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Effects of soil properties and heavy metals with a bioactive compound, acteoside, in *Acanthus ebracteatus* from three different habitats of eastern part of Thailand

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Abstract

Acanthus ebracteatus Vahl is a mangrove medicinal plant widely distributed along the coastal regions of Thailand. It is recognized as a rich natural source of acteoside, a phenylethanoid glycoside and is discovered in the anti-inflammatory, antioxidant, and hepatoprotective activities. However, the information availability regarding the influence of environmental factors, particularly soil properties and heavy metal accumulation, on acteoside biosynthesis is limited. The aims of this study were to investigate the effects of soil physicochemical characteristics and heavy metal concentrations on acteoside content in *A. ebracteatus* collected from three distinct habitats in eastern Thailand: Chachoengsao, Chonburi, and Rayong. Soil samples were analyzed for pH, organic matter, and macronutrient contents, including nitrogen (N), and phosphorus (P), as well as concentrations of As, Cd, Hg, and Pb using inductively coupled plasma – optical emission spectrometer (ICP–OES). Acteoside levels in methanolic leaf extracts were quantified by high-performance liquid chromatography coupled with diode-array detection (HPLC-DAD). Correlations between soil parameters and acteoside content were evaluated. Based on our searching literature found that an association between soil heavy metal levels and acteoside concentration in *A. ebracteatus* is demonstrated. The results revealed significant variations in soil composition among the sampling sites. The highest acteoside accumulation was observed in plants from the Chachoengsao habitat, which was characterized by near-neutral soil pH (7.1 ± 0.2) and relatively elevated concentrations of Hg (0.06 ± 0.00 mg kg^{-1}) and Pb (20.07 ± 0.81 mg kg^{-1}). A strong positive correlation was identified between acteoside content and soil Hg and Pb concentrations. These findings indicated that soil mineral composition and heavy metal availability played a crucial role in modulating acteoside biosynthesis in *A. ebracteatus*. The results provide valuable insights for phytochemical quality control, environmental monitoring, and the sustainable cultivation of this medicinal plant within the Eastern Economic Corridor (EEC) of Thailand.

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1. Introduction

Medicinal plants are valuable natural resources that produce a wide range of bioactive secondary metabolites with therapeutic potential. The accumulation and variation of these compounds are influenced by environmental factors such as soil composition, temperature, salinity, and exposure to heavy metals [1 – 3]. Soil acts as a reservoir of essential minerals and trace elements, and its physicochemical properties can significantly affect plant metabolism and the biosynthetic pathways responsible for secondary metabolite production [4, 5]. Understanding how these environmental variables influence phytochemical profiles is therefore essential for optimizing the yield and quality of bioactive compounds and ensuring the consistency of medicinal plant products [6].

Acanthus ebracteatus Vahl, commonly known as Sea Holly or "Nguak Pla Mo" in Thai, is a perennial mangrove species widely distributed along the coasts and estuarine areas of Southeast Asia [7]. In traditional medicine, it has been used in Thai and Chinese medicine to treat rheumatism, inflammation, liver disorders, and skin diseases [8]. Phytochemical investigations have revealed that *A. ebracteatus* contains diverse classes of secondary metabolites, including alkaloids, flavonoids, lignans, and phenylethanoid glycosides [9, 10]. Among these, acteoside or some time called verbascoside is the predominant and pharmacologically active constituent, exhibiting anti-inflammatory, antioxidant, neuroprotective, and hepatoprotective effects [11]. Because of its strong pharmacological properties, acteoside is often used as a marker compound for quality evaluation of *A. ebracteatus* extracts and related herbal formulations according to the Thai Herbal Pharmacopia, 2022 [12, 13].

The Eastern part of Thailand, encompassing provinces such as Chachoengsao, Rayong, and Chonburi, is characterized by diverse ecological zones ranging from coastal mangroves to brackish wetlands and inland freshwater areas. In addition, this region is a dynamic ecotone characterized by diverse anthropogenic activities, ranging from intensive agriculture and aquaculture to large-scale industrial operations. These activities contribute to the accumulation of heavy metals in coastal sediments, posing significant physiological challenges to local flora [14]. Among the native species, *A. ebracteatus*, a mangrove herb thriving in these ecotones, serves as an ideal model to examine soil–metal interactions in coastal ecosystems. Specifically, this study focuses on Chachoengsao, Rayong, and Chonburi provinces. These sites were strategically selected due to their location within Thailand's Eastern Economic Corridor (EEC), a zone known for high industrial output and agricultural runoff, which increases the environmental load of heavy metals such as Mercury (Hg), Lead (Pb), and Arsenic (As) [15]. These habitats differ markedly in soil pH, salinity, organic matter, and concentrations of heavy metals such as Fe, Zn, Pb, Hg, Cu, and Cd, differences influenced by both natural geochemical processes and anthropogenic activities such as agriculture and aquaculture [16]. Variations in soil composition and contamination levels can lead to distinct chemical profiles in medicinal plants, even within the same species [4]. Previous studies have shown that heavy metals can act either as micronutrient cofactors that enhance secondary metabolite production or as stress factors that induce oxidative responses, thus altering biosynthetic pathways [17]. Heavy metals are recognized as abiotic stressors that induce oxidative stress in plants through excessive generation of reactive oxygen species (ROS), which subsequently activate stress-responsive signal transduction pathways involved in phenylpropanoid metabolism. This regulation may enhance the biosynthesis of phenolic glycosides, including acteoside, as part of the plant's adaptive defense mechanism [18 - 19] .

Acanthus ebracteatus, a mangrove-associated herb naturally thriving in coastal ecotones, represents an appropriate model species for examining interactions between soil properties, heavy metal exposure, and secondary metabolite accumulation in eastern Thailand. Under heavy metal stress, phenolic compounds such as acteoside may act synergistically with antioxidant enzyme systems, including peroxidase, catalase, and polyphenol oxidase, to mitigate oxidative damage and maintain cellular homeostasis [18 – 20].

However, only limited research has examined the influence of soil physicochemical and heavy metal properties on acteoside biosynthesis in *A. ebracteatus*. Considering the ecological diversity of its natural habitats, understanding this relationship is important for identifying environmental cues that regulate metabolite accumulation. Such knowledge would support the development of sustainable cultivation systems, standardized harvesting protocols, and regional quality markers for herbal raw materials. Therefore, the present study aimed to investigate the impact of soil properties and heavy metal concentrations on acteoside accumulation in *A. ebracteatus* collected from three different habitats, Chachoengsao, Chonburi, and Rayong areas, of the Eastern part of Thailand. The outcomes are expected to contribute to the scientific foundation for bioresource utilization and value addition under the principles of Thailand's Bio-Circular-Green (BCG) economy [21]. These insights could also guide environmental biomonitoring and the standardization of phytopharmaceutical resources in line with the BCG economy framework.

2. Materials and Methods

Materials

Site description, soil sampling, and soil sample

After a general distribution survey of *Acanthus ebracteatus* around Eastern Economic Corridor (EEC) region of Thailand, the soils were collected in three different locations: Chachoengsao (N13º 30′ 46.646″ E100º 59′ 59.719″), 2) Chonburi (N13º 26′ 40.002″ E100º 59′ 37.06″), and 3) Rayong (N12º 44′ 16.775″ E101º 40′ 55.167″) in October 2024. The area of each sampling site was ten quadrats of 1 m × 1 m were randomly established at each site. A soil sample 30 cm in depth was taken in each quadrat using a small spade at 10 cm from selected plant. Soil samples were dried by oven at 50 °C for 3 days and sieved (< 2 mm) after that soil samples were taken to laboratory for chemical analysis (Fig 1).

Plant material

For each sampled plant, leaves and twigs were collected from approximately 20 cm above the ground at the same time and the same sites where the soil samples were obtained. Plant species were identified Mr.Pitphiboon Thanphuthon, Department of Thai Traditional Medicine, Faculty of Science, Ramkhamhaeng, Thailand and the voucher specimen (RRU–SH 048) was deposited at the Faculty of Science and Technology, Rajabhat Rajanagarindra University, Thailand (Fig. 2). The plant materials were dried in a hot-air oven at 50 °C for 48 h. The dried samples were then cut into small pieces, ground into a fine powder using an herbal grinder, and stored at 4 °C until further analysis of secondary metabolites.

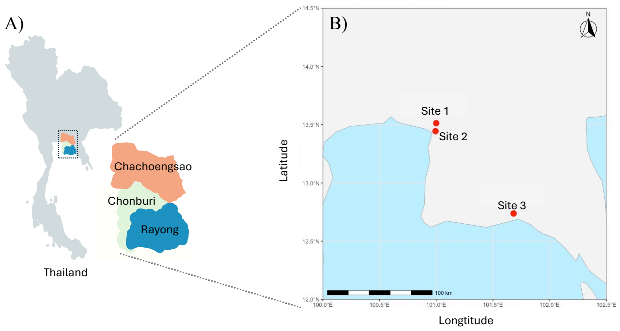

Fig. 1 (A) Geographic locations of Chachoengsao, Chonburi, and Rayong provinces in the eastern region of Thailand. (B) Detailed map showing the three sampling sites along the eastern coastal area, with latitude and longitude indicated.

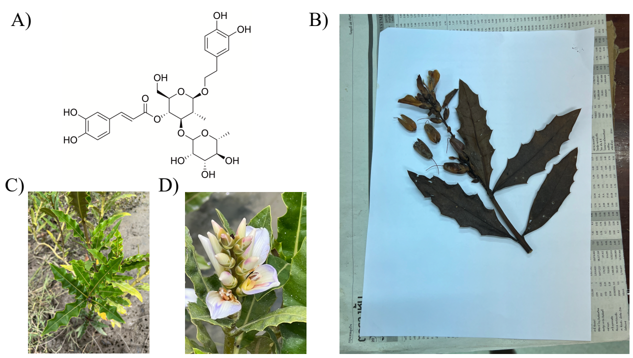

Fig. 2 (A) Chemical structure of acteoside. (B) Dried herbarium specimen of *A. ebracteatus* showing leaves and reproductive parts. (C) Leaf morphology of *A. ebracteatus*. (D) Inflorescence and flower of *A. ebracteatus*.

Instrumentation

Heavy metals (As, Cd, Hg, and Pb) were analysed using an inductively coupled plasma – optical emission spectrometer (ICP-OES) (PerkinElmer Optima 8000). Chemical profiling and quantification of the bioactive compounds were performed on an HPLC system equipped with a Waters 600 pump and controller and a 717 autosampler, fitted with a Waters 996 photodiode array (PDA) detector set at 254 nm. The pH of water and soil was measured with a digital pH meter (DI–1000 INDEX) at 20 cm dept for pH water and in a 2.5:1 water/soil ratio for pH soil.

Chromatographic separation for HPLC profiling and quantification was carried out using a reversed-phase Nova-Pak C18 column (4 µm, 150 × 3.9 mm). A binary mobile phase comprising deionised water (A) and acetonitrile (B) was applied under gradient elution, with 90% A held for 3 min and then decreased linearly to 10% A over 30 min at a flow rate of 1.0 mL min^{-1}.

Reagents, Chemicals, and Glassware

Hydrochloric acid (HCl), nitric acid (HNO_3), sulfuric acid (H_2SO_4), perchloric acid ($HClO_4$), and hydrogen peroxide (H_2O_2) were purchased from Merck (Germany). Stock standard solutions of 1000 mg L^{-1} metals (As, Cd, Hg, Pb, and K) were prepared were purchased from PerkinElmer. Analytical HPLC grade acetonitrile (ACN) was obtained from Honeywell Burdick & Jackson (USA), and commercial grade methanol was obtained from RCI Labscan™ (USA) was used for plant extraction. The acteoside standard (98% purity) was purchased from AK Scientific, Inc. To avoid the heavy metal concentration error from equipment, each glassware washed with detergent and tap water and then soaked with 10% HNO_3 for 24 h follow by wash with DI-H_2O and dry the cleaned glassware in hot air oven for 90 min before use.

Procedure

Soil organic carbon (OC) was estimated following the method of Walkley and Black [22]. Soil organic matter (OM) was calculated by multiplying the soil organic carbon by 1.72. For macronutrients, the traditional analysis of total nitrogen (TN) and total phosphorus (TP) contents in soil was carried out using Kelvin-distillation titration is used for TN determination, sodium hydroxide melting-molybdenum antimony anti colorimetric method is used for TP determination.

For heavy metal determination, soil sample (n = 5 per site) were dried for a week. Each site of soil sample was combined, grounded using grinder machine, and sieved using a 2 mm mesh size. The sample solution was prepared using 5:1:1 triacid mixture of 70% HNO_3, 70% H_2SO_4 (Merck, Germany), and 65% $HClO_4$ (Merck, Germany). A triacid mixture of 15 mL was added to each beaker, which already contained 1 g of the dried material. At a temperature of 80 °C, each combination was allowed to digest until a clear solution was formed. To analysis the heavy metals, the digested samples were first allowed to cool, then filtered, and then diluted to a volume of 50 mL using deionized water. An inductively coupled plasma-optical emission spectrometer (ICP–OES) was used to determine the concentrations of selected metals including As, Cd, Hg, and Pb) that were present in the digested solution [23].

For plant extract, 10 g of each plant was collected (n = 5 per site). The sample were dried and grounded using grinder machine. The plant material was extract with over methanol solvents (50 mL) under Soxhlet apparatus for 8 h. After removal solvents using aspirator and dried the material using vacuum pump, the crude extracts of each plant material were obtained.

For HPLC profiling and quantification, separation was achieved on a Nova–Pak C18 column (4 µm, 150 × 3.9 mm) using a gradient of DI–H_2O (A) and ACN (B) (gradient system (A in B); 90% A for 3 min, 90–10 % A for 30 min: flow rate; 1 mL min^{-1}). Samples and standards were dissolved in methanol prior to analysis.

Statistical Analysis

Data were presented as means ± standard deviations from three replicates. One-way analysis of variance (ANOVA) was performed for all datasets, followed by Tukey's post hoc test at a 95% confidence level using SPSS (SPSS Inc., Chicago, IL, USA). Pearson's correlation coefficients were calculated and visualized using the R "corrplot" package to evaluate the relationships between acteoside and soil parameters [24].

3. Results and Discussion

Soil Physicochemical Properties Across Sampling Sites

Soil analysis from the three habitats, Chachoengsao, Chonburi, and Rayong provinces showed in Table 1. The results revealed that the significant differences in physicochemical characteristics were obtained. The pH values ranged from 6.8 ± 0.1 to 7.2 ± 0.0, indicating neutral conditions, with Chachoengsao showing the most neutral soil environment. Organic matter content and macronutrients (N and P) also varied, with Chachoengsao soils exhibiting the highest organic carbon (2.99 ± 0.19%), total nitrogen concentration (0.29 ± 0.01%), and phosphorus 0.11 ± 0.02 (mg kg^{-1}), potentially contributing to enhanced plant metabolic activity. It is noteworthy that Rayong samples displayed lower nutrient availability, consistent with sandy coastal soils typical of mangrove habitats.

Soil physicochemical properties play a fundamental role in regulating nutrient availability, microbial activity, and plant metabolic processes, thereby influencing plant growth and the biosynthesis of secondary metabolites. In the present study, soils collected from Chachoengsao, Chonburi, and Rayong provinces exhibited generally neutral pH values (6.8–7.2), which are considered optimal for nutrient solubility and microbial–mediated nutrient cycling in most terrestrial ecosystems.

Among the three sites, Chachoengsao soils demonstrated the highest levels of soil organic carbon (OC), soil organic matter (OM), total nitrogen (TN), and phosphorus. Elevated OC and OM contents are indicative of greater organic inputs and enhanced microbial activity, which collectively promote nutrient mineralization and improve soil structure. From an environmental chemistry perspective, organic matter acts as a key regulator of soil cation exchange capacity and nutrient retention [25]. As results described above, it was markedly higher in Chachoengsao soils. This enriched nutrient status may create suitable conditions for plant metabolic activity, potentially supporting higher fluxes through primary metabolic pathways and providing sufficient precursors for secondary metabolite biosynthesis.

TN content varied substantially across sites, with Chachoengsao (0.29 ± 0.01%) soils exhibiting Chonburi (0.22 ± 0.02%). On the contrary, TN content found in Chachoengsao is nearly five–fold higher TN compared to Rayong soils (0.06 ± 0.02%). Nitrogen is a critical element for amino acid synthesis, enzyme production, and nucleic acid metabolism. In plants, nitrogen availability has been shown to influence not only biomass accumulation but also the qualitative and quantitative profiles of specialized metabolites, especially phenolics, and flavonoids [26]. Therefore, the higher nitrogen status observed in Chachoengsao soils may partially explain site-specific differences in plant chemical composition observed in downstream metabolomic analyses.

Phosphorus concentrations were relatively low across all sites but showed site–specific variability. Given phosphorus's strong affinity for soil minerals and organic matter, its bioavailability is closely linked to soil chemistry rather than total concentration alone. In neutral soils, phosphorus availability is often maximized; however, low organic matter inputs, as observed in Rayong soils, may limit phosphorus mobilization via organic ligand complexation and microbial activity.

By comparison the study regarding the soil physicochemical properties across the Eastern coast of the Gulf of Thailand by Thongra–ar et al. [16], and Lui et al. [27] found that pH value from Chachoengsao province showed more natural than both areas. These finding agreed well with our observation data. In addition, the OM (%) from surrounding area of Bangpakong which belongs to Chachoengsao province gave OM (%) value as 2.5 (data from Thongra–ar et al. [16]) which was lower comparable to our data (5.19 ± 0.31). Interestingly, Thongra–ar et al. revealed the surrounding area of Leam Chabang belonging to Chonburi province (1.7) and Map Ta Phut belonging to Rayong province (1.5) which showed the OM (%) data lower than Bangpakong in Chachoengsao province (2.5). It is notable that the OM values from previous data with our data are the same trend.

Thus, the observed spatial variability in soil physicochemical properties reflects differences in parent material, land use history, and ecosystem type across eastern Thailand. These variations are environmentally significant, as they establish distinct biogeochemical niches that influence plant nutrient acquisition strategies and metabolic outcomes. The integration of soil chemistry data with plant metabolomic profiles is therefore essential for interpreting site-dependent variation in bioactive compound production and for identifying optimal habitats for the sustainable cultivation or conservation of medicinal plants. Nitrogen and phosphorus are primarily involved in primary metabolic processes related to plant growth; however, their relationships with acteoside accumulation were not statistically significant in this study. This indicates that environmental stress factors may play a more prominent role in regulating acteoside biosynthesis in *Acanthus ebracteatus*.

Table 1 Soil physicochemical properties across three sampling sites.

Site	pH	OC (%)	OM (%)	TN (%)	P (mg kg^{-1})
Chachoengsao	7.1 ± 0.2	2.99 ± 0.19	5.19 ± 0.31	0.29 ± 0.01	0.11 ± 0.02
Chonburi	7.2 ± 0.0	2.39 ± 0.06	4.11 ± 0.10	0.22 ± 0.02	0.04 ± 0.01
Rayong	6.8 ± 0.1	1.90 ± 0.17	3.19 ± 0.29	0.06 ± 0.02	0.08 ± 0.00

Heavy Metal Accumulation in soils

Heavy metal concentrations varied significantly among the three sites was summarized as shown in Table 2 . Mercury (Hg) and Lead (Pb) levels were notably higher in Chachoengsao, with mean concentrations of 0.06 ± 0.00 mg kg^{-1} and 20.07 ± 0.81 mg kg^{-1}, respectively, whereas Asenic (As) were elevated in Chonburi (31.53 ± 0.19 mg kg^{-1}). However, Cadmium (Cd) was not detected in all areas. The variation in metal content likely reflects differences in anthropogenic input and sediment deposition patterns along the eastern coast. These findings align with previous reports indicating that industrial and agricultural runoff in Chachoengsao contributes to trace metal accumulation in soils reported by Thongra–ar et al. [16].

Heavy metal stress can induce a range of adverse effects that disrupt cellular processes at multiple metabolic levels, ultimately leading to reduced plant productivity. In response to such environmental stressors, phenolic compounds play essential molecular and biochemical roles in plants. Enhanced biosynthesis of phenolic compounds under heavy metal stress contributes to plant defense by mitigating oxidative damage and maintaining cellular redox balance [28].

Some available evidence [27 - 31] indicated that heavy metals, including Hg and Pb, primarily induce oxidative stress in plants and disrupt photosynthetic physiology. These effects are expected to activate secondary metabolite defense mechanisms, particularly the biosynthesis of phenolics, flavonoids, and tannins, owing to their roles in reactive oxygen species (ROS) scavenging and metal chelation. Although the effect of secondary metabolite profiling with heavy metal responses in mangrove plant species remains limited, field studies conducted along pollution gradients consistently report elevated phenolic and flavonoid contents, along with enhanced antioxidant and metal-chelating capacities in mangrove tissues from metal-impacted sites. Furthermore, mechanistic studies examining mangrove responses to other heavy metals support the conclusion that phenolic metabolism including phenolic acids is a key tolerance pathway. Collectively, these findings provide a strong rationale for investigating heavy metal–induced upregulation of the phenylpropanoid pathway and identifying targeted phenolic markers in mangrove plants growing in metal–contaminated habitats.

Table 2 Heavy Metal Accumulation in Soils across three sampling sites.

Site	As (mg kg^{-1})	Cd (mg kg^{-1})	Hg (mg kg^{-1})	Pb (mg kg^{-1})
Chachoengsao	12.55 ± 0.09	not detected	0.06 ± 0.00	20.07 ± 0.81
Chonburi	11.18 ± 0.02	not detected	0.04 ± 0.00	17.61 ± 0.39
Rayong	31.53 ± 0.19	not detected	0.03 ± 0.02	15.78 ± 0.08

Acteoside Content and Quantification

For the calibration curve, the data was linear over the range of 0–20 µg g^{-1}. The concentration of acetoside was calculated from the equation as peak area(Y) = 658042 [acteoside, µg g^{-1} (X)] – 212785 with R^2 = 0.998. For the limit of detection (LOD) and quantification (LOQ) determined from 3 times blank, concentrations of 1.07 µg g^{-1} and 3.23 µg g^{-1} of acteoside, respectively, were found in this method.

HPLC–DAD analysis confirmed the presence of acteoside in all leaf and stem extracts, with an average retention time of approximately 13.5 min (Fig. 3) under the gradient conditions described in the Experimental section. Quantitative analysis revealed marked differences in acteoside concentrations among the three habitats (Table 3). Plants collected from Chachoengsao exhibited the highest acteoside levels in both plant parts, with concentrations of 27.1 ± 0.34 µg g^{-1} dry weight (DW) in leaves and 23.9 ± 0.09 µg g^{-1} DW in stems. In contrast, leaf extracts from Chonburi and Rayong contained lower acteoside levels, at 8.53 ± 0.08 µg g^{-1} DW and 1.83 ± 0.25 µg g^{-1} DW, respectively. Conversely, stem extracts showed an opposite trend, with higher acteoside contents in Rayong (0.67 ± 0.06 µg g^{-1} DW) than in Chonburi (0.48 ± 0.03 µg g^{-1} DW). These findings suggest that acteoside biosynthesis in *Acanthus ebracteatus* is strongly influenced by habitat-specific soil properties and may be modulated by stress responses associated with trace metal exposure.

Correlation Between Soil Properties and Acteoside Concentration

Pearson correlation analysis revealed significant relationships between soil properties, heavy metal and acteoside contents (Fig. 4). Notably, Pb exhibited significant positive correlations with OC (r = 0.999, $p < 0.05$) and (r = 0.999, $p < 0.05$). Additionally, acteoside content in leaves exhibited a strong positive correlation with Hg content in soil (r = 0.997, $p < 0.05$). Although very high correlation coefficients ($r \approx 0.99$) were observed between certain environmental variables and acteoside content, these values should be interpreted cautiously due to the limited number of sampling sites in the present study. Such high correlations likely reflect strong associations within a small dataset and do not necessarily imply robust or causal relationships. Larger-scale studies with expanded sampling are required to validate these correlations and assess their statistical robustness. These findings indicate that certain heavy metals especially Hg may induce stress-related secondary metabolite production, consistent with the metal-induced oxidative stress hypothesis [31- 32]. This trend supports the concept that specific environmental stressors can modulate polyphenol glycoside synthesis through activation of defense-related pathways.

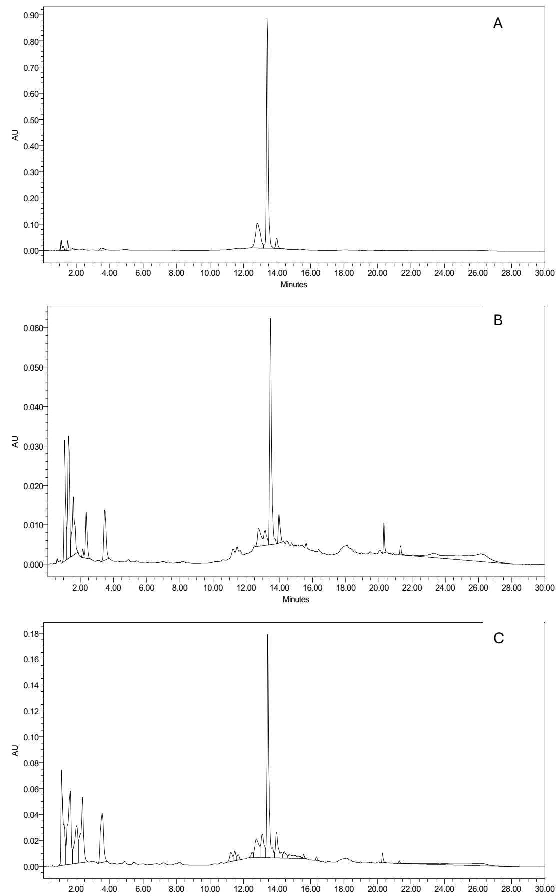

Fig. 3 HPLC chromatogram of acteoside standard (A) and leaf (B) and stem (C) extracts of *A. ebracteatus* from Chachoengsao.

Environmental and Ecological Implications: Soil Heavy Metals and Acteoside Production

Soil heavy metals constitute persistent environmental stressors in coastal and wetland ecosystems, including mangrove habitats. At low to moderate concentrations, metals such as Hg and Pb typically induce oxidative stress signaling rather than acute phytotoxicity. In response, plants undergo metabolic reprogramming, particularly through activation of the phenylpropanoid and phenylethanoid pathways, which play central roles in the biosynthesis of phenolic compounds [4, 27-32]. Although a related species, *Acanthus ilicifolius* [20], has been investigated for heavy metal bioaccumulation in different plant tissues, direct evidence linking heavy metal exposure to enhanced acteoside accumulation in *A. ebracteatus* was limited. To the best of our knowledge, this study provides the first report demonstrating an association between soil heavy metal levels and acteoside concentration in *A. ebracteatus*. Similar stress-induced modulation of acteoside or related phenylethanoid glycosides has been reported in other plant species, including *Orobanche laxissima* [33], *Scrophularia striata* [34], and *Petroselinum crispum* [35], supporting the role of heavy metal stress in regulating phenolic metabolite biosynthesis.

Table 3 Acteoside concentration in leaves and stems from *A. ebracteatus* across three sampling sites.

Site	Acteoside concentration ($\mu g\ g^{-1}$ DW)	
	Leaves	Stems
Chachoengsao	27.10±0.34	23.91±0.09
Chonburi	8.53±0.08	0.48±0.03
Rayong	1.83±0.25	0.67±0.06

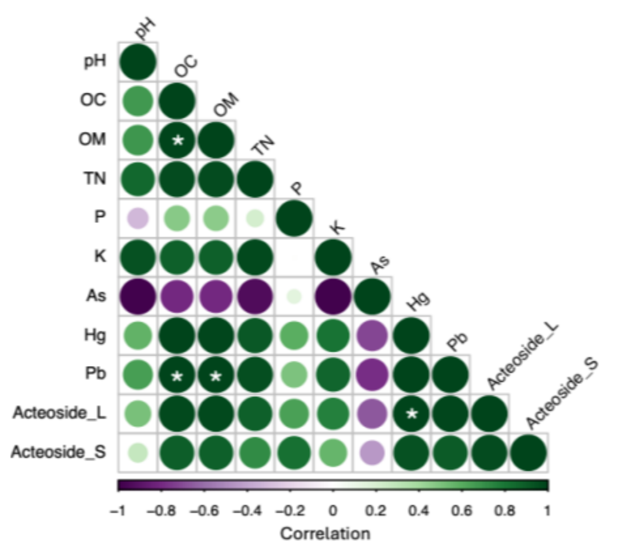

Fig. 4 Pearson correlation matrix showing relationships among soil properties, heavy metals, and acteoside contents. Circle size and color indicate the strength and direction of correlations. *Represent statistically significant correlations ($p < 0.05$).

Although the Chachoengsao site exhibited higher soil N and P levels alongside increased acteoside accumulation, the present field-based data do not allow definitive separation of nutrient-driven effects from heavy metal-induced stress responses. Elevated nutrient availability may enhance metabolic capacity and precursor supply for phenylpropanoid-related pathways, while simultaneous exposure to trace heavy metals may impose oxidative stress that further stimulates phenolic glycoside biosynthesis. Therefore, acteoside accumulation observed in this study is most plausibly explained by a combined effect of soil fertility and environmental stress under natural ecological conditions. Therefore, acteoside accumulation observed in this study is most plausibly explained by a combined effect of soil fertility and environmental stress under natural ecological conditions. This observation provides practical implications for the cultivation of medicinal plants in controlled environments, where soil quality management could optimize phytochemical yield.

Implications for Sustainable Cultivation and Conservation

This study contributes to understanding how environmental heterogeneity affects phytochemical diversity within the Eastern Economic Corridor (EEC) of Thailand. Identifying soil parameters influencing metabolite production supports sustainable resource management and standardization of herbal raw materials. Cultivation practices that balance soil fertility and metal content may improve acteoside yield while ensuring ecological safety. Furthermore, integrating environmental chemistry with phytochemical research offers valuable insights into adaptive mechanisms of coastal medicinal plants, essential for conservation and pharmacological innovation.

4. Conclusion

This study revealed that soil physicochemical properties and heavy metal availability significantly influence acteoside accumulation in *Acanthus ebracteatus* across coastal habitats of eastern Thailand. Variations in soil pH, nutrient status, and trace metal concentrations were closely associated with differences in metabolite content, with the highest acteoside levels observed at the Chachoengsao site. Positive correlations between acteoside concentration and soil Hg and Pb suggest that controlled environmental stress may enhance secondary metabolite biosynthesis.

These findings provide an environmental chemistry framework for understanding plant–soil–metal interactions affecting phytochemical quality. From a policy perspective, the results support the integration of soil monitoring and environmental risk assessment into medicinal plant cultivation within the Eastern Economic Corridor (EEC). Such strategies are essential for ensuring sustainable production, phytochemical standardization, and environmental safety of high-value medicinal plants in industrializing coastal regions.

5. Suggestions

For suggestion and future perspectives, the research should include more broader habitat sampling and seasonal monitoring to clarify spatial and temporal influences of soil properties and heavy metals on acteoside biosynthesis in *Acanthus ebracteatus*. Incorporating metabolomic and molecular approaches would further elucidate biosynthetic regulation under environmental stress. From a policy perspective, developing soil quality benchmarks and cultivation guidelines within the Eastern Economic Corridor (EEC) would support sustainable medicinal plant production while mitigating risks associated with heavy metal accumulation.

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7. Declaration of generative AI in scientific writing

AI techniques were utilized for enhancing readability and language quality improvement.

8. CRediT author statement

Phakawan Kongchantree: Conceptualization; Design; Methodology; Validation; Writing -Original draft preparation.

Chakkree Lekklar: Software; Validation; Statistical analysis.

Sataporn Deeying: Conceptualization; Supervision; Constructive comments

Pitthaya Hinbuddee: Design; Methodology.

Sakchai Hongthong: Design; Supervision; Reviewing and Editing.

Amornrassamee Jinnarak: Conceptualization; Design; Methodology; Writing -Original draft preparation; Reviewing and Editing

9. Research involving human and animals rights

Not applicable

10. Ethics Approval and Consent to Participate

Not applicable

11. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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