

Basal Area, Soil, and Elevation Factors Affecting The Essential Oils Yield, Geranyl Acetate, and D-Limonene of *Zanthoxylum acanthopodium*

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Abstract

Andaliman (*Z. acanthopodium*) is an aromatic Non-Timber Forest Product (NTFP) plant species that contains essential oils (EOs), which have various benefits for cuisine, medicine, and cosmetics. This study is essential because it is not yet known what biotic factors (basal area) and abiotic factors (soil and elevation) influence the EOs yield, geranyl acetate, and D-limonene as skin anti-aging. This study investigated whether the basal area, soil, and elevation factors influence the EOs yield, geranyl acetate, and D-limonene content of Andaliman fruit. The biotic and abiotic factors were measured from 9 sample plots representing the Andaliman habitats in North Sumatra, Indonesia. A vegetation analysis was conducted to derive the Andaliman basal area of each sample plot. Soil samples were taken randomly in each plot and analyzed for their physicochemical (pH, C, SOM, N, CNR, P, K, Ca, Mg, CEC, BS, SA, DU, CL, WC, and BD) contents. Elevation gradients were measured on each plot. The EOs of Andaliman fruit include yield, geranyl acetate, and D-limonene as skin anti-aging. The data were analyzed using Partial Least Square Regression for Generalized Linear Models (PlsRglm) to determine which biotic and abiotic factors influenced the EOs yield, geranyl acetate, and D-limonene. This study confirmed that clay soil texture was the main factor influencing EOs of Andaliman fruit yield, geranyl acetate, and D-limonene. Apart from soil factors, basal area, and elevation factors were also essential to consider when cultivating Andaliman fruit as a source of skin anti-aging substances.

Keywords: Andaliman, Aromatic plant, Stem diameter, Nutrients, Altitude, Plsrglm, Clay

Introduction

Andaliman (*Z. acanthopodium*) is an aromatic Non-Timber Forest Product (NTFP) plant species that is essential for conservation, economy, and sociocultural aspects [1]. Andaliman's natural habitat is in forests, especially in mountain forests. This plant is naturally distributed in South, East and Southeast Asia including India, Nepal, Sikkim, East Pakistan, China, India, Myanmar, and Thailand. In Indonesia, Andaliman is distributed across North Sumatra and Aceh [2]. However, Andaliman that produces essential oils (EOs), is still limited in its fruit use as a spice [3] and traditional farming is implemented in Andaliman cultivation [4]. Some NTFP species as aromatic plants that have been developed in Indonesia are agarwood (*Aquilaria* spp. and *Gyrinops* spp.), benzoin (*Styrax* spp.), sandalwood (*Santalum album* L.), and cajuput (*Melaleuca cajuputi* Powell) [5]. The population of agarwood in Indonesia 3.4 million trees with dominant population located in central Kalimantan (24.7 %), North Sumatra (17.9 %) [6]. On the other hand, in North Tapanuli Regency, North Sumatra Province have benzoin plantation covers 22,670 ha and produces 321.3 kg/ha/year resin on average or 4.247 tonnes/year [7]. Sandalwood production from East Nusa Tenggara (NTT) Province, a province in the eastern part of Indonesia, was 38.26 % on average during 1989/1990 - 1993/1994 and 12.17 % during 1995/1996 - 1999/2000 [8], sandalwood oil is generally used in stick incense products, incense cones, soaps, and wood bracelets/prayer beads [5]. Cajuput produced by Perhutani 41,649 tonnes of cajuput leaves and sold 322 tonnes of cajuput oil in Indonesia IDR 85 billion in 2019 [9].

The Andaliman fruit, contains α -sanshool, almost identical with Szechuan pepper contains hydroxy- α -sanshool that causes a tingling trigeminal sensation, pungency, and numbing in the mouth [10-14], it is used by the Batak tribe (a North Sumatran ethnic group) as a unique spice for their dishes. Andaliman fruit have been reported to have multiple benefits for cuisine such as arsik (cooked golden fish), tombur (a condiment used with grilled fish), naniura (fresh raw goldfish which is than fermented), napinadar (fish or grilled meat), beef meatball, terites (medicinal food of a traditional Batak Karo), sambal (additional food from crushed chilies and andaliman), saksang (meat cooked with the blood of slaughtered animals or ordinary spices without blood), and mie gomak (straight noodles) [4,11,15-18]. Additionally, the EOs and chemical compounds of Andaliman can be used for various medicines and cosmetics. The 2 active compounds of the 63 chemical components contained in Andaliman, estragole (15.46 %) and eucalyptol (10.94 %) are reported to prevent malaria and anopheles mosquitoes [19], the methanol extract possess cytotoxic properties with IC_{50} 19.14 $\mu\text{g}/\text{mL}$ against murine P388 leukemia cells [15], inhibit the growth of WiDr and HCT-116 cells (colon cancer cells) *in vitro* with IC_{50} μg 95.61 and 94.64 $\mu\text{g}\cdot\text{mL}^{-1}$ via apoptosis pathway [20], the bark of *Z. acanthopodium* var. timbor have anti-inflammatory effects based on TLC-based generally useful estimation of solvent systems (GUESS) method and high-performance countercurrent chromatography (HPCCC) in combination. Three lignins were enriched by HPCCC with an off-line inhibition mode of nitric oxide production in lipopolysaccharide-stimulated RAW264.7 macrophage cells, using petroleum ether-ethyl acetate-methanol-water (3:2:3:2) as the solvent system [21], and the chemical component EOs of Andaliman leaves, beta-caryophyllene can be used as antiSARS-Cov-2 agents [3]. The geranyl acetate and D-limonene are the 2 dominant chemical compounds in the essential oil of andaliman fruit [11,15,22-24]. The latest study confirmed that geranyl acetate and D-limonene are the most potent chemical compounds of Andaliman fruit EOs as skin anti-aging from North Sumatra Province, Indonesia [25]. The potential of Andaliman fruit EOs as a source of skin anti-aging substances can be optimized as early as the cultivation phase. The high selling price of Andaliman fruit is also an attraction for cultivating this spice plant [26].

The existence of the Andaliman plant is unique because it is challenging for the plant to cultivate outside its natural habitat, include in breeding and regeneration [1,4,18,27-30]. This indicates that the

Andaliman has an ecological preference for living in its in situ habitat. The inability of Andaliman to live outside its natural habitat could potentially threaten the existence of this plant species. Many factors influence the threat to the existence of Andaliman in nature, including decreasing land area, widespread conversion of agricultural land into other functions, low seed germination capacities, undiscovered cultivation techniques, specific natural distribution habitat around Lake Toba, habitat fragmentation, and illegal logging [4,27,29]. Species may respond differently to similar environmental variations in determining ecological preferences [31]. It is essential to know the ecological preferences of Andaliman, especially basal area, soil factors, and elevation that influence EOs yield, geranyl acetate and D-limonene, to support its cultivation development potential as skin anti-aging.

The previous study on the ecology of Andaliman are confirmed that the soil seed bank from the Andaliman habitat contained many seeds from the Compositae, Melastomataceae, Poaceae, Solanaceae, Cannabaceae, Leguminosae and Lamiaceae families [32]. Environmental factors that were reported to affect the growth of Andaliman include light, surface run-off, land openness, normalized difference moisture index (NDMI), normalized difference vegetation index (NDVI), digital elevation model (DEM), rainfall, slope, elevation, land cover, soil type, and critical land [1,28,29]. To the best of our knowledge, there has been no previous study on biotic factor (basal area) and abiotic factors include soil (i.e., pH, C, SOM, N, CNR, P, K, Ca, Mg, CEC, BS, SA, DU, CL, WC, and BD) and elevation that influence the content of Andaliman fruit EOs, which have the potential as skin anti-aging agents (yield, geranyl acetate, and D-limonene). A previous study showed that soil factors generally influenced yield and EOs compounds in kilemo (*Litsea cubeba*) plants [33]. On the other hand, elevation, soil chemistry and growing conditions influenced the chemical components of *Zanthoxylum armatum* leaf EOs in Nepal [34].

The current study used basal area, soil, elevation, EOs yield, geranyl acetate and D-limonene of Andaliman fruit to determine the relationship between variables. The importance of the basal area is closely related to the measurement of plants diameter at the breast height (± 1.30 m above the ground), which indicates tree growth, making it more reliable and essential for forest ecology management [35,36]. The main functions of soils are biomass production, storage and filtration of water, storage and recycling of nutrients, habitat for biological activity, and carbon storage related to the cultivation of the plant [37]. Elevation influences aromatic plants' yield content and EO compounds [38-42]. The EOs are produced by oil glands [11]. This study aimed to determine how the basal area, soil factors and elevation influence the EOs yield, geranyl acetate, and D-limonene content of Andaliman fruit.

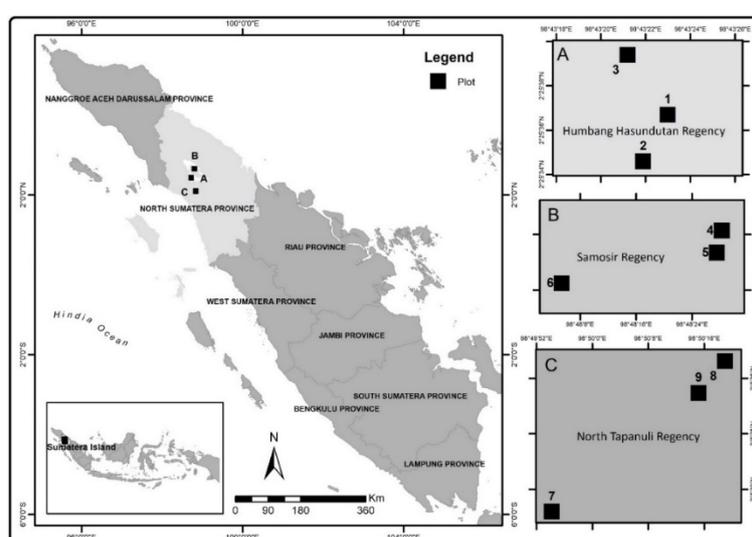
Materials and methods

Study area

The study area was the 3 regencies of North Sumatra Province, near Toba Lake area, Indonesia: Humbang Hasundutan (HH), Samosir (S) and North Tapanuli (NT). The elevation range was 1,500 - 1,800 masl (meters above sea level) (**Figure 1**). This elevation range is included in the mountain forest ecosystem [43]. **Table 1** shows the elevation, latitude and longitude of the sample plots in the study area [1,4,25,28,29].

Table 1 The elevation, latitude and longitude of the study area in North Sumatra Province, Indonesia.

Plot	Location	Elevation (masl)	Latitude/Longitude
1	HH1	1,787	N: 02 25'36.7" E: 098 43'23.0"
2	HH2	1,799	N: 02 25'34,6" E: 098 43'21.9"
3	HH3	1,800	N: 02 25'39.4" E: 098 43'21.2"
4	S4	1,642	N: 02 39'12.6" E: 098 48'28.3"
5	S5	1,612	N: 02 39'09.4" E: 098 48'27.6"
6	S6	1,604	N: 02 39'05.0" E: 098 48'05.4"
7	NT7	1,543	N: 02 05'16.8" E: 098 49'54.2"
8	NT8	1,551	N: 02 05'38.5" E: 098 50'19.0"
9	NT9	1,532	N: 02 05'33.9" E: 098 50'15.2"

**Figure 1** Location map of the Andaliman plots in North Sumatra Province, Indonesia.

Stand structure

The stand structure of Andaliman was assessed through a vegetation analysis in 9 sample plots using the purposive sampling method, a convenience sampling type. For experimental research on small intersectional identity groups, many purposive samples may be fit for use because they trade off design-based representativeness against obtaining a sample size sufficiently large to estimate an experimental effect size [44] powerfully. Selection of plot locations is based on the following criteria: 1) plots are made at locations where Andaliman grows are found, 2) plots are made at locations where measurements are possible [29], 3) plots was created in the Andaliman natural habitat at different elevation levels (1,500 -1,600, 1,600 - 1,700, and 1,700 - 1,800 masl), in each elevation level there are 3 plots. The sample plot is square with the size of each of 9 sample plot was $20 \times 20 \text{ m}^2$ [45]. The sample plots were purposively established in the field to cover the variability of Andaliman habitats in North Sumatra. All Andaliman plants in each sample plots were measured for stem diameter ($\pm 1.30 \text{ m}$ above the ground) using diameter tape and total height using pull meter and vertex series III. Measurement of environmental factors included latitude, longitude and the elevation using Global Positioning System (GPS) of each sample plot. Andaliman specimens were collected by selecting the plant material, do not mix each individual plant, cutting twigs, leaves, flowers and fruit in 1 specimen number from the end with a standard herbarium size of $42 \times 26.4 \text{ cm}^2$. Each individual plant was prepared in triplicate, labeled, then placed in old newspaper, 60-80 % alcohol was used to preserve the

specimens until they were later dried [46]. Furthermore, the specimen collections were identified in the Herbarium Bogoriense.

Soil samples

The sampling of soils using disturbed and undisturbed soil samples methods [47]. The disturbed soil samples were taken randomly at 5 points and then a composite sample was produced; 1 kg of each soil sample in each plot, was put into a black plastic bag using shovel. The undisturbed soil samples were taken using a random sample ring at 3 points in each plot, which were not composited. Disturbed soil samples were analyzed for soil chemical properties including pH (pH) using the potentiometric method [48]; Carbon (C) using the Walkey and Black / Gravimetric method; Soil Organic Matter (SOM) using the Walkey and Black / Gravimetric method; Nitrogen (N) using the Kjeldahl method; Carbon Nitrogen Ratio (CNR) using the Walkey and Black / Gravimetric and Kjeldahl methods; Phosphor (P) using the Olsen and Bray I methods; Potassium (K) used the N NH₄OAc pH 7.0 method; Calcium (Ca) using the N NH₄OAc pH 7.0; Magnesium (Mg) using the N NH₄OAc pH 7.0; Cation Exchange Capacity (CEC) using the N NH₄OAc pH 7.0 method, and BS using the N NH₄OAc pH 7.0 method. However, soil physical properties include sand (SA), silt (DU) and clay (CL) using the pipet methods and water content (WC) using gravimetric methods. Furthermore, the gravimetric method analyzed undisturbed soil samples for Bulk Density (BD) content. Soil analysis assessment was classified using the criteria of Eviati and Sulaeman [49] (Table 2).

Table 2 The criteria of soil analysis assessment.

Soil Parameter	Criteria										
	Very Sour (SM)	Sour (M)	Slightly Sour (AM)	Neutral (N)	Slightly Alkali (SA)	Alkali (A)	Very Low (SR)	Low (R)	Medium (S)	High (T)	Very High (ST)
pH	< 4.5	4.5 - 5.5	5.5 - 6.5	6.6 - 7.5	7.6 - 8.5	> 8.5					
C (%)							< 1	1 - 2	2 - 3	3 - 5	> 5
N (%)							< 0.1	0.1 - 0.2	0.21 - 0.5	0.51 - 0.75	> 0.75
CNR							< 5	5 - 10	11 - 15	16 - 25	> 25
P (mg/Kg)							< 15	15 - 20	21 - 40	41 - 60	> 60
K (cmol(+)/kg)							< 0.1	0.1 - 0.3	0.4 - 0.5	0.6 - 1.0	> 1
Ca (cmol(+)/kg)							< 2	2 - 5	6 - 10	11 - 20	> 20
Mg (cmol(+)/kg)							< 0.3	0.4 - 1	1.1 - 2.0	2.1 - 8.0	> 8
CEC (cmol(+)/kg)							< 5	5 - 16	17 - 24	25 - 40	> 40
BS (%)							< 20	20 - 40	41 - 60	61 - 80	> 80

The EOs

Grown-up Andaliman plants in 10 varieties that already bear fruit in the vegetation plot take the ripe fruit 200 g, air dry in triplicate for each Andaliman variety. EOs of Andaliman fruit was produced through hydrodistillation for 6 h. Sodium sulfate anhydrous pro-analysis was used to separate EOs and water; meanwhile, in the N-hexan pro-analysis, Merck was used to dilute EOs of Andaliman fruit before GC-MS analysis [25]. Furthermore, GC-MS analysis was used to obtain the identity of the chemical compounds in the EOs of Andaliman fruit. A heatmap in the Orange data mining software used for grouping and showing the abundance of chemical compounds of 10 varieties. Based on the GC-MS analysis, the dominant and characteristic compounds of the EOs of the Andaliman fruit varieties were selected as the test ligands, while the control ligand used was ascorbic acid. The Protein Data Bank was used to obtain the 3-dimensional crystal structure of hyaluronidase (2PE4), tyrosinase (5M8R), collagenase (2TCL) and elastase (3F19) as receptor. Molecular Docking was carried out using the software AutoDockTools 1.5.7. The ligand with the

highest negative affinity energy was acquired from the outcome of the molecular docking analysis. The highest negative affinity energies of the 10 dominant compounds and the identification of the compounds with each receptor were then analyzed admetSAR and visualized to decide the chemical compounds that are prospective candidates as skin anti-aging agents. The specific chemical compounds chosen in this study are geranyl acetate and D-limonene, which have the potential to be used as skin anti-aging substances. Detailed procedures are explained in the previous study [25].

Statistical analysis

The independent variables in this study were basal area (BA), 16 soil factors (i.e., pH, C, SOM, N, CNR, P, K, Ca, Mg, CEC, BS, SA, DU, CL, WC, and BD), and elevation. The dependent variables were yield (Y), geranyl acetate (G) and D-limonene (L) of the in situ Andaliman habitats. The vegetation analysis method was used to calculate the basal area (m^2ha^{-1}) of the Andaliman in each plot [50]. The data were analyzed using Partial Least Square Regression for Generalized Linear Models (PlsRglm) [51,52]. The PlsRglm is commonly used to analyze small amounts of data with collinear predictors, and it provides a cross-validation technique to test the significance of each predictor in the data set [51]. In this study, the PlsRglm was used to determine which biotic and abiotic factors influence the EOs of Andaliman fruit. The PlsRglm was carried out using 3 main steps: 1) Fitting regression models to the data and choosing 4 PLS components, 2) bootstrapping the model with 1,000 replications and saving the model coefficients and 3) calculating bias-corrected and accelerated bootstrap (BCa) and confidence intervals (CI). The analysis was implemented in R Studio (4.3.2.), using the readxl, plsRglm and plsdo packages [53].

Results and discussion

This study continues the study of compounds in the Andaliman fruit varieties with most potential as skin anti-aging using the molecular docking method that has been carried out previously [25]. This study found that the Andaliman habitats had altitude ranges of 1,500 - 1,800 masl, which enriched the information of previous study that Andaliman was found maximal at an altitude of around of 1,600 masl [29]. There were challenges during the field data collection because the distribution of Andaliman was scattered with limited areas, so this study used a limited number of samples. However, the plot coverage in this study of 9 plots measuring $20 \times 20 \text{ m}^2$ (0.36 ha) is wider than previous study using 11 plots measuring $10 \times 10 \text{ m}^2$ (0.11 ha) [29]. Still, we found a relationship pattern between basal area, soil factors, and elevation that influence Andaliman fruit EOs, especially yield, geranyl acetate and D-limonene as skin anti-aging.

The soil characteristics of Andaliman habitat

The soil properties where Andaliman grows have various characteristics (**Table 3**). The Humbang Hasundutan location (1,700 - 1,800 masl) has relatively higher soil contents than the altitude below, especially C, SOM, CNR, P, and WC, respectively. On the other hand, the soil on Samosir Island (1,600 - 1,700 masl) tends to have higher N, CEC, and BD contents than other locations. Meanwhile, North Tapanuli (1,500 - 1,600 masl) has higher pH, Ca, Mg, and BS. Macronutrients are essential nutrients that plants need in large quantities, such as C, H, O, N, P, K, S, Ca, and Mg. N plays a role in building protein, building green leaves and triggering vegetative growth. The presence of P is needed to build proteins, make up Adenosine Monophosphate (AMP), Adenosine Diphosphate (ADP), Adenosine Triphosphate (ATP), transfer energy and trigger the generative or reproductive period. On the other hand, K acts as a catalyst. Ca is a constituent of Ca-Oxalate and Ca-Pectate and also generates cell turgor. Mg is needed for chlorophyll composition, enzyme activation, seed formation and oil synthesis [54].

Table 3 The soil properties of Andaliman habitats in North Sumatra Province, Indonesia.

Variable	Location					
	HH	Criteria	S	Criteria	NT	Criteria
pH	4.85 ± 0.18	M	4.38 ± 0.28	SM	5.63 ± 0.56	AM
C (%)	7.20 ± 3.79	ST	6.35 ± 3.78	ST	4.29 ± 3.26	T
SOM (%)	12.38 ± 6.52		10.92 ± 6.50		7.38 ± 5.61	
N (%)	0.32 ± 0.14	S	0.36 ± 0.08	S	0.26 ± 0.23	S
CNR	23.00 ± 6.08	T	16.67 ± 6.43	T	17.33 ± 2.52	T
P (mg/Kg)	37.88 ± 7.94	S	3.34 ± 1.98	SR	12.64 ± 11.50	SR
K (cmol(+)/kg)	0.42 ± 0.43	S	0.34 ± 0.11	S	0.42 ± 0.24	S
Ca (cmol(+)/kg)	2.65 ± 2.28	R	1.12 ± 0.98	SR	3.59 ± 2.74	R
Mg (cmol(+)/kg)	0.61 ± 0.64	R	0.51 ± 0.25	R	1.22 ± 0.79	S
CEC (cmol(+)/kg)	15.35 ± 4.93	R	21.59 ± 2.85	S	13.78 ± 6.00	R
BS (%)	22.40 ± 14.76	R	9.47 ± 7.12	SR	34.57 ± 13.35	R
WC (%)	13.36 ± 5.98		5.04 ± 0.34		6.78 ± 3.85	
BD (g/cc)	0.34 ± 0.29		0.51 ± 0.07		0.47 ± 0.15	

Criteria for soil analysis assessment: (SM = Very Sour, M = Sour, AM = Slightly Sour, N = Neutral, SA = Slightly Alkali, A = Alkali, SR = Very Low, R = Low, S = Medium, T = High and ST = Very High [49]).

The influence of basal area, soil and elevation factors on Andaliman EOs

The PlsRglm results are shown in **Table 4**, and BCa Bootstrap CI of regressor's coefficients with dependent variables of yield, geranyl acetate and D-limonene are shown in **Figure 2**. Environmental conditions, including elevation, soil, and growing conditions play an essential role in plants producing EOs yield and compounds [34]. Plant biochemical processes are influenced by various chemical elements in the soil found in the plant rhizosphere and included in the enzyme composition [55]. Therefore, the composition of EOs can be influenced by soil chemistry [56]. The composition of EOs is also influenced by the altitude at which they grow [57]. Basal area is positively related to plant density [58,59]. In conditions of high plant density, it will trigger stress in plants [60] and this stress in plants affects to EOs [61,62]. Therefore, soil factors, elevation, and basal area are essential in determining the yield and chemical components of EOs.

Table 4 The PlsRglm output.

Independent Variable	Dependent Variable					
	Y (R ² = 97.97)		G (R ² = 86.44)		L (R ² = 79.96)	
	Coefficients	95 % BCa CI	Coefficients	95% BCa CI	Coefficients	95 % BCa CI
BA	0.1073*	(0.02, 0.24)	0.3542*	(0.28, 0.60)	0.0742	(-0.10, 0.56)
pH	0.2941*	(0.19, 0.49)	0.0659	(-0.19, 0.27)	-0.3389*	(-0.53, -0.21)
C	-0.0552	(-0.16, 0.00)	-0.1802*	(-0.37, -0.12)	0.1251*	(0.01, 0.26)
SOM	-0.0551	(-0.16, 0.00)	-0.1814*	(-0.37, -0.12)	0.1252*	(0.01, 0.26)
N	-0.0158	(-0.13, 0.14)	-0.0937	(-0.25, 0.08)	0.0290	(-0.15, 0.29)
CNR	-0.0571	(-0.33, 0.04)	-0.1246	(-0.57, 0.07)	0.2123*	(0.03, 0.39)
P	0.0645	(-0.07, 0.20)	-0.0741	(-0.44, 0.15)	-0.1171	(-0.64, 0.07)
K	-0.0459	(-0.16, 0.04)	-0.0281	(-0.39, 0.54)	0.2873*	(-0.02, 0.99)
Ca	0.1498*	(0.06, 0.28)	-0.1664*	(-0.30, -0.15)	-0.1237	(-0.30, 0.25)
Mg	0.1409*	(0.05, 0.22)	0.0637	(-0.15, 0.32)	-0.1344	(-0.29, 0.14)

Independent Variable	Dependent Variable					
	Y ($R^2 = 97.97$)		G ($R^2 = 86.44$)		L ($R^2 = 79.96$)	
	Coefficients	95 % BCa CI	Coefficients	95% BCa CI	Coefficients	95 % BCa CI
CEC	-0.1672*	(-0.25, -0.12)	0.0819	(-0.02, 0.28)	0.1108	(-0.08, 0.21)
BS	0.1790*	(0.11, 0.28)	0.0846	(-0.13, 0.33)	-0.0756	(-0.22, 0.29)
SA	0.0193	(-0.17, 0.12)	0.3208*	(0.18, 0.78)	0.0598	(-0.02, 0.22)
DU	-0.1814*	(-0.26, -0.11)	-0.1410*	(-0.30, -0.08)	-0.1622	(-0.74, 0.17)
CL	-0.2615*	(-0.45, -0.19)	-0.3117*	(-0.51, -0.28)	0.4509*	(0.17, 1.41)
WC	0.0525	(-0.09, 0.24)	-0.5978*	(-1.05, -0.37)	-0.0018	(-0.30, 0.55)
BD	-0.0656	(-0.23, 0.09)	-0.3899*	(-0.81, -0.04)	0.3590	(-0.08, 0.96)
Elevation	-0.1589*	(-0.45, -0.03)	0.0978	(-0.19, 0.46)	0.2343*	(0.03, 0.55)

* Significant at 5 % level, Y = yield, G = geranyl acetate, L = D-limonene and R^2 = coefficient of determination.

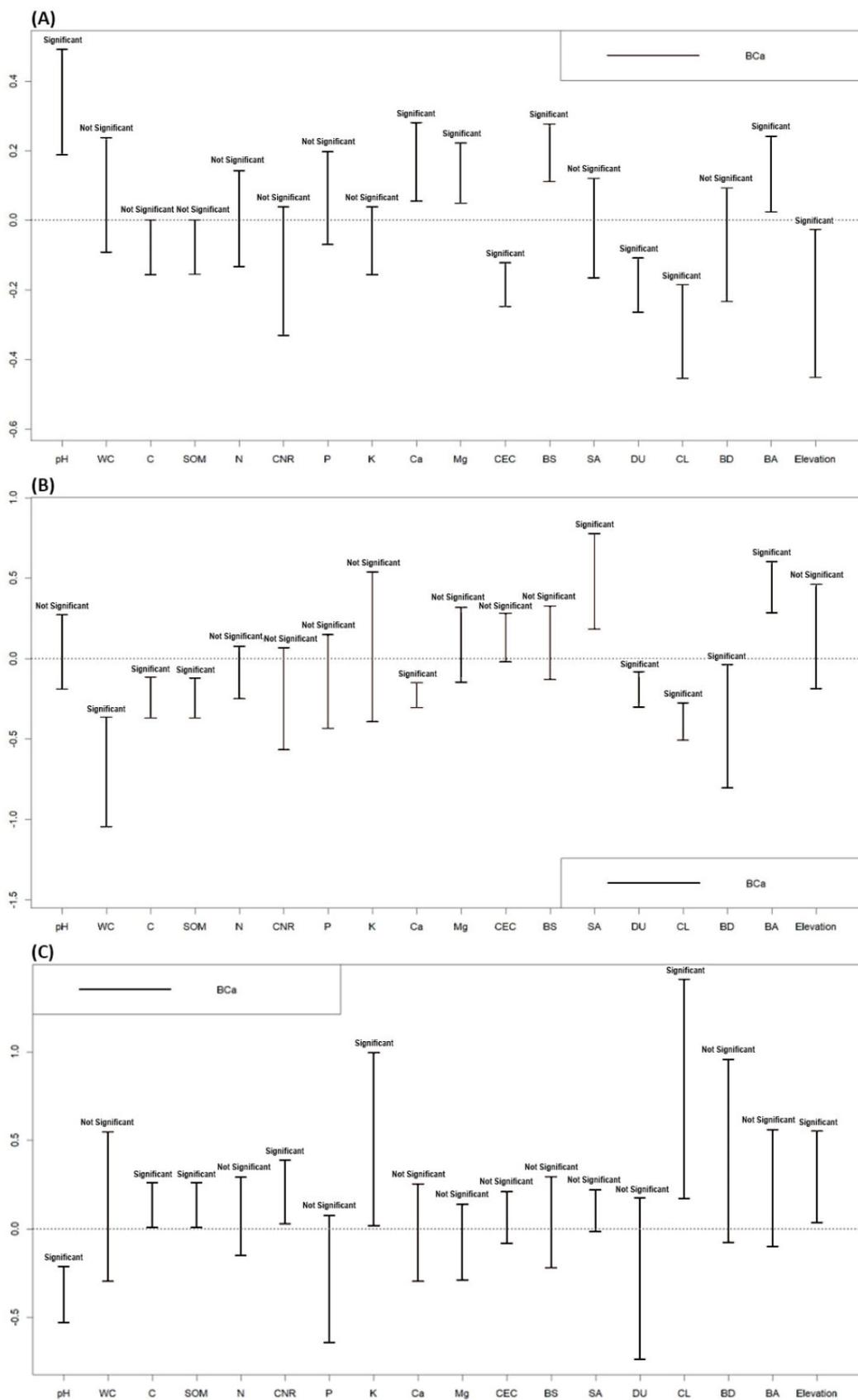


Figure 2 BCa Bootstrap CI of regressor's coefficients with dependent variables of yield (A), geranyl acetate (B), and D-limonene (C).

The factors that significantly influence the yield of Andaliman fruit EOs in this study are BA, pH, Ca, Mg, CEC, BS, DU, CL, and elevation. The BA was positively related to the yield of Andaliman fruit EOs. The BA of Andaliman in North Sumatra ranges between 0.18 ± 0.11 - 1.26 ± 0.82 m²ha⁻¹. It is higher than the BA of the *Zanthoxylum zanthoxyloides* in Nigeria 0.82 ± 0.01 m²ha⁻¹ [63]. Variation in BA increment depends on tree-specific factors, inter-tree relationships and the environment, including soil factors [36]. Andaliman plants generally have a low density and are rarely found in nature. Therefore, Andaliman cultivation requires setting appropriate plant spacing. Andaliman soil generally has an acidic pH [64]. In this study, the lower the pH condition, the lower the yield of Andaliman fruit EOs. Low pH conditions will generally affect the availability of nutrients in the soil. It also affects soil weathering, which is the process of releasing structural nutrients from soil minerals. The release of structural nutrients is more potent at low pH because the H⁺ ions which are abundant in the soil system, will act as an attacking agent that can destroy mineral structures so that they can release nutrients into groundwater [54].

The Ca and Mg contents were positively related to the yield of Andaliman fruit EOs. The application of Ca and Mg can increase the yield of EOs but does not effect on the EO compounds of *Origanum vulgare* ssp. *hirtum* plant [65]. The yield of Andaliman fruit EOs was negatively related to CEC. Overall uptake and translocation from soil to plant systems play an essential role in CEC [66]. However, high CEC does not always impact the high availability of other soil nutrients [67]. This might affect the yield of Andaliman fruit EOs. The BS plays vital role in soil fertility assessment and soil taxonomic classification, but BS can cause low actual CEC values [68]. This explains the positive relationship between BS and yield, while the opposite is true for CEC. Soil texture (DU,CL) is negatively related to yield. CL soil texture has high water holding capacity but poor drainage. Therefore, clay soil can cause water accumulation and puddles, which may disrupt root growth and cause Andaliman plants to not grow properly. The DU soil texture has good drainage so that root easily penetrate it, but it does not have a good water holding capacity so plants are susceptible to drought. The other study explained that soil texture is key factors that strongly influences the yield of *Thymus migricus* EOs [69]. The elevation factor has a negative significant relationship on yield. This study shows that the highest yield was obtained from Andaliman fruit EO that located at the lowest altitude, 1,500 - 1,600 masl. This finding is in line with the other study that reported there was a decrease in EOs yield caused by an increase in altitude [70]. Therefore, for the successful planting of Andaliman plants that have skin-anti aging potential, it is necessary to consider the elevation factor.

Geranyl acetate and D-limonene are aromatic components in Andaliman fruit [22]. The geranyl acetate and D-limonene compounds in Andaliman fruit from North Sumatra are 29.87 and 26.49 %, respectively. These 2 compounds are important compounds as skin anti-aging agents [25]. Geranyl acetate also has biological activity as an insecticidal and repellent against *Ulomoides dermestoides*. This compound is found in other plant species, *Cymbopogon citratus* (12.4 %) [71], *Dracocephalum moldavica* (9.14 - 33.06 %) [72], and the aerial parts of *Thapsia minor* plant (82.3 - 83 %) [73]. D-limonene is a monocyclic monoterpene, abundant in citrus family plants. The application of D-limonene is generally used for flavor and fragrance, but it also plays essential role in preventing several degenerative and chronic diseases. This treatment effect is because D-limonene has biological activity as an antioxidant, antidiabetic, anti-cancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune-modulatory, anti-fibrotic and anti-genotoxic which is used for health [74].

The chemical compounds of Andaliman fruit EOs that have the potential as skin anti-aging, which are geranyl acetate and D-limonene is significantly influenced by different factors. The geranyl acetate content in Andaliman fruit EOs is influenced by BA, WC, C, SOM, Ca, SA, DU, CL, and BD. On the other hand, the D-limonene content is influenced by pH, C, SOM, CNR, K, CL, and Elevation. Although these 2

Andaliman fruit EOs compounds are generally impacted by different factors, there are also the same factors, C, SOM, and CL.

This study confirms that increasing BA has a positive effect on the geranyl acetate so increasing BA positively affects. The water content is negatively related to the EOs content of geranyl acetate. Water stress can significantly increase the content of EOs compounds in *Tymus eriocalyx* plants in Iran [75]. On the other hand, water content can increase nutrient content and plant production [76]. Aromatic plants can survive various adverse conditions on marginal land. Carbon sequestration in the soil and increased biomass yield can be obtained from recycling aromatic plant distillation waste on marginal land [77]. SOM influences soil properties (aggregation, porosity and CEC), thus determining the soil's ability to provide desired results, including plant productivity and soil functional capacity [78].

This study shows that Ca is negatively related to the content of geranyl acetate EOs. Increasing Ca (NO₃)₂ levels gradually reduces the EOs content of the *Artemisia dracunculus* plant [79]. SA and DU soil texture in this study significantly affected the components of geranyl acetate EOs. This is in line with another study that explains soil texture influences the content of EO compounds in the purple basil plant [80]. BD negatively affects the geranyl acetate component in Andaliman fruit EOs. BD fluctuates with soil structural conditions. Study in India shows a negative relationship between BD and all soil nutrients except sandy soil [81]. Soil pH is one of the soil factors that significantly affects the EOs composition of the aromatic plant *Z. armatum* [34]. CNR has a positive effect on the content of the D-limonene compound in Andaliman fruit EOs. Increasing potassium and elevation will increase the D-limonene content in Andaliman fruit EOs. Potassium and elevation are also the main factors influencing the composition of *Juniperus rigida* EOs [39].

The contents of N and P, essential macronutrients, had no significant effect on the overall response of EOs (yield, geranyl acetate and D-limonene). On the contrary, another study stated that N and P fertilizers significantly increased EOs content of *Z. armatum* 10.1 - 40.2 % [82]. This is because the soil nutrient content that plays a role in oil synthesis is Mg [54]. CL soil texture is a factor that significantly influences all responses (yield, geranyl acetate, and D-limonene) of Andaliman fruit EOs. This is in line with another study that stated that the components of *Litsea cubeba* EOs are influenced by clay soil texture factors [33]. This is because clay can cause puddles, excess water in plants triggers stress and increases EOs content as a defense mechanism. Clay soil is also rich in nutrients, so nutrients can be absorbed optimally by Andaliman fruit plants to produce EOs that can be used as skin anti-aging.

Conclusions

Soil factors, especially clay soil texture substantially impacts the Andaliman fruit EOs as a whole (yield, geranyl acetate, and D-limonene). Planting Andaliman with a clay soil texture is recommended to produce Andaliman fruit, which has the potential as a source of for skin anti-aging substances. The basal area has a significant effect on yield and geranyl acetate but not on D-limonene. On the other hand, elevation factors have a significant effect on yield and D-limonene, but not geranyl acetate. Apart from soil factors, basal area and elevation factors are also essential factors that need to be considered for cultivating Andaliman fruit as skin anti-aging substances.

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