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ORIGINAL RESEARCH ARTICLE

Ecotoxicological insight of phytochemicals, toxicological informatics, and heavy metal concentration in *Tridax procumbens* L. in geothermal areas

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ABSTRACT

BACKGROUND AND OBJECTIVES: *Tridax procumbens* L. is a plant that grows abundantly in the Ie-Seu'um geothermal area in Aceh Province, Indonesia. The objective of this study is to determine metabolite compounds from *Tridax procumbens* plants in a geothermal area using qualitative and quantitative analyses. In addition, the contents of six heavy metals in plants and their toxicology were assessed using an in silico approach.

METHODS: The ethanolic extract of *Tridax procumbens* was analyzed qualitatively using reagents to determine the contents of secondary metabolites such as flavonoids, alkaloids, tannins, steroids, triterpenoids, and saponins. In addition, quantitative analysis was conducted using gas chromatography–mass spectroscopy to obtain the chromatograms and mass spectra of the metabolite compounds of the ethanolic extract of *Tridax procumbens*, which were used in computational toxicology analysis using a simplified molecular input system in a predictor server. Atomic absorption spectrometry was conducted to confirm the contents of six heavy metals harmful to medicinal plants.

FINDINGS: The results showed that *Tridax procumbens* from the *Ie-Seu'um* geothermal area, Aceh, has secondary metabolites such as flavonoids, saponins, steroids, and tannins, with phytol from diterpenoid group having the highest content (32.72 percent). Toxicological analysis showed that the compounds in the ethanolic extract of *Tridax procumbens* were nontoxic or inactive in five toxicity parameters. The other results of the heavy metal analysis showed the dominance of chromium among the other six metals tested (copper, not detected; cadmium, 0.91 ± 0.03 milligram per kilogram; zinc, 3.50 ± 0.03 milligram per kilogram; iron, 4.65 ± 0.02 milligram per kilogram; lead, 6.42 ± 0.05 milligram per kilogram; and chromium, 13.81 ± 0.07 milligram per kilogram.

CONCLUSION: This study highlights the unique secondary metabolite composition of *Tridax procumbens* under such extreme conditions and underscores the potential implications of heavy metal accumulation in plants in geothermal areas.

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INTRODUCTION

Heavy metal contamination is a potential problem in geothermal regions. Effluents containing heavy metals from geothermal energy sources are rare and difficult to interpret because of interference from geysers and other naturally existing thermal phenomena (Sabadell and Aaxtmann, 1975; Sabilillah *et al.*, 2023). Geothermal fluids are prone to having elevated chemical concentrations, which can have a negative effect on nearby water sources. One of the hydrothermal areas in Aceh Province, Indonesia, is *Ie-Seu'um* (local name: hot air), which is reported to have the highest arsenic-containing hot springs with levels of 166.73 ± 0.0081 microgram per liter (µg/L), (Irnawati *et al.*, 2021). *Ie-Seu'um* is a hot spring which is one of the manifestations of Mount Seulawah Agam with temperatures up to 86.09 degress Celsius (°C), (Idroes *et al.*, 2019). The examination of the chloride–bicarbonate–sulfate (Cl–HCO_3–SO_4) triangle diagram and Piper diagram indicates that the hydrological characteristics and prevailing chemical composition of the fluids in *Ie-Seu'um* are primarily chloride based, with significant amounts of sodium, potassium, and chloride ions (Idroes *et al.*, 2019). The potential for pollution caused by trace heavy metals originating from geothermal sources is considerable, but available field measurements are limited. The Seulawah Agam mountain is known as one of the most active volcanoes in Aceh Province, Indonesia. Aceh Province is well known for its many geothermal sites, including *Ie-Seu'um*, *Ie-Jue*, and *Ie-Brouk* in the Aceh Besar District. Surface manifestations of geothermal locations include thermal springs, gases or solfatara, streaming earth, altered rock, and phreatic eruptions. Geological phenomena have the ability to change ambient environmental conditions, such as temperature, moisture levels, and humidity (Idroes *et al.*, 2019). The flora found in these geothermal regions possesses distinct characteristics and synthesizes metabolic chemicals that are associated with various health advantages (Nurasikin *et al.*, 2020). Limited study has been conducted on plants in geothermal areas in Aceh Province. However, the findings of existing studies indicate the promising potential of these plants as candidates for pharmaceutical applications. The investigation of the possibility of utilizing weeds from geothermal zones as complementary-based therapy warrants further exploration. Currently, published research on the exploration of geothermal medicinal plants, particularly plants from the Asteraceae family, is limited. The Asteraceae family, also known as Compositae, is considered to be among the most extensive families of flowering plants, encompassing a remarkable assemblage of over 1600 genera and approximately 25.000 species distributed across various regions of the world (Rolnik and Olas, 2021). Asteraceae has a high biodiversity that spreads across all regions in all conditions and continents, except Antarctica (Funk *et al.*, 2005). Many species of the Asteraceae family have therapeutic properties and have been used in traditional medicine for a long time. Certain members of this family have been cultivated for over three millennia, largely for its nutritional and medicinal utilities (Rolnik and Olas, 2021). One member of this family is the plant *Tridax procumbens* L., described by Linnaeus in 1753, with various pharmaceutical and traditional uses (Ingole *et al*., 2022). *T. procumbens* is native to Mexico and is found southward throughout Central America and the majority of South America, demonstrating its robust capacity for proliferation and dissemination due to its extensive natural occurrence over tropical and subtropical regions worldwide (PPQ, 2018). *T. procumbens* is a perennial plant with light green color, 15–40 centimeter (cm) height, roots that emerge from nodes, stems that emerge from the base of the wood, and ovate to lanceolate hairy leaves that are 4–30 millimeter (mm) long (Powell, 1965). In the *Ie-Seu'um* geothermal area, *T. procumbens* can be seen around hot springs and surrounding areas (Fig. 1). The soil conditions in the geothermal area surrounding the spring of *Ie-Seu'um* exhibit temperatures ranging from approximately 27.16°C to 36.13°C (Irnawati *et al.*, 2021). The findings of the study by Chauhan and Johnson (2008) indicated that *T. procumbens* exhibits optimal seed germination ability in temperature conditions of 35°C/25°C and 30°C/20°C. In addition, the research suggests that the seed germination of *T. procumbens* is significantly enhanced under light conditions compared to dark conditions, with a range of 58 percent (%) to 70% germination observed. This observation implies that *T. procumbens* plants thrive in geothermal environments because of their adaptive traits that enable them to tolerate soil conditions characterized by elevated temperatures.

Fig. 1: *T. procumbens* plants

Medicinal plants are often used over a long period to improve health, but this can lead to the accumulation of harmful heavy metals in the body, which can cause various health problems if not managed properly (Kandić *et al*., 2023; Samimi and Mansouri, 2023). *T. procumbens* plants are used in traditional medicine, but research on its chemical content is limited. Currently, research using computational or *in silico* techniques is growing rapidly. Various research using *in silico* approaches such as quantitative relationship structure analysis (Noviandy *et al.*, 2023) and molecular docking to molecular dynamics (Tallei *et al*., 2020). This currently study we conducted research that included i) qualitative (using reagents) and quantitative (using gas chromatography–mass spectroscopy (GC–MS)) phytochemical analyses of plant constituents, ii) the assessment of the heavy metal (i.e., lead (Pb), copper (Cu), chromium (Cr), cadmium (Cd), iron (Fe), and Zinc (Zn)) concentrations in the ethanolic extract of *T. procumbens*, and (iii) toxicological informatics studies using an *in silico* approach. The objective of the study is to investigate and analyze the chemical composition of *T. procumbens*, a plant species found in the *Ie-Seu'um* geothermal area, Aceh. Specifically, the study aims to achieve the following objectives: confirmation of secondary metabolites, identification of dominant bioactive compounds and their toxicology using an *in silico* approach, and analysis of the heavy metal concentration of the ethanolic extract *T. procumbens*. This study was conducted in Aceh Province, Indonesia, in 2023.

MATERIALS AND METHODS

Plant preparation and extraction

T. procumbens was obtained from the manifestation geothermal of Seulawah Agam *Ie-Seu'um*, which is located at 05°32'50" north (N), 95°32'45" east (E) (Fig. 2). The plant parts that were collected include branches, leaves, and flowers. The voucher for the species was given the number B-2238 in National Research and Innovation Agency, Indonesia. To prepare the extracts, various plant parts were mixed, cleaned, air-dried at room temperature, and then ground into powder. Maceration at a ratio of 1:10 weight: volume (w:v) for three days resulted in the production of an ethanolic extract. Immediately after that, extract was dried using a rotary evaporator (Butchi Rotavapor®, Switzerland), followed by a vacuum filtering process to obtain a dry extract.

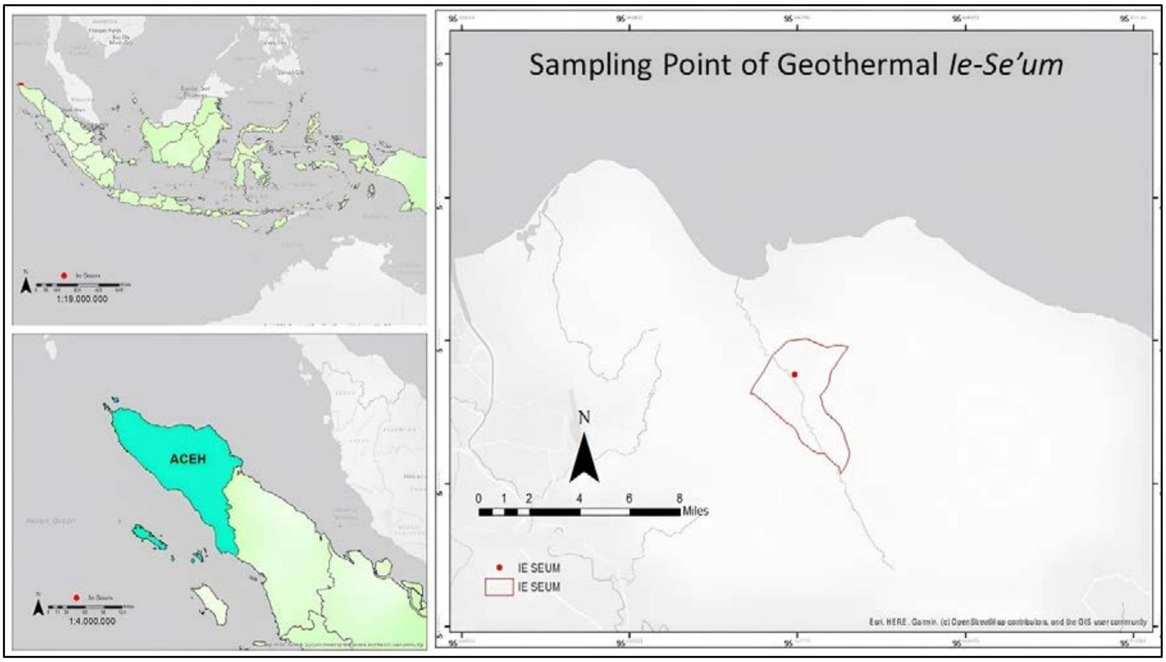


Fig. 2: Geographical location of the study area in *Ie-Seu'um,* Indonesia

Phytochemical analysis

Phytochemical analysis was conducted on six classes of secondary metabolites, namely, flavonoids, tannins, saponins, terpenoids, steroids, and alkaloids. The method used in this study was based on the method used by Harbone (1987), with slight modifications.

Gas chromatography–mass spectroscopy (GC–MS) analysis

GC–MS was conducted using a TRACE 1310 GC and single quadrupole (iSQ) 7000 equipped with a TraceGOLD TG-35MS column- 30 meter (m) × 0.25 millimeter (mm) × 0.25 micrometer (µm); Thermo Fisher Scientific, Inc., United States of America) to examine the ethanolic extracts of *T. procumbens*. The temperature of the injector was kept constant at 250°C, and the ion source temperature was adjusted to 250°C. The programmed temperature of the column was set to gradually increase from 60°C to 280°C at a rate of 10°C/minutes. Helium was used as the carrier gas at a flow rate of 1 µL/min. Mass spectra were acquired using an energy level of 75 electron volt (eV) while scanning a range of 400 to 500 atomic mass units (Amu).

Heavy metal analysis

The samples for heavy metal analysis were prepared by placing plant samples that weighed up to 1.00 ± 0.05 gram (g) in a tube vessel, adding 10 ± 0.1 milliliter (mL) of pure nitric acid (HNO_3) (Merck, Darmstadt, Germany), and then placing the tube vessel in a microwave to destruct the sample. The destruction was carried out at 180°C for 10 minutes and then the analyte was cooled to room temperature. After the destruction step was completed, the analyte was transferred to a 25 mL volumetric flask and diluted with demineralized water. The test sample solution was analyzed using an atomic absorption spectrophotometer (AAS) (PinAAcle 900H PerkinElmer, Waltham, MA, USA). The calibration curves for the metals Pb, Cd, Cu, Cr, Fe, and Zn were established using specific wavelengths of 217.0, 220.8, 324.8, 357.9, 248.1, and 213.9 nanometer (nm), respectively. These wavelengths were employed in accordance with the guidelines from Association of Official Analytical Collaboration (AOAC) International (AOAC official method, 2022).

Method validation

Method validation was conducted to determine

Table 1: Method validation

No.	Parameters	Heavy metals					
		Pb	Cd	Cu	Cr	Fe	Zn
1	Slope	0.0402	0.2422	0.0991	0.0519	0.0526	0.0419
2	Intercept	0.0001	0.0050	−0.0005	1.77×10^{-5}	0.0007	0.0101
3	R^2	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
4	LoD	0.018	0.016	0.022	0.019	0.021	0.019
5	LoQ	0.049	0.056	0.067	0.042	0.065	0.066
6	Precision (%RSD)	1.07	1.09	1.09	1.03	0.98	1.12
7	Recovery (%R)	100.99	100.35	100.29	100.49	100.33	100.82

Table 2: SMILES structure of metabolite compounds

SMILES structure	Compound CID
CC1=CC(N=N1)(C2=CC=CC=C2)C3=CC=CC=C3	605783
C[C@@H](CCC[C@@H](C)CCC/C(=C/CO)/C)CCCC(C)C	5280435
CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C	10446
CCCCC/C=C\C(C)CCCCCCOC(=O)C	5363222
CCCCCCCCCCCCCCCC(=O)OC	8181
CC(C)(C)C1=CC(=CC(=C1O)C(C)(C)C)CCC(=O)OC	62603
CCCCCCCCCCCCCCCC(=O)O	985
CCCCCCC/C=C\CC#CC#C[C@H](C=C)O	5469789
CCCCC/C=C\C=C\CCCCCCCCC(=O)O	5282800
CC/C=C\C/C=C\C/C=C\CCCCCCCC(=O)O	5280934

the reliability of the research results (Winarsih *et al*., 2023). To achieve linearity, six different concentration levels of each metal were determined and expressed using the coefficient of determination (R^2). Method precision was determined by performing measurements six times on one concentration of standard solution to percent of relative standard deviation (%RSD), and the value of recovery was determined by performing a spike on the sample percent of recovery (%R). The values for the limits of detection (LoD) and quantification (LoQ) for each metal were determined based on the standard curve, and the measurement uncertainty for each metal was computed using the LINEST function in Microsoft Excel based on the standard deviation of concentration (Sc). As shown in Table 1, method validation exhibited a significant level of linearity and sensitivity, demonstrating acceptable levels of recovery and precision. In addition, every experiment displayed a strong R^2 of 0.9999. This finding suggests that the calibration curve exhibited a strong association between the standard concentration and the instrument response.

Toxicology computational analysis

The chemical compounds that were successfully identified using GC–MS then underwent verification for their presence in the PubChem database (Rachmawati *et al.,* 2022). Data regarding the PubChem identifier using the compound identifier (CID), chemical formula, simplified molecular input line-entry system (SMILES), and two-dimensional structure were collected (Kim *et al*., 2023). Then, toxicology computational analysis was conducted using a ProTox-II system (Banerjee *et al.,* 2018). Toxicology assessments were conducted using the SMILES structure presented in Table 2. Four toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity) and one organ toxicity (hepatotoxicity) were subjected to probability calculations with values ranging from 0 to 1, and the final results were either active or inactive.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Qualitative analysis commonly entails the identification of specific compounds or chemical qualities using diverse reagents, which are

Table 3: Phytochemical analysis using qualitative methods

No	Secondary metabolites	Results
1	Alkaloids	–
2	Flavonoids	+
3	Saponins	+
4	Steroids	+
5	Triterpenoids	–
6	Tannins	+

(–) absent; (+) present

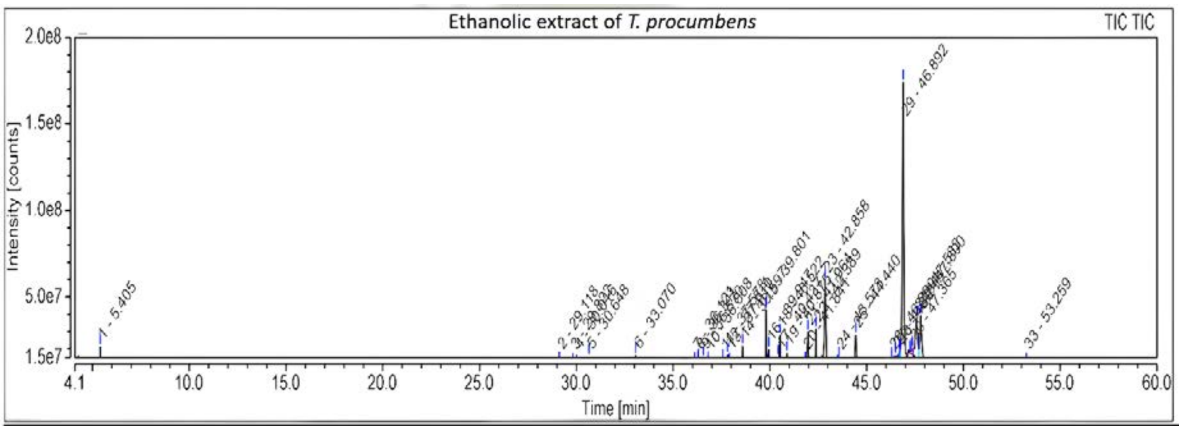


Fig. 3: Total ion chromatogram of *T. procumbens* extract

specialized chemicals employed for testing purposes (Rachmawati *et al.*, 2022). Phytochemical analysis showed that *T. procumbens* plants from the *Ie-Seu'um* geothermal area in Aceh Province have secondary metabolites such as flavonoids, saponins, steroids, and tannins (Table 3). These results corroborate that there is diversity in the content of secondary metabolite compounds in these plants. In the study conducted by Ingole *et al*. (2022), it was found that the metabolite compounds derived from *T. procumbens* include alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, essential oils, carbohydrates, carotenoids, and various other chemicals. The study by Christudas *et al.* (2012) showed that the phytochemical screening of *T. procumbens* using different solvents such as petroleum ether, chloroform, and ethanol extract showed the presence of alkaloids, tannins, steroids, purines, carbohydrates, and proteins. The diversity of the active compounds found in *T. procumbens* may hold promise as a complementary medicine. Recent studies have shown that *T. procumbens* extracts have several pharmacological activities, including antihyperuricemic, antioxidant, antibacterial, and antifungal activities (Andriana *et al.*, 2019).

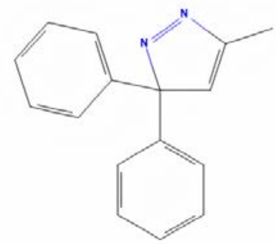
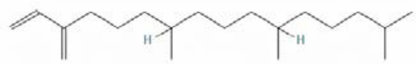
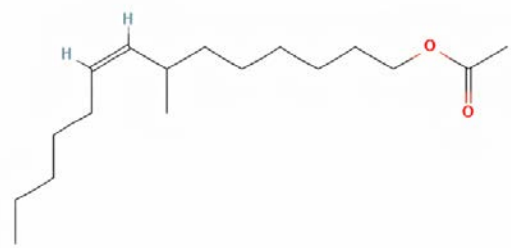
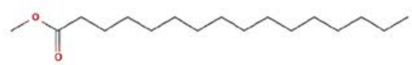
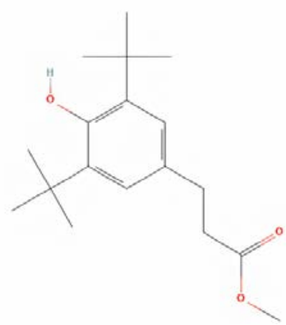
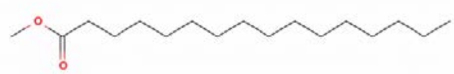
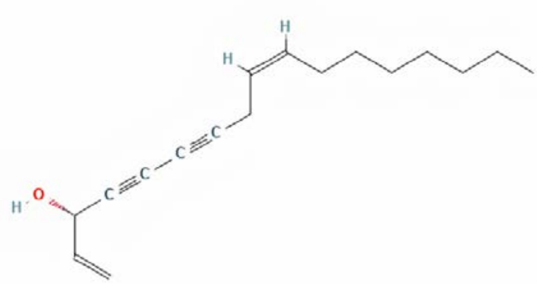
Quantitative phytochemical analysis

Gas chromatography–mass spectroscopy analysis was performed using a nonpolar column. The results showed 32 peaks in the total ion chromatogram analysis with a running time of 60 min (Fig. 3). The results showed one highest peak with a retention time of 46.98 min known as phytol. Another study also found phytol in *T. procumbens* mainly in the leaf essential oil with a percentage of 0% to 7.2% (Coulibaly *et al.,* 2020).

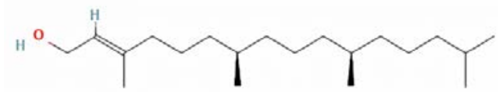
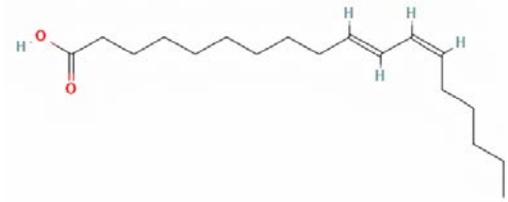
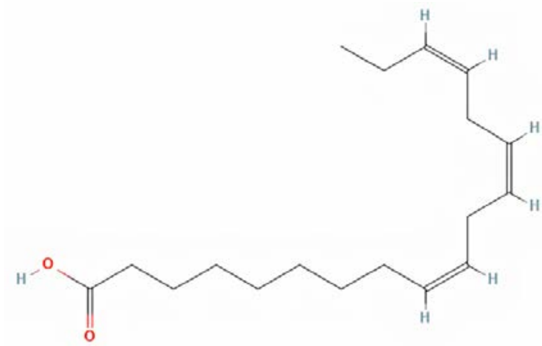
In addition, metabolite compounds with a percentage area >2% are classified in Table 4. Terpenoids (neophytadiene and phytol) and fatty acids (hexadecanoic acid methyl ester, n-hexadecanoic acid, 10(E),12(Z)-conjugated linoleic acid, and 9,12,15-octadecatrienoic acid, (Z,Z,Z)) dominated these results.

As shown in Table 4, the compound with the highest relative percentage was phytol. Phytol is an isoprenoid alcohol with 20 carbon atoms and

Table 4: Chemical profiling of the *T. procumbens* extract using GC–MS

Compound name	Retention time (min)	Relative area (%)	Molecular formula	Chemical structure
3,3-Diphenyl-5-methyl-3H-pyrazole	30.648	3.12	$C_{16}H_{14}N_2$	
Neophytadiene	39.801	4.14	$C_{20}H_{38}$	
7-Methyl-Z-tetradecen-1-ol acetate	40.522	2.78	$C_{17}H_{32}O_2$	
Hexadecanoic acid, methyl ester	41.964	3.32	$C_{17}H_{34}O_2$	
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	42.389	3.73	$C_{18}H_{28}O_3$	
n-Hexadecanoic acid	42.858	9.23	$C_{16}H_{32}O_2$	
(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	44.44	3.72	$C_{17}H_{24}O$	

Continued Table 4: Chemical profiling of the *T. procumbens* extract using GC–MS

Compound name	Retention time (min)	Relative area (%)	Molecular formula	Chemical structure
Phytol	46.892	32.72	$C_{20}H_{40}O$	
10(E),12(Z)-Conjugated linoleic acid	47.582	7.94	$C_{18}H_{32}O_2$	
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	47.8	6.81	$C_{18}H_{30}O_2$	

1 double bond ($C_{20}H_{40}O$) that is known to have the highest peak area with a value of 32.72%. Phytol is a compound of the terpenoid group, that is, acyclic diterpenoids, which also metabolize chlorophyll in plants; therefore, it is widely available in nature. Chlorophyll is the most abundant photosynthetic pigment in higher plants (Gutbrod *et al.,* 2019). During the aging process, chlorophyll is hydrolyzed to release phytol and free chlorophyllide in a ratio of 1:1 (Ischebeck *et al.,* 2006). As shown in the results of the GC–MS analysis, the most abundant compound was phytol. It can be connected that *T. procumbens* weed plants in the *Ie-Seu'um* geothermal area have a high chlorophyll content and will be useful for the surrounding environment because it increases oxygen levels in the air. Phytol is also known as the precursor of synthetic vitamin E and vitamin K (Santos *et al.,* 2013), which are known to be toxic to breast cancer cells, that is, Michigan Cancer Foundation-7 (MCF7), and have the potential to provide antioxidant and antinociceptic effects (Pejin *et al.,* 2014). Pharmacokinetic analysis of phytol was found to have good characteristics as a drug candidate and can be used against enzyme targeted *Staphylococcus aureus* (Maulydia *et al.,* 2023). Other substances in Table 2 support the uniqueness of *T. procumbens* growing in severe environments, such as in geothermal areas, because geographical origin influences the makeup of bioactive compounds (Imelda *et al.,* 2024).

Heavy metal concentrations

Metals can be categorized into two groups based on their relevance to human metabolism: essential and nonessential. Metals such as Fe, Cu, and Zn play crucial roles in human metabolism, but excessive amounts of these metals can lead to hazardous effects (Samimi and Nouri, 2023). Metals such as Pb, Cd, and mercury (Hg) lack nutritional and helpful properties for metabolism and can exert hazardous effects on living organisms even when present in extremely low amounts (Varol and

Table 5: Heavy metal contents of the ethanolic extract of *T. procumbens*

Sample	Heavy metal concentrations ± Sc milligram per kilogram (mg/kg)					
	Pb	Cu	Cr	Cd	Fe	Zn
Ethanolic extract of *T. procumbens*	6.42 ± 0.05	N.D.	13.81 ± 0.07	0.91 ± 0.03	4.65 ± 0.02	3.50 ± 0.03

N.D.= not detected

Table 6: Heavy metal limits and adverse effects

Metals	WHO recommended limits for toxic metals in herbal medicines and products	Possible adverse effects on the human body	Sources
Cu	-	Nausea, vomiting, and diarrhea	Balali-Mood *et al.,* 2021
Cd	0.3 mg/kg	Kidney dysfunction, prostate, breast cancer, osteomalacia, and reproductive deficiencies	Naseri *et al.,* 2021
Cr	-	Respiratory irritation, lung damage, and cancer	Naseri *et al.,* 2021
Fe	-	Nausea, vomiting, and diarrhea	Balali-Mood *et al.,* 2021
Zn	-	Nausea, vomiting, and diarrhea	Balali-Mood *et al.,* 2021
Pb	10 mg/kg	Developmental delays in children, anemia, and damage to the nervous system	Naseri *et al.,* 2021

Sunbul, 2020; Sulistyowati *et al.,* 2022; Samimi *et al.,* 2023; Nurhasanah *et al.,* 2023; Samimi, 2024). Heavy metals can be present in small amounts in soil and plants because living organisms need certain metals to perform metabolic processes. According to the validated assay method, this study presents the quantities of the heavy metals found in the ethanolic extract of *T. procumbens* leaves taken from *Ie-Seu'um*, as shown in Table 5.

The validated methodology was utilized to assess the amounts of Pb, Cu, Cr, Fe, Zn, and Cd in the geothermal area known as *Ie-Seu'um* by employing AAS. As shown in Table 5, the heavy metal concentrations in the ethanolic extract of *T. procumbens* in *Ie-Seu'um* were Cu, not detected; Cd, 0.91 mg/kg; Zn, 3.50 mg/kg; Fe, 4.65 mg/kg; Pb, 6.42 mg/kg; and Cr, 13.81 mg/kg. Of the six metals found in the ethanolic extract of *T. procumbens*, chromium (Cr: electron configuration: [Ar] $3d^5 4s^1$) had the highest concentration of 13.81 mg/kg. Cr is a trace element that is essential for the metabolism of glucose. Research conducted on both humans and animals has shown evidence for the crucial significance of small quantities of Cr(III) 50 µg/day to 200 µg/day in regulating proper glucose metabolism. The administration of substances through the oral route does not pose a significant risk of toxicity (Dayan and Paine, 2001). Although Cr(III) interacts with biomolecules such as deoxyribonucleic acid, its limited capacity to traverse cell membranes has been linked to its diminished biological and toxicological effects. Currently, conclusive research that establishes the indispensability of chromium in biomolecular or physiological mechanisms is lacking (Pavesi and Moreira, 2020). Shamsul and Mangaonkar (2010) reported that *T. procumbens* plants from India have the highest amounts of Cu at 29.96 ppm; Pb, 6.48 ppm, and Cd, 0.46 ppm. Existing literature does not provide any specific information regarding the impact of heavy metals present in *T. procumbens* L. on human health. Additional investigation is required to ascertain the potential health implications associated with the consumption of *T. procumbens* L. that is contaminated with heavy metals. According to the guidelines provided by the World Health Organization (WHO), there are specific restrictions imposed on the presence of hazardous metals such as Cu and Pb in medicines and herbal products. The suggested limits set by WHO state that the permissible levels of Cu should not exceed 0.3 mg per/kg and the maximum allowable concentration of Pb should not exceed 10 mg/kg (WHO, 2007). On the basis of the available evidence, the concentrations of the metals present in the ethanol extract of *T. procumbens* did not exceed the set limits of Cu (not detected) and Pb (6.42 ±

Table 7: Toxicology prediction of metabolite compounds in *T. procumbens*

Compound name	Hepatotoxicity		Carcinogenicity		Immunotoxicity		Mutagenicity		Cytotoxicity		LD_{50} (mg/kg)	Toxicity class
	Pred.	Prob.	Pred.	Prob.	Pred.	Prob.	Pred.	Prob.	Pred.	Prob.		
3,3-Diphenyl-5-methyl-3H-pyrazole	Inactive	0.51	Active	0.59	Inactive	0.99	Inactive	0.54	Inactive	0.76	1000	4
Phytol	Inactive	0.79	Inactive	0.76	Inactive	0.99	Inactive	0.97	Inactive	0.85	5000	5
Neophytadiene	Inactive	0.79	Inactive	0.73	Inactive	0.99	Inactive	0.98	Inactive	0.81	5050	6
7-Methyl-Z-tetradecen-1-ol acetate	Inactive	0.76	Active	0.50	Inactive	0.88	Inactive	0.98	Inactive	0.74	3460	5
Hexadecanoic acid, methyl ester	Inactive	0.58	Inactive	0.55	Inactive	0.99	Inactive	0.98	Inactive	0.73	5000	5
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	Active	0.55	Inactive	0.60	Inactive	0.96	Inactive	0.90	Inactive	0.77	5000	5
n-Hexadecanoic acid	Inactive	0.52	Inactive	0.63	Inactive	0.99	Inactive	1	Inactive	0.74	900	4
(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	Inactive	0.69	Inactive	0.61	Inactive	0.69	Inactive	0.95	Inactive	0.80	8000	6
10(E),12(Z)-Conjugated linoleic acid	Inactive	0.55	Inactive	0.64	Inactive	0.98	Inactive	1	Inactive	0.71	3200	5
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Inactive	0.54	Inactive	0.63	Inactive	0.99	Inactive	0.95	Inactive	0.71	10000	6

Pred = Prediction
Prob = Probability
LD_{50} = Lethal dose 50%

0.05 mg/kg). These metal contents as chemical compounds may also have harmful effects on the human body if taken in large quantities (Samimi and Shahriari Moghadam, 2021). Some of these illnesses are chronic, with gastrointestinal issues being the most frequent (Table 6).

Similar findings regarding the presence of heavy metals in *Chromolaena odorata* (local name: *seurapoh*) leaves were reported by Abubakar *et al*., (2023), who found Cd (0.0219±0.005) and Pb of 0.0181 to 0.0356 mg/kg, but no As or Hg. The ability of plants to absorb and utilize heavy metals in their metabolism may explain the discrepancy in the results of the two analyses. *T. procumbens* has been shown to possess significant bioremediation capabilities, particularly in the removal of heavy metals such as Cr, Cu, Pb, and Cd from soil that has been contaminated by industrial effluents (Govarthanan *et al.,* 2016). *T. procumbens* is also reported to have the ability to absorb heavy metals in large quantities. The desiccated leaves of *T. procumbens* can remove 91% of copper from a synthetic metal solution at pH 5.0 and 250 rpm with constant stirring. This adsorbent is an excellent choice for the adsorption of heavy metal ions in wastewater stream, including copper ions (Karthika *et al.,* 2010). Due to its favorable characteristics, *T. procumbens* has potential as a viable option for the implementation of phytoremediation initiatives in regions characterized by significant levels of heavy metal contamination. It is essential to note that the risks associated with heavy metal contamination in geothermal regions can vary depending on the location and quantity of contamination. Consequently, it is essential to conduct field measurements and research to better comprehend the environmental issue and its potential health dangers. This research is needed to explore the potential and safety of plants in geothermal areas if they are to be used as traditional medicines.

Ecotoxicological analysis

The GC–MS analysis revealed the abundance of compounds in the ethanol extract of *T. procumbens.* Some of these compounds are hazardous to other organisms, such as humans, animals, microbes, and plants. Ecotoxicology of secondary metabolite compounds in plants investigates the potential impact of these compounds on ecosystems and the creatures that inhabit them. Ecotoxicology is one of the fields of science that studies the impact of chemicals or other pollutants on the environment and living organisms. One approach in measuring the toxicity impact of these substances is using an *in silico* approach (CDESCS, 2014). The *in silico* approach in ecotoxicology refers to the use of software and computers to predict the potential impact of a substance on organisms and ecosystems without the need to conduct direct trials on living organisms (Benfenati, 2013). *T. procumbens* is frequently used as a traditional medicine, and the toxicological discussion in this paper refers to the human body. In this particular study, the ecotoxicology of the secondary metabolites from the ethanolic extracts of *T. procumbens* was analyzed using a ProTox-II system. The ProTox-II model utilizes a combination of molecular similarity, fragment propensities, most frequent features, and machine learning techniques, specifically employing a cross-validation method known as fragment similarity-based cluster. This approach involves the use of 33 different models to predict a range of toxicity endpoints, including acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes in Tox21 pathways, and toxicity targets (Banerjee *et al.,* 2018). Table 7 shows the results of the toxicity prediction against five toxicity targets, showing the dominance of nontoxic. The results of the hepatotoxic analysis showed that the compound 3,5-bis(1,1 dimethylethyl)-4-hydroxy-,methyl ester has an active potential with a probability of 0.55. Next, 3,3-diphenyl-5-methyl-3H-pyrazole with potential toxicity because it is carcinogenic with a probability of 0.59. The results of other tests show that all compounds have nontoxic potential in immunotoxicity, mutagenicity, and cytotoxicity. Toxicity examination also supported by the results of the lethal dose 50% (LD_{50}), which is the dose that causes 50% of subjects to die from a chemical after exposure in milligrams per kilogram of body weight. Toxicity classes are defined according to the globally harmonized chemical labeling classification system (GHS) ranging from class I (fatal) to class VI (nontoxic). Secondary metabolites from *T. procumbens* showed class 4 (harmful if swallowed $300 < LD_{50} \leq 2000$), class 5 (may be harmful if swallowed $2000 < LD_{50} \leq 5000$), and class 6 (nontoxic $LD_{50} > 5000$). Research on acute oral toxicity studies in albino mice has been conducted on extracts of *T. procumbens*, and the results showed that they did not cause acute toxicity or severe liver damage (Burgos-Pino *et al.,* 2023). This supports the evidence that *T. procumbens* extracts are safe to consume at controlled doses. According to the findings of this investigation, toxicological informatics has been demonstrated to have the capability to determine the toxicity of the chemicals present in the ethanol extracts of *T. procumbens.* Understanding the aforementioned subject matter is of utmost significance because of its potential to offer comprehensive understanding of the adverse effects of metabolite compounds from plants on human health resulting from the consumption of traditional medicine and exposure to the environment.

CONCLUSION

T. procumbens is a plant species that can be found in the *Ie-Seu'um* geothermal area in Aceh Province, Indonesia. The primary purpose of this research is to determine whether this plant possesses secondary metabolites and to evaluate the plant's potential for usage in medical and therapeutic applications. Secondary metabolites are chemical substances produced by plants, and they frequently participate in a wide variety of biological processes. The research demonstrated that *T. procumbens*, which was collected from the geothermal region specified, contains secondary metabolites. These secondary metabolites include tannins, steroids, and saponins. It is important to note the presence of these chemicals because, in many cases, diverse pharmacological and therapeutic qualities are linked to them. The discovery of these secondary metabolites indicates that *T. procumbens* may have the potential for a variety of uses in the fields of medicine and therapy. Traditional medicine, medicines, and various other healthcare-related applications could all fall under this category of potential uses. The research highlights the presence of the diterpenoid phytol as one of the secondary metabolites that were found. The highest concentration of phytol was 32.72%. It is known that phytol possesses bioactive qualities, and the fact that there is a large amount of it in *T. procumbens* suggests that it may play a significant role in the pharmacological actions of the plant. This lends credence to the hypothesis that phytol-rich extracts of *T. procumbens* may provide a number of advantageous health effects. In addition, an investigation of the presence of heavy metals in *T. procumbens* was conducted using samples taken from the geothermal region, and a number of heavy metals were found. This aspect of the research is very important because it helps in determining whether *T. procumbens* is safe to use and whether it is suitable for a variety of applications. Understanding the presence of heavy metals is vital, particularly if the plant will be used for medical or nutritional purposes, because excessive amounts of heavy metals can be harmful to one's health. It is imperative to emphasize the significance of conducting studies of this nature to establish a cohesive connection between the state of a plant and the possible toxicity associated with its metabolite composition. The subsequent procedure involves performing tests on the biological activity of *T. procumbens* plants to verify their therapeutic potential and enhance their efficacy for medicinal purposes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, ethical issues such as plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	degrees Celsius
AAS	Atomic absorption spectrometry
AOAC	Association of Official Analytical Collaboration
Amu	Atomic mass units
Cd	Cadmium
CDESCS	Committee on the Design and Evaluation of Safer Chemical Substitutions
CID	Compound identifier
$Cl–HCO_3–SO_4$	Chloride–bicarbonate–sulfate
cm	Centimeter
Cr	Chromium
Cu	Copper
E	East
eV	Electron volt
Fe	Iron
g	Gram
GC–MS	Gas chromatography–mass spectroscopy
GHS	Globally harmonized chemical labeling classification system
Hg	Mercury
HNO_3	Nitric acid
iSQ	Single quadropole
LD_{50}	Lethal dose 50%
LoD	Limits of detection
LoQ	Limits of quantification
m	Meter
MCF7	Michigan cancer foundation-7
mg/kg	Milligram per kilogram
Min	Minute
mL	Milliliter
mm	Millimeter
N	North
N.D.	Not detected
nm	Nanometer
Pb	Lead
pH	Power of hydrogen
Ppm	Part per million
Pred	Prediction
Prob	Probability
R	Recovery
R^2	Coefficient of determination
rpm	Rotation per minutes
RSD	Relative standard deviation
Sc	Standard deviation of concentration
SMILES	Simplified molecular input line-entry system
w:v	Weight:volume
WHO	World Health Organization
Zn	Zinc
µg/L	Microgram per liter
µm	Micrometer

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