Chromolaena odorata Linn leaf extract – Geothermal versus nongeothermal: Phytochemical, antioxidant, and cytotoxicity screenings

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Abstract

Chromolaena odorata Linn, a popular yet underutilized ethnomedicinal plant, is hypothesized to possess higher bioactive phytoconstituents when it grows in geothermal areas. In this study, the comparison of ethanolic extract from geothermal and nongeothermal *C. odorata* leaves was carried out based on the phytochemical profile, antioxidant activity, and cytotoxicity. The leaf extracts were produced from a maceration using ethanol 96%, where the products were identified using reagents and gas chromatography–mass spectrometry (GC‑MS). Antioxidant activities of both samples were measured based on their 2,2‑diphenyl‑1‑picrylhydrazyl (DPPH) scavenging activities. Cytotoxicity was determined by brine shrimp lethality test using *Artemia salina*. Phenols were found to be more abundant in geothermal sample based on the qualitative screening and GC‑MS analysis (i.e. higher relative abundance of phytol – 3.97%). DPPH antioxidant was higher in geothermal sample than in nongeothermal sample (median inhibitory concentration =13.04 ± 3.35 mg/L vs. 41.09 ± 4.13 mg/L, respectively). Geothermal sample was noncytotoxic (median lethal concentration [LC_{50}] =2139.30 mg/L), whereas the nongeothermal sample had low cytotoxicity (LC_{50} = 491.48 mg/L). Taken altogether, geothermal *C. odorata* leaves contain higher bioactive compounds with potent antioxidant activities.

Key words: 2,2‑diphenyl‑1‑picrylhydrazyl, antioxidant, cytotoxicity, gas chromatography–mass spectrometry, siam weed

INTRODUCTION

Chromolaena odorata Linn is a medicinal plant known as *Seurapoh* by those living in Aceh Province, Indonesia. As a part of traditional concoctions, the ethnomedicinal plant was popular for its usage in managing the common cold, fever, and stomachache.[1] It is also common, especially among Vietnamese communities, to use the crushed leaves of *C. odorata* to treat open wounds, burn wounds, skin infection, and rashes.[2] Among researchers, the plant has been suggested to possess potential therapeutic properties, including analgesic, antipyretic, antimicrobial, diuretic, anti‑inflammatory, antioxidant, and antiulcer.[3,4] In Aceh Province, Indonesia, the plant is found to be abundant and often underutilized. This plant is considered weed that massively grows in geothermal areas, becoming one of its vegetative components.[5,6]

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It is worth mentioning that geothermal activities yielded extremely high temperatures and different mineral compositions to the surrounding environment. Such conditions have been reported to affect the biosynthesis of secondary metabolites of plants.[7,8] A study revealed the correlation between the soil temperature and components of terpenes and phenolic acids in plants grow in geothermal areas.[9] Many researchers have stipulated on higher efficacy of geothermal plants as therapeutic agents, but only a little evidence supports this claim. Moreover, not all plants inhabiting geothermal areas are affected by extreme conditions.[9] Previous studies only reported the phytochemical and bioactivity profiles of *C. odorata* but were unable to provide a direct comparison between those collected from geothermal and nongeothermal areas. Therefore, the objective of this study was to decipher the differences of the sample collected from the two locations in terms of their phytoconstituents and *in vitro* bioactivities (represented by antioxidant activity and cytotoxicity).

MATERIALS AND METHODS

Materials

In this study, ethanol 96%, methanol 99.8%, dimethyl sulfoxide, ascorbic acid (Vitamin C), carboxymethyl cellulose, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) that were priorly purchased from Merck (Selangor, Malaysia) in the analytical grade were used without any pretreatment. The leaf samples of *C. odorata* Linn were obtained from geothermal (Ie Suum) and nongeothermal (Lhoknga) locations. The characteristics of these two locations as geothermal and nongeothermal locations have been determined previously.[5,10] The time of the sample collection was in June 2021 or during the dry season. The taxonomic appraisal was performed at the Biology Laboratory of Universitas Syiah Kuala, Banda Aceh, Indonesia, with voucher number: 179/UN11.1.8.4/TA.00.03 / 2023. The geographical coordinates for Ie Suum are 5°321′51′′ N and 95°32′53′′ S, whereas Lhoknga – 5°29′01′′ N and 95°14′35′′ S. The map indicating the locations where the samples were obtained as presented in Figure 1.

Sample extraction

On collection, samples were washed-clean using distilled water with a flowing current and then air-dried for 7 days. The dried leaves (1.5 kg) were crushed to produce the simplicial powder. Each of the geothermal and nongeothermal samples was macerated with ethanol 96% for 72 h, and the filtrate was collected after completion. A vacuum rotary evaporator was employed to concentrate each extract.

Identification of phytoconstituents

Qualitative screening of the major phytoconstituents was carried out using Liebermann–Burchard reagent (steroids), $FeCl_3$ (phenolics), Mg powder (flavonoids), a combination of gelatin and sulfuric acid (tannins), Mayer reagent (alkaloids), Dragendorff's reagent (alkaloids), and Wagner reagent (alkaloids). As for saponins, its presence was determined by the formation of stable foam upon a shaking in distilled water. Further determination of the phytoconstituents was carried out on gas chromatography-mass spectrometry (GC-MS) QP2020 NX Shimadzu (Kyoto, Japan). To determine the total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC), calibration curves constructed from 100 to 200 mg/L of gallic acid, quercetin, and tannic acid were used, respectively. The TPC, TFC, and TTC of the extract were presented as gallic acid equivalent (GAE), quercetin equivalent (QE), and tannic acid equivalent (TAE) per dry extract, respectively.

2,2-diphenyl-1-picrylhydrazyl inhibition assay

First, DPPH solution 0.4 mM was prepared from its powder form (7.9 mg) that was dissolved in a volumetric flask (50 mL) using methanol. The extract sample was varied in concentrations with a range of 20–100 mg/L, where the solution of each concentration was mixed with DPPH 0.4 mM (1 mL) and added with methanol until the total volume became 5 mL. Homogenization of the mixture was carried out on a vortex mixer before being incubated for 30 min at 37°C. The absorbance was measured afterward on a UV-Vis spectrophotometer at a wavelength of 517 nm. In this analysis, Vitamin C was used as the positive control. The results were expressed as inhibition percentage (%), and median inhibitory concentration (IC_{50}) was measured for each extract.

Brine shrimp lethality test

The brine shrimp lethality test (BSLT) was used in this study to investigate the cytotoxicity profile of the *C. odorata* leaf extracts. The protocols used have been reported previously.[11]

RESULTS AND DISCUSSION

Phytochemical profile

Results from the qualitative analysis of the major groups of the phytoconstituents in *C. odorata* leaf extracts from geothermal or nongeothermal areas have been presented in Figure 2. The presence of phenolics is more pronounced in geothermal samples, but nongeothermal seems to have a more portion of flavonoids. Saponins and steroids are more intense in geothermal sample, whereas terpenoids in nongeothermal samples. Tannins and alkaloids are observable in both geothermal and nongeothermal samples with similar apparent color intensity. Such differences in the phytochemical compositions explain the results from our previous preliminary study, where the infrared spectra of both samples possessed different characteristics.[10] The presence of bioactive compounds in geothermal plants has been witnessed in several previously published reports,

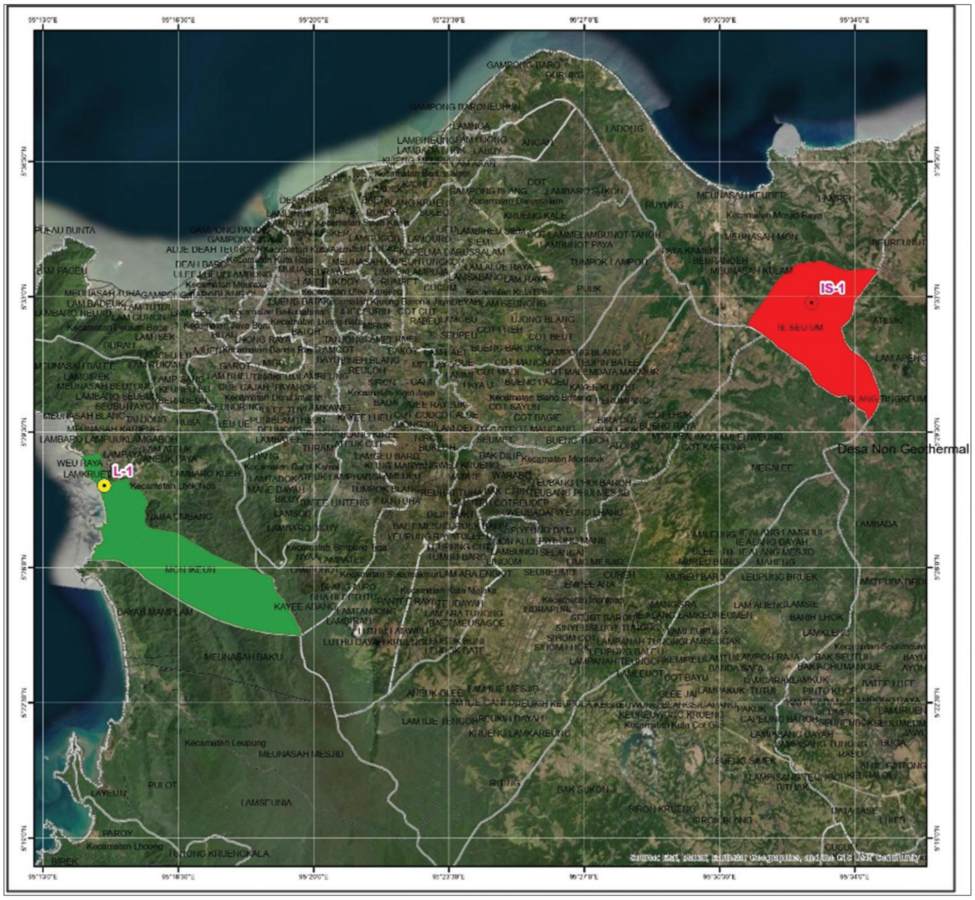

Figure 1: Sampling locations of *Chromolaena odorata* leaves in geothermal area Ie Suum (IS-1) and non-geothermal area Lhoknga (L-1) indicated by red and green colors, respectively

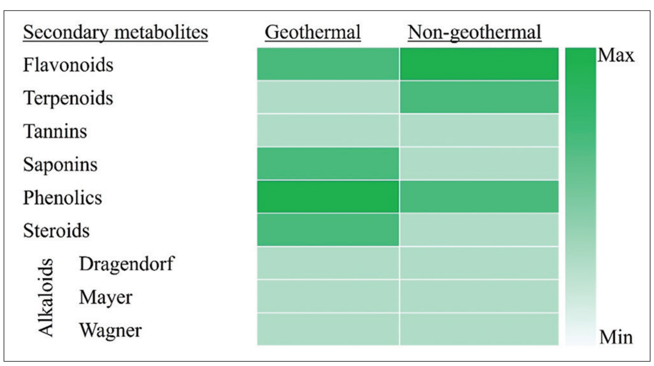

Figure 2: Secondary metabolites of *Chromolaena odorata* leaf extracts (geothermal and nongeothermal) detected through qualitative phytochemical test. Color intensity is indicative to the quantity of the phytocompounds

including those investigating *Calotropis gigantea*[12,13] and *Vitex pinnata*.[14,15]

Phytochemical contents identified in geothermal or nongeothermal *C. odorata* extract by the GC-MS have been presented [Table 1]. Squalene (or all-trans squalene) was observable in both geothermal and nongeothermal samples in relatively high abundance (6.94% and 2.67%). Squalene could act as antioxidant and anti-inflammatory agents, exerting medicinal benefits to organ damage, imbalance oxidative stress, and dysregulation of the immune response.[16-18] Hexadecanoic acid and its derivative hexadecanoic acid methyl ester were predominantly found in both extracts. Hexadecanoic acid (commonly known as palmitic acid) has the activity to attenuate inflammatory factors.[19,20] Phytol was observed in geothermal and nongeothermal samples with relative abundance of 2.58% and 1.36%, respectively. The chlorophyll component, phytol, is also among the anti-inflammatory compounds identified in the extracts.[21,22]

Phytocompounds such as 9,17-octadecadienal and cyclopropaneoctanal, 2-octyl were only found and present with high relative-abundance in geothermal sample. As in nongeothermal sample, 9,12-octadecadienoic acid (5.98%), 5-hydroxy-4',7-dimethoxyflavanone (4.99%), and (IS,6R,9S)-5,5,9,10-tetramethyltricyclo (7,3,0,0[1,6]) dodec-10 (11)-en (4.41%) were only exclusively found therein. Interestingly, (+)-longiflene or commonly known as longifolene was found in nongeothermal sample (1.10%). Longifolene has been reported for its potent activity against fungi and tumor cells.[23,24]

Table 1: Phytochemical profiles of ethanolic extract from *Chromolaena odorata* leaves collected from geothermal and nongeothermal areas

Geothermal			Nongeothermal		
Compound	**Simi-larity (%)**	**Area (%)**	**Compound**	**Simi-larity (%)**	**Area (%)**
Squalene	99	6.94	Hexadecanoic acid	99	5.58
Hexadecanoic acid	99	6.18	All-trans-squalene	99	2.67
Hexadecanoic acid methyl ester	99	1.89	Hexadecanoic acid methyl ester	99	1.53
9,17-octadecadienal	98	5.84	9,12-octadecadienoic acid	97	5.98
Cyclopropaneoctanal, 2-octyl	95	3.27	5-hydroxy-4',7-dimethoxyflavanone	95	4.99
Phytol	94	1.39	Octadecanal	95	1.15
(R)-(-)-14-methyl-8-hexadecyn-1-ol	93	1.71	Phytol	91	1.36
2-aminoethanethiol hydrogen sulfate (ester)	91	1.22	4A-methyldecahydro-2H-benzo (A) cyclohepten-2-one	86	1.03
1-hexadecyene	90	1.02	(+)−longiflene	83	1.10
Phytol	87	2.58	(IS,6R,9S)-5,5,9,10-tetramethyltricyclo (7,3,0,0[1,6]) dodec-10 (11)-en	80	4.41
Noephytadiene	83	1.13			

The phytochemical profile was determined by GC-MS and comparing the spectral data with the compound library. GC-MS: Gas chromatography-mass spectrometry

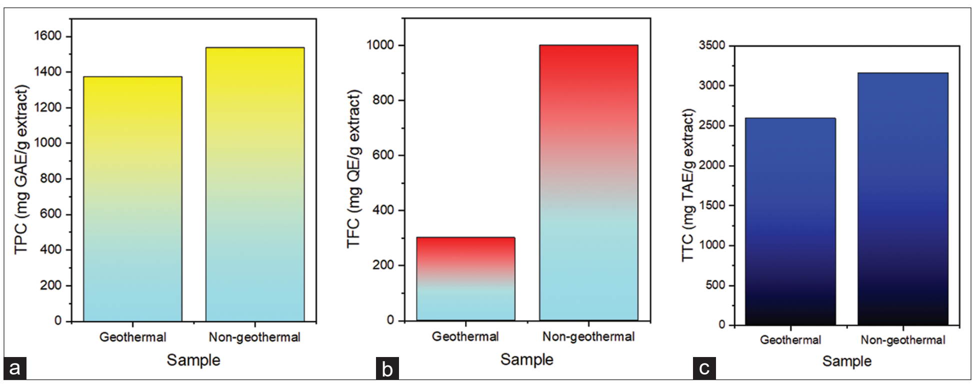

Figure 3: TPC (a), TFC (b), and TTC (c) of the ethanolic extract of *Chromolaena odorata* leaves collected from geothermal and nongeothermal locations. GAE: Gallic acid equivalent, QE: Quercetin equivalent, TAE: Tannic acid equivalent, TFC: Total flavonoid contents, TPC: Total phenolic contents, TTC: Total tannin contents

Total phenolic, flavonoid, and tannin content

The TPC, TFC, and TTC of each extract are presented in Figure 3. The TPC was found to be 1373.75 mg GAE/g extract in the geothermal sample and 1536.25 mg GAE/g extract – in nongeothermal sample. A significant contrast between the two samples was observed in the TFC, where the quantity reached 301.09 and 1000 mg QE/g extract for geothermal and nongeothermal, respectively. As for TTC, 1373.75 mg TAE/g extract was found in geothermal and 1536.25 – nongeothermal. In conclusion, the portions of TPC, TFC, and TTC are quantitatively more higher in nongeothermal extract. These findings are not in line with the qualitative screening presented previously, which could be attributed to the molecular structures (such as the length of the aliphatic carbon chain).

In comparison with other previously published studies, the TPC and TFC of *C. odorata* observed in this study were tremendously higher.[25] Differences in sampling locations, strains, and extraction methods could be the reason of this disagreement. Extraction using hydroalcohol has been suggested to be efficient in yielding phenolic compounds including flavonoids and tannins.[26]

2,2-diphenyl-1-picrylhydrazyl antioxidant activity

DPPH inhibition and IC_{50} values of geothermal and nongeothermal *C. odorata* leaf extracts have been presented in Figure 4. Interestingly, at concentration as low as 20 mg/L, the geothermal extract exerted antioxidant activity against free radical DPPH that was similar to that of Vitamin C (53.76 and 55.52%, respectively). The IC_{50} yielded by the geothermal sample was 13.04 ± 3.35 mg/L, whereas nongeothermal sample was 41.09 ± 4.13 mg/L. The antioxidant activity of geothermal sample was significantly higher at $P < 0.01$ as compared with that of nongeothermal sample. As a comparison to the positive control, Vitamin C had IC_{50} of 3.66 ± 2.17 mg/L against free radical DPPH. It is worth mentioning that the higher antioxidant activities in the geothermal sample were resulted from the presence

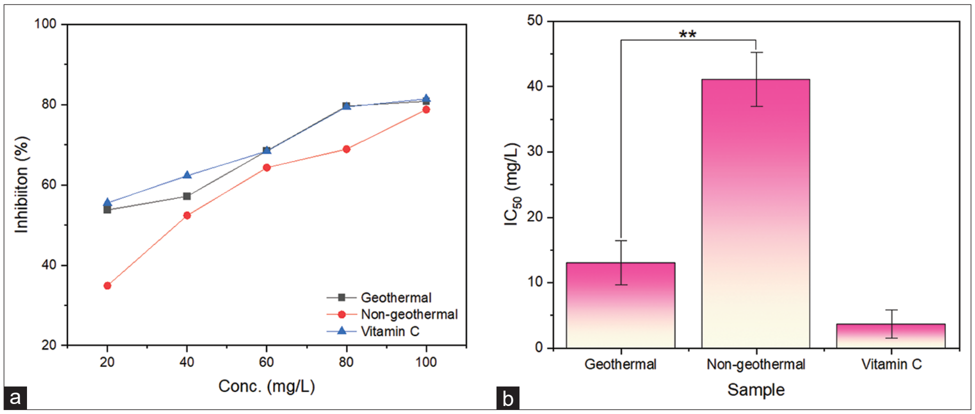

Figure 4: 2,2-diphenyl-1-picrylhydrazyl inhibition (a) and median inhibitory concentration (b) of *Chromolaena odorata* leaf extracts collected from geothermal and nongeothermal areas. Statistically significant at $^{**}P < 0.01$ based on independent *t*-test

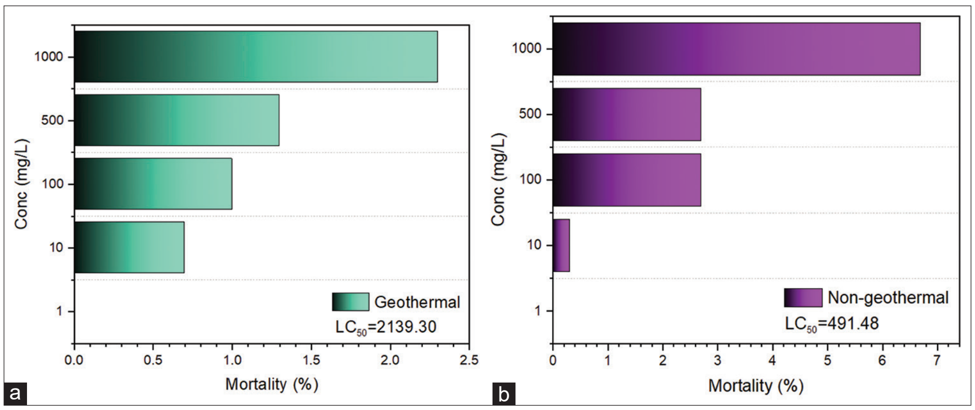

Figure 5: BSLT results for *Chromolaena odorata* leaf extracts collected from geothermal (a) and non-geothermal (b) areas

of compounds with conjugated unsaturated carbon which could neutralize free radicals through electron donor, which is further increased by the hydroxyl and carbonic acid functional groups.

Cytotoxicity profile

Results from the BSLT for geothermal and nongeothermal samples have been presented [Figure 5]. Based on this analysis, the geothermal sample is less cytotoxic as compared to its nongeothermal counterpart, with median lethal concentration (LC_{50}s) of 2139.30 mg/L and 491.48 mg/L, respectively. It suggests that geothermal *C. odorata* leaf extract ideally would not cause cytotoxic side effects. However, it is worth noting that the geothermal samples are likely to be contaminated by cadmium, as reported previously.[6]

As for the nongeothermal sample, it has a potential as antiproliferative agent owing to its high cytotoxicity. A previous study suggests the correlation between high LC_{50} in BSLT with anti-leukemia activity.[11] In line with the finding from GC-MS analysis, the nongeothermal sample exclusively possessed observable contents of (+)-longifolene – an antitumor compound.[23,24]

CONCLUSION

Phytoconstituents contained in the ethanolic extracts of *C. odorata* leaves collected from geothermal and nongeothermal areas are different which is probably associated with the effect from the geothermal activities. The presence of flavonoids was indicated to be higher in the nongeothermal sample as observed by apparent color intensity in the qualitative screening. This was further confirmed by higher TFC value in the quantitative analysis using quercetin. However, there was a disagreement when it comes to TPC, which could be attributed to the component of the phenolic group itself. Stronger antioxidant activity, based on DPPH inhibition assay, was observed in geothermal sample. Such potent antioxidant activity might be attributed to the presence of squalene, phytol, and palmitic acid observed in the GC-MS analysis. Further, geothermal extract was found to be noncytotoxic. Yet, some concerns should be paid to the nongeothermal sample as it showed cytotoxicity against *Artemia salina*, though the activity was low.

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Conflicts of interest

There are no conflicts of interest.

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