

NIST 05 Mass Spectral library. Peak area was shown in percentage.

1,1-diphenyl-2-picryl hydrazyl radical scavenging assay

One hundred microliters of various concentrations of the extract, standard chlorogenic acid, and positive controls (butylated hydroxytoluene [BHT] and quercetin) in ethanol were added to 100 μ l of DPPH (120 μ m in ethanol) in 96 well microplate.^[11] The plate was incubated at room temperature for 30 min in the dark. The absorbance was measured at 517 nm. Each sample was done in triplicate. Percent scavenging activity was calculated:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance control} - \text{Absorbance sample}]/\text{Absorbance control}}{\text{Absorbance control}} \times 100.$$

β -carotene bleaching assay

This assay was performed in 96 well plate. One milliliter of β -carotene solution (0.2 mg/ml in chloroform) was added with 20 μ l of linoleic acid and 200 μ l of Tween 20 in 96 well microplate.^[12] Chloroform was removed at 40°C under vacuum. Ultra-pure water (50 ml) was added and shaken to form an emulsion. Aliquots (200 μ l) of the emulsion were transferred into the 96 well plates containing 10 μ l of the various concentrations of extract, standard chlorogenic acid, or positive controls (BHT and quercetin) and heated at 50°C. Absorbance at wavelength of 470 nm was recorded at 30 min intervals for 120 min. Each sample was performed in triplicate. The antioxidant activity was evaluated.

$$\% \text{ Antioxidant activity} = (1 - [A_0 - A_{120}]/[C_0 - C_{120}]) \times 100$$

Where A_0, A_{120} : The absorbance values measured at 0 time and end time of sample
 C_0, C_{120} : The absorbance values measured at 0 time and end time of control.

RESULTS

Quantitative analysis of chlorogenic acid

The yield of ethanolic extract of *C. odorata* leaves was 27.46 ± 2.21 g/100 g by dry weight. The quantitative analysis of chlorogenic acid in the leaf extracts was performed by TLC-densitometry and TLC image analysis using ethylacetate:water:formic acid (17:3:2) as mobile phase. TLC chromatogram under UV 254 is shown in Figure 2. TLC densitogram scanned in the range of 200–700 nm is shown in Figure 3. The chlorogenic acid content of *C. odorata* leaves determined by TLC-densitometry and TLC image analysis were 7.39 ± 0.40 and 7.31 ± 0.77 g/100 g of dry leaves, respectively. The chlorogenic acid contents by two methods were not significantly different ($P > 0.05$) using paired *t*-test.

Method validation

The method validation consisted of the specificity, accuracy, precision, LOD, LOQ, and robustness. The

specificity was confirmed by comparing UV spectrum of the peak in standard chlorogenic acid and all samples. The result showed the maximum absorbance at a wavelength of 330 nm [Figure 2]. The validity of TLC densitometry and TLC image analysis is presented in Table 1. The polynomial calibration curves ranged from 0.15 to 1.35 μ g/spot [Figures 4 and 5]. The recovery was determined to evaluate the accuracy by spiking known three concentrations of chlorogenic acid in a sample. The recovery values of both methods were between 84.69% and 103.99%. The repeatability and the intermediate precision were determined in the same day and in three

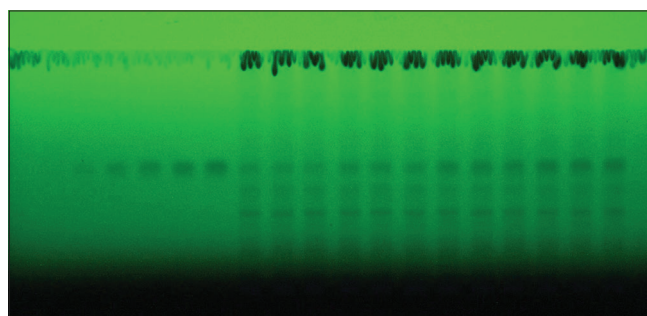


Figure 2: The thin-layer chromatography plate under ultraviolet 254 nm; standard chlorogenic acid (track 1–5), and *Chromolaena odorata* leaves extracts from 10 different locations

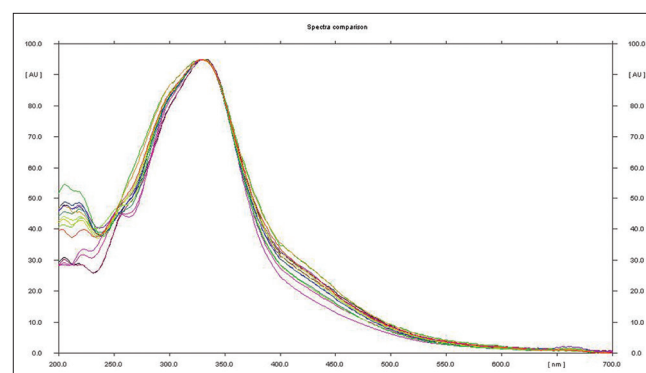


Figure 3: The absorption spectra of chlorogenic acid in standard and sample bands

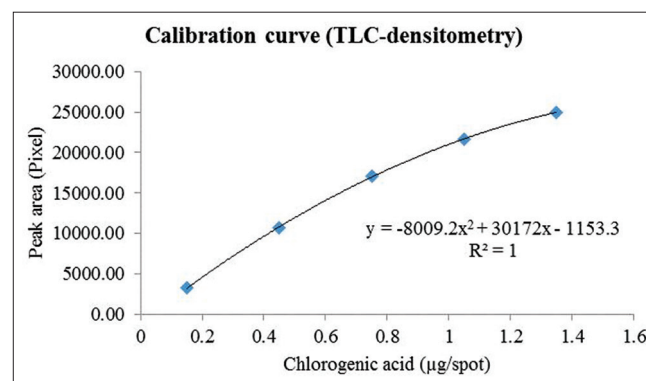


Figure 4: The calibration curve of chlorogenic acid in *Chromolaena odorata* by thin-layer chromatography-densitometry

different days. The repeatability and the intermediate precision of both methods were <10% relative standard deviation (RSD). The LOD and LOQ of TLC-densitometry and TLC image analysis were calculated by the residual standard deviation of a regression line and were found to be 0.002 and 0.007 µg/spot, respectively. The robustness studied by changing the mobile phase ratio showed the values of 0.99% RSD for TLC-densitometry and 2.62% RSD for TLC image analysis.

Gas chromatography mass spectrometry analysis

The essential oils of *C. odorata* leaves were analyzed by GC/MS and at least 20 compounds were detected as shown in Table 2. Pregeijerene, dauca-5, 8-diene,

α-pinene, (E)-caryophyllene, and β-pinene were found as major components of the essential oil. Their quantities were 40.60%, 16.75%, 9.67%, 6.11%, and 5.37%, respectively.

1,1-diphenyl-2-picryl hydrazyl radical scavenging assay

The results demonstrated that the ethanolic extract of *C. odorata*, chlorogenic acid, BHT, and quercetin showed the radical scavenging activity with IC₅₀ of 72.23, 10.59, 32.55, and 3.82 µg/ml, respectively [Figure 6].

β-Carotene bleaching assay

C. odorata ethanolic extract at 1 mg/ml showed 35.61% antioxidant activity compared to 90.88 and 87.18% of BHT and quercetin at the same concentration, respectively. The antioxidant activities of these extract and positive controls demonstrated the dose-response relationship. On the contrary, chlorogenic acid expressed a reciprocal relationship with 20.8–0.3% antioxidant activities at the concentrations of 0.125–1 mg/ml [Figure 7].

Table 1: Method validity of thin-layer chromatography-densitometry and thin-layer chromatography image analysis of chlorogenic acid content in *Chromolaena odorata* leaves

Parameter	TLC-densitometry	TLC image analysis
Accuracy: Percentage of recovery (%)	85.94-94.13	84.69-103.99
Precision: Repeatability (%RSD)	0.55-1.34	0.41-5.55
Precision: Intermediate precision (%RSD)	2.00-3.25	1.05-9.02
Limit of detection (µg/spot)	0.002	0.002
Limit of quantitation (µg/spot)	0.007	0.007
Robustness (%RSD)	0.99	2.62

TLC: Thin-layer chromatography, RSD: Relative standard deviation

Table 2: The chemical constituents of *Chromolaena odorata* essential oil

Retention time (min)	Compound name	Area (%)	Kovat's index
6.67	α-pinene	9.67	939
7.87	Sabinene	1.10	975
7.99	β-pinene	5.37	979
8.38	Myrcene	1.54	990
9.77	Sylvestrene	0.77	1030
10.45	(E)-β-ocimene	2.20	1050
14.26	Geijerene	1.90	1143
20.35	Cogeijerene	0.80	1285
20.60	Pregeijerene	40.60	1287
25.96	(E)-caryophyllene	6.11	1419
27.33	α-humulene	1.77	1454
28.44	Dauca-5,8-diene	16.75	1472
28.98	γ-muurolene	0.86	1479
29.04	Bicyclogermacrene	1.65	1500
29.37	α-bulnesene	0.67	1509
29.77	γ-cadinene	0.85	1513
30.08	Δ-cadinene	3.21	1523
31.07	Elemol	0.77	1549
34.55	α-muurolol	1.25	1646
35.01	α-cadinol	2.19	1654

DISCUSSION

TLC-densitometry is a quantitative TLC method with high reliability due to selective optical characteristics of tested compound. Wavelength specific light absorption or light emission and its intensity are related to the amount of the compound. TLC-densitometric analysis of chlorogenic acid in *C. odorata* leaves was developed and found to be valid. Furthermore, the quantitation of TLC chromatogram can be performed using image analysis which is inexpensive and convenient technique. The image analysis is processed by acquiring a digital image of TLC chromatogram using simple digital camera computing the pixel intensity, converting to corresponding peak, and calculating peak area using ImageJ free software.^[13] The quantitative analysis of chlorogenic acid in *C. odorata* leaves by TLC image analysis was also developed and its validity was demonstrated. The contents of chlorogenic acid in *C. odorata* leaves by two methods were not statistically significant different. Therefore, TLC image analysis could be used as an alternative method instead of TLC-densitometry.

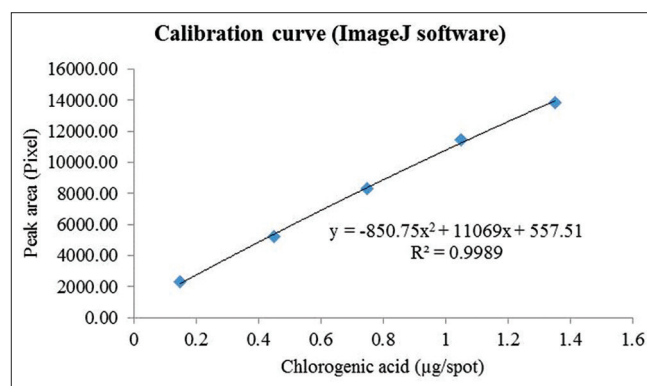


Figure 5: The calibration curve of chlorogenic acid in *Chromolaena odorata* by thin-layer chromatography image analysis

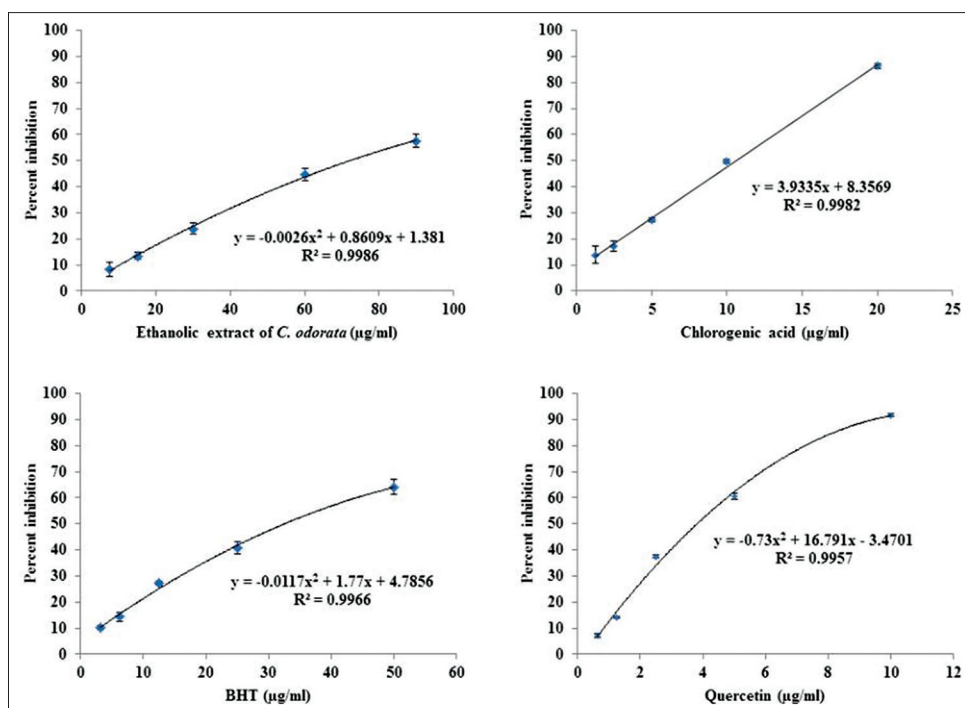


Figure 6: 1,1-diphenyl-2-picryl hydrazyl scavenging activities of ethanolic extract of *Chromolaena odorata* leaves, chlorogenic acid, butylated hydroxytoluene, and quercetin

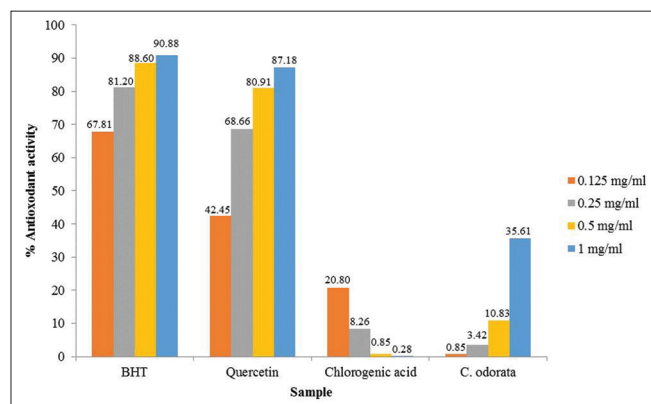


Figure 7: The antioxidant activity of *Chromolaena odorata*, butylated hydroxytoluene, quercetin, and chlorogenic acid by β -carotene bleaching assay

For GC/MS analysis, the chemical constituents of *C. odorata* essential oil in this study were in accordance with the previous study in Thailand which reported the constituents of the essential oil from aerial parts as pregeijerene, α -pinene, germacrene D, β -caryophyllene, and β -pinene.^[14] Likewise, the previous study in Nigeria showed that the main constituents in the stem essential oil were α -pinene, β -pinene, caryophyllene, bicyclo (7.2.0) undec-4-ene, and germacrene D.^[15] However, the chemical compositions of samples from different parts and localities expressed the individual amount of components.^[16]

DPPH is a stable free radical that is used to evaluate the ability of compounds to act as free radical scavengers or hydrogen

donors that cause decoloration of DPPH and to estimate the antioxidant activity.^[17] In this study, the ethanolic extract of *C. odorata* could scavenge DPPH free radical with IC_{50} of 72.23 μ g/ml. The scavenging activity of *C. odorata* was less potent than quercetin, chlorogenic acid, and BHT with IC_{50} of 3.82, 10.59, and 32.55 μ g/ml, respectively. The previous study also reported DPPH radical scavenging activity of *C. odorata* and showed nearly IC_{50} value.^[18]

In β -carotene bleaching assay, linoleic acid produces hydrogen peroxide. The bleaching of yellow color of β -carotene is due to peroxide free radicals. The rate of β -carotene bleaching can be delayed with the presence of antioxidants. In this study, the ethanolic extract of *C. odorata* at a concentration of 1 mg/ml showed antioxidant activity of only 35.61% compared to BHT and quercetin, which showed the antioxidant activity of 90.88% and 87.18%, respectively. The bleaching inhibitory activities of *C. odorata*, BHT, and quercetin were concentration-dependent, whereas chlorogenic acid expressed reciprocal relationship. In the previous study, phenolic acids presented in foods were reported to act as pro-oxidants such as caffeic, chlorogenic, and ferulic acids.^[19,20] Pro-oxidants are chemicals that induce oxidative stress, either by generating reactive oxygen species or inhibiting antioxidant systems.^[21]

CONCLUSION

The present study indicated that pregeijerene, dauca-5,8-diene, (E)-caryophyllene, β -pinene, and α -pinene

were found as major components of the essential oil from *C. odorata* leaves in Thailand. The chlorogenic acid content of *C. odorata* leaves was established and TLC image analysis could be used for chlorogenic acid quantitation. Furthermore, the ethanolic extract of *C. odorata* leaves showed less potent of both free radical scavenging activity and inhibitory activity of β -carotene bleaching compared to the standard BHT.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Liogier HA. Descriptive Flora of Puerto Rico and Adjacent Islands. Vol. 5. San Juan: University of Puerto Rico Press; 1997. p. 436.
- Howard RA. Flora of the Lesser Antilles, Leeward and Windward Islands. Vol. 6. Jamaica Plain: Arnold Arboretum, Harvard University; 1989. p. 658.
- Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST. Phenolic Compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. *Biol Pharm Bull* 2001;24:1373-9.
- Cáceres A, Menéndez H, Méndez E, Cohobón E, Samayoa BE, Jauregui E, *et al.* Antigonorrhoeal activity of plants used in Guatemala for the treatment of sexually transmitted diseases. *J Ethnopharmacol* 1995;48:85-8.
- Triratana T, Suwannuraks R, Naengchomnong W. Effect of *Eupatorium odoratum* on blood coagulation. *J Med Assoc Thai* 1991;74:283-7.
- Chakraborty AK, Rambhade S, Patil UK. *Chromolaena odorata* (L.): An overview. *J Pharm Res* 2011;4:573-6.
- Thang PT, Patrick S, Teik LS, Yung CS. Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage. *Burns* 2001;27:319-27.
- Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T, *et al.* *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *Int J Pharm* 2011;403:136-8.
- ICH Harmonized Tripartite Guideline. "Validation of Analytical Procedures: Text and Methodology, Q2 (R1)," in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Geneva; 2005.
- World Health Organization. "Determination of volatile oils," in quality control method for medicinal plant materials. Vol. 10. Geneva: WHO; 2011. p. 37-40.
- Brand WW, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebenson Wiss Technol* 1995;28:25-30.
- Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK. Evaluation of antioxidant activities and antimutagenicity of turmeric oil: A byproduct from curcumin production. *Z Naturforsch C* 2002;57:828-35.
- Tie-xin T, Hong W. An image analysis system for thin-layer chromatography quantification and its validation. *J Chromatogr Sci* 2008;46:560-4.
- Pisutthanan N, Liawruangrath B, Liawruangrath S, Baramée A, Apisariyakul A, Korth J, *et al.* Constituents of the essential oil from aerial parts of *Chromolaena odorata* from Thailand. *Nat Prod Res* 2006;20:636-40.
- Olusegun OS, Musa M. Composition of stem essential oil of *Chromolaena odorata* (L.) from Nigeria. *Int J Herb Med* 2014;2:65-7.
- Tonzibo ZF, Wognin E, Chalchat JC, Guessan YT. Chemical investigation of *Chromolaena odorata* L. King Robinson from Ivory Coast. *J Essent Oil Bearing Plants* 2007;10:94-100.
- Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol* 2011;48:412-22.
- Srinivasa Rao K, Chaudhury PK, Pradhan A. Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. *Food Chem Toxicol* 2010;48:729-32.
- Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J Agric Food Chem* 2000;48:3597-604.
- Sakihama Y, Cohen MF, Grace SC, Yamasaki H. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. *Toxicology* 2002;177:67-80.
- Puglia CD, Powell SR. Inhibition of cellular antioxidants: A possible mechanism of toxic cell injury. *Environ Health Perspect* 1984;57:307-11.