

THE CHEMOMETRIC APPROACHES FOR DPPH RADICAL SCAVENGING ACTIVITIES MEASUREMENT OF *Cananga odorata* L. AND ITS CORRELATION WITH ENVIRONMENTAL PARAMETERS

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ABSTRACT

Cananga odorata L. (CO), belonging to the Annonaceae family, has been widely reported to have pharmacological effects and is a good source of phenolics, flavonoids, and saponins, which contribute to its antioxidant activity. Using chemometric approaches, this study investigated the impact of environmental parameters such as location height, soil physicochemical characteristics, and heavy metal content on total phenolic content (TPC) and total flavonoid content (TFC), as well as their correlation with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of CO leaf extracts. Fresh leaves and soil samples were gathered from 25 distinct locations. Following the post-harvest process, the grounded leaves were macerated with 70% ethanol for 72 hours, and the resulting dry-thickened extract was further examined. The soil was analyzed for heavy metal concentrations and physicochemical properties. The findings showed that CO samples grow at an altitude of 8-865 m above sea level, with pH-values of soil ranging from 5.51 to 7.44, with the most prominent soil texture being loamy soil. Soil sample from Banjarsari-Surakarta had the highest organic carbon content (7.80). In addition, the KR Bogor-Bogor CO sample included the greatest quantities of TPC and TFC. The extract from KR Cibinong-Bogor demonstrated the strongest DPPH radical scavenging activity, with an IC₅₀ value of 42.14 µg/mL. The loading plot of PCA demonstrated a positive correlation between altitude and total P on TPC, TFC, and DPPH. This study found that the various leaf extracts of CO have remarkable phytochemical properties that could be affected by specific environmental factors, implying a high bioactive component content and potential as novel antioxidant sources.

Keywords: *Cananga odorata*, loading plot, DPPH, chemometric approaches, total phenolics content.

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Published first online October 08, 2025

Published final November 30, 2025

INTRODUCTION

Cananga odorata L., known as "ylang-ylang", is an evergreen perennial tropical tree belonging to a family of Annonaceae. CO is taken into account as one of the interesting medicinal plants due to its pharmacological activities including antioxidant and antibacterial activities toward some micro-organisms. In traditional medicine, CO has been reported to be used widely for treating some diseases including asthma, malaria, and pneumonia. In addition, this plant is well-known for its fragrant essential oil, which is extensively used in cosmetics, perfumery, and aromatherapy preparations (Pavela *et al.*, 2021) (Romano *et al.*, 2024). The essential oils coming from

this plant have been approved as Generally Recognized as Safe (GRAS) by the Flavour and Extract Manufacturers Association (FEMA) to be used as a flavoring agent and adjuvant in the food industry (Tan *et al.*, 2015).

The extract, fractions, and phytochemical compounds isolated from this plant are also reported to have antibiofilm, antidiabetic, antifertility, anti-melanogenesis, sedative, and anxiolytic effects as reviewed by (Rahmawati *et al.*, 2024). These activities are related to the bioactive compounds present in this plant, especially phenolics and flavonoid compounds responsible for antioxidant activities (Gharsallah *et al.*, 2023). GC-MS analysis revealed the following CO

chemical constituents: p-methoxy toluene, benzyl alcohol, linalool, methyl benzoate, citronellol, geraniol, eugenol, geranyl acetate, ylangene, caryophyllene, humulene, γ -muurolene, germacrene D, α -farnesene, and δ -cadinene, benzoic acid, 2-hydroxy-2-methylbutyl ester, amyl salicylate, diethyl phthalate, cis-isoeugenol, α -cadinol, cedryl acetate, benzyl benzoate, phenylacetic acid, 2-methylphenyl ester, benzoic acid, and 2-hydroxy phenylmethyl ester (Denkova *et al.*, 2023; Mrani *et al.*, 2024). The environmental and agricultural factors can significantly affect the phytochemical profiles of CO. Climate, including temperature, humidity, and rainfall, plays a crucial role in the growth and development of this plant, affecting the bioactive compounds' concentration and composition. Soil characteristics include pH, organic carbon (C), total phosphorus (P), total nitrogen (N), and heavy metals content can influence the health of the plants and the quality of phytochemicals produced. Additionally, the altitude at which CO is grown can lead to variations in the concentration of phytochemicals, potentially impacting biological activities (Mrani *et al.*, 2024). Indeed, the study on the impacts of soil quality and altitude on the compositions of phytochemicals especially phenolics and flavonoids, and their correlation with biological activities is very urgent.

The plant including CO is a good source of phenolics, flavonoids, and saponins contributing to antioxidant activity. Antioxidants are any materials capable of delaying oxidation reactions through several mechanisms including radical scavenging activities, lipid peroxidation inhibition, chelating agents, and synergists. Among these mechanisms, the radical scavenging activity is the most reported due to its simplicity and rapidity (Rohman *et al.*, 2019). The natural-based antioxidants are related to the TPC and TFC, therefore, in this study the radical scavenger activity of CO correlated with TPC and TFC (Mistriyani *et al.*, 2018). The data coming from the soil quality and altitude are very huge, therefore the use of multivariate data analysis or chemometric approaches is unavoidable. The chemometric analysis has been successfully used for the correlation between antioxidant activities and TPC-TFC (Widodo *et al.* 2019) and for the correlation between environmental factors and essential oil contents (Nugraheni *et al.*, 2024). In this study, the impact of environmental parameters namely location height, soil physicochemical characteristics, and heavy metal content on TPC and TFC, as well as their correlation with the DPPH radical scavenging activity of CO leaf extracts have been highlighted.

MATERIALS AND METHODS

Chemicals: The current study employed ethanol (E-Merck), ethanol 70% (technical grade), distilled water (Ikapharmindo), Folin-Ciocalteu reagent (Sigma-Aldrich), sodium carbonate (E-Merck), AlCl_3 (E-Merck),

acetate buffer of pH 3.6, quercetin (Sigma-Aldrich), gallic acid (Sigma-Aldrich), and L-(+)-ascorbic acid (E-Merck).

Preparation of plant extracts: Fresh CO leaf samples were collected in 2024 at close intervals from several growing areas on the Indonesian island of Java, with altitudes ranging from 5-900 meters above sea level. The samples went through post-harvest processing, which included washing, sorting, drying, and grinding. A 40-mesh powder was extracted using the maceration process with 70% ethanol solvent with a ratio of 1:10, for 72 hours (Roanisca and Mahardika, 2020; Irwan *et al.*, 2024). The acquired extract was evaporated using a water bath, followed by an oven at 40°C until dry or thick, stored in a glass sample bottle in the fridge at 4-8°C, and ready for testing (Dini *et al.*, 2019).

The soil sample collection: The sampling of soil where CO grows was carried out using the disturbed soil sampling approach, as described in earlier research (Zhao *et al.*, 2010; Simmons, 2023). Samples were collected from plant areas at a depth of 0-20 cm for approximately 0.5-2 kg. The sample was stored in the zip-lock plastic bag, dried at 70°C, and ready to be used for further analysis.

Soil physicochemical analysis: The physical analysis of soil was carried out by evaluating the soil texture as well as the percentages of sand, clay, and dust. Soil chemical analyses including pH value, percentage of organic carbon (C), total phosphorus (P), and total nitrogen (N) were carried out using the pH meter method, Walkley-Black Method, Kjeldahl Method, and HNO_3 and HClO_4 extraction methods, respectively using procedures used by Pratiwi *et al.* (2022) and Saputra *et al.* (2020).

Heavy metal analysis of soil samples: The heavy metal composition of soil samples including 32 elements (Pb, Zn, Fe, Cu, Ni, Ti, Ca, Mo, Zr, Sr, Rb, Se, As, W, Co, Cr, V, Sb, Sn, Cd, Ag, Pd, Nb, Bi, Th, U, Hg, Sc, K, S, Ba, Cs, Te) were analyzed using a Portable Thermo X-ray Fluorescence (XRF) instrument (Scientific Niton type XL3t 500 Analysers) using SiO_2 as calibration standard (Lukita *et al.*, 2025). Since several metals were not detected due to their low levels, only a few metals would be displayed in the results.

TPC determination: The TPC of CO extracts were determined using the Folin-Ciocalteu reaction (Cicco *et al.* 2009; Widodo *et al.* 2019). The standard curve equation was prepared by correlating the concentration series of gallic acid (0-32 $\mu\text{g}/\text{mL}$) and absorbance values measured using a UV-Vis spectrophotometer at 732 nm. The standard curve equation derived is $Y=0.0352X-0.0223$ ($R^2=0.9985$). The tested sample was prepared by dissolving extract in ethanol (1 mg/mL) and homogenized with a sonicator for 15 minutes. The extract

solution (40 μL) was mixed with 360 μL of distilled water and 10 μL of 10% Folin-Ciocalteu reagent, then homogenized, left for 2 minutes, add 500 μL of 5% sodium carbonate and incubated at 40° for 20 minutes. A total of 150 μL of sample solution was added into a microplate, and the absorbance was read using a UV vis spectrophotometer at 732 nm. TPC was calculated as mg gallic acid equivalent per gram sample (mg GAE/g extract).

Analysis of TFC: Determination of TFC was carried out according to (Widodo *et al.* 2019). using quercetin as reference standard. The standard curve equation was prepared by correlating quercetin at concentrations ranging from 0 to 80 $\mu\text{g/mL}$ (*x-axis*) and absorbance values (*y-axis*). The standard curve was obtained with the equation $Y=0.0231X-0.0006$ ($R^2=0.9991$). To prepare the sample, 10 mg of extract was dissolved in 10 mL of ethanol (1000 $\mu\text{g/mL}$) and homogenized using vortex and sonicator for 10 minutes. To prepare a blank solution, 350 μL of distilled water and 250 μL of acetate buffer with pH 3.6 were mixed, and ethanol was added until a volume of 1 mL. To prepare the test sample solution, 350 μL of distilled water, 250 μL of acetate buffer, and 150 μL of AlCl_3 were mixed, and ethanol was added to a volume of 1 mL. A microplate was filled with 150 μL of each sample and blank solution, and the absorbance values were measured by UV-vis spectrophotometer at 421 nm. TFC was expressed as quercetin equivalent ($\mu\text{g QE/mL}$ sample).

DPPH radical scavenging activity assay: DPPH radical scavenging activity was determined according to Sharma and Bhat (2009). The sample solution had a final volume of 1.0 mL with an extract concentration of 0-100 $\mu\text{g/mL}$, and DPPH concentration at 70 μM . The absorbance measurements were taken at 515 nm after 25-60 minutes (depending on operating time optimization). The percentage of inhibitory activity is calculated using the following formula:

$$\frac{(A_0-A_1)/A_0}{1} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the test extract or standard. The antiradical activities were expressed as IC_{50} using (L-+)-ascorbic acid at a concentration of 0-5 $\mu\text{g/mL}$ as positive control.

Chemometric Analysis: Minitab version 18 (Minitab LLC Corp., USA) was used to perform the chemometric analysis of principal component analysis (PCA). The PCA score plot was used to classify CO extract samples based on characteristics such as clay, organic C, dust, N-total, pH, sand, P total, altitude, TPC, TFC, and DPPH radical scavenging activity. While the loading plot of PCA was utilized for the correlation between variables including clay, organic C, dust, N-total, pH, sand, P-total, altitude, TPC, TFC, and DPPH radical scavenging activities of CO extract samples.

RESULTS AND DISCUSSION

The present study explored the environmental aspects, namely location height, soil physicochemical properties, and heavy metal content on TPC and TFC, as well as their correlation with the DPPH radical scavenging activity of CO leaf extracts. The leaf samples of CO were collected from diverse growing areas on Java Island (Indonesia), at elevations ranging from 8 to 865 meters above sea level. Differences in the height of a plant's growth location have been shown to effect both plant development and secondary metabolite content. Altitude is an important environmental factor that influences ecological niches by affecting the composition and diversity of microbial communities in soil ecosystems. Altitudinal gradients produce significant variability in vegetation, with different plant groupings influencing soil organic matter and root exudates. In natural ecosystems, elevation effects soil properties such as pH, organic content, and nutrient availability (Reyes-Ardila *et al.*, 2024). Soil, with its physical, chemical, and biological characteristics has a significant influence on plant production (Jakišić *et al.*, 2024). Organic matter contributes significantly to the chemical, physical, and biological properties of soil (Moradi *et al.*, 2024). The physical characteristics of the soil examined in this study include soil texture, which shows the fineness or roughness of soil and is determined by the percentages of sand, clay, and dust. According to the United States Department of Agriculture (USDA) concerning soil texture classification, loamy soil texture dominates CO growth in contrast to other soil texture classifications, accounting for 52% (Moreno-Maroto and Alonso-Azcárate, 2022). Loam soil containing roughly equal proportions of sand, silt, and clay, has good permeability and drainage, and retains nutrients and moisture effectively, making it well-suited for plant growth (Zohry, 2022). The finer the texture of the soil, the more micropores are produced and filled with water and air (Isra *et al.*, 2019).

The soil chemical properties highlighted include pH, organic C level, total N, total P, and heavy metal compositions. Soil pH, often known as soil acidity, measures the concentration of hydrogen ions in soil. Soil pH significantly affects soil fertility and plant viability (Kusuma *et al.*, 2014). Plants thrive best in pH levels ranging from 6.5 to 7.5. In this environment, nutrients have good solubility in water, thus they are more easily absorbed by soil (Karamina *et al.*, 2018). Soil pH analysis in this investigation yielded findings ranging from 5.51 to 7.44 with the lowest and highest pH observed in successive soil samples from Turen-Malang and Imogiri-Bantul, while the remaining 60% was in the appropriate pH range, implying that most of our CO sample plants were growing in the optimal soil pH condition. Table 1 revealed the altitude and physicochemical properties of

soil where CO grows. Soil pH has an impact on other soil chemical parameters such as organic carbon content, total N, and total P. The greatest total P level was 0.53 and belonged to soil samples from Ngerong-Magetan with an ideal pH of 7.02 (Table 1). In low soil pH conditions, phosphorus will react with iron and aluminium ions to generate iron phosphate and aluminium phosphate, which are difficult to dissolve in water and cannot be absorbed

by plants. Likewise, if the pH is excessively alkaline, phosphorus will combine with calcium ions to produce calcium phosphate, which is difficult to dissolve and cannot be used by plants (Siswanto, 2019). Applying organic fertilizer generates significant amounts of phosphorous, nitrogen, and other vital components (Huang *et al.*, 2025).

Table 1. The altitude and soil physicochemical analysis results

Sample code	East longitude	South Latitude	Altitude (masl)	Silt (%)	Clay (%)	Sand (%)	Soil texture	pH	Organic C	Total P	Total N
1	112.68353	-7.53505	8	40.00	18.19	41.81	Loamy	7.05	1.26	0.12	0.42
2	110.37595	-7.91590	33	27.27	18.41	54.32	Sandy loam	7.44	2.60	0.17	0.35
3	110.82195	-7.56945	93	26.29	21.88	51.83	Loamy	6.07	7.80	0.14	0.56
4	113.24049	-8.05795	94	38.58	21.88	39.54	Loamy	6.56	2.16	0.12	0.28
5	110.89963	-6.99393	125	30.44	16.09	53.47	Sandy loam	7.22	3.96	0.24	0.56
6	110.89928	-6.99379	129	33.42	18.05	48.53	Loamy	7.16	3.98	0.15	0.60
7	110.90132	-6.99293	129	22.11	31.43	46.46	Sandy clay loam	7.17	4.00	0.14	0.50
8	106.85573	-6.48940	137	25.13	53.92	20.95	Clay	6.01	3.82	0.16	0.45
9	110.67550	-7.53687	197	26.11	14.36	59.53	Sandy loam	6.99	1.64	0.29	0.46
10	112.11654	-8.03258	202	31.93	22.68	45.4	Loamy	6.00	2.38	0.16	0.36
11	112.12995	-8.03556	220	40.33	17.61	42.06	Loamy	6.89	1.60	0.19	0.20
12	112.13292	-7.96820	249	28.44	22.89	48.67	Loamy	7.43	1.90	0.17	0.20
13	110.23673	-7.63980	258	25.38	44.99	29.63	Clay	6.93	2.20	0.19	0.20
14	110.23621	-7.63963	259	27.85	44.23	27.91	Clay	6.51	2.62	0.09	0.29
15	110.23618	-7.63972	260	29.67	23.87	46.46	Loamy	6.42	1.34	0.17	0.20
16	106.80452	-6.59988	272	20.29	36.93	42.78	Clay loam	5.99	2.32	0.10	0.32
17	112.65211	-8.20016	352	25.75	33.09	41.16	Clay loam	5.51	3.08	0.13	0.35
18	112.71996	-7.79392	404	30.98	21.45	47.57	Loamy	6.53	1.62	0.15	0.24
19	112.71245	-7.79734	457	30.44	34.54	35.02	Clay loam	6.34	3.22	0.15	0.34
20	111.12537	-7.79641	520	38.4	23.51	38.09	Loamy	6.98	2.22	0.21	0.25
21	112.70000	-7.79714	528	33.64	23.04	43.33	Loamy	6.21	2.50	0.23	0.35
22	110.42633	-7.62376	651	33.05	19.57	47.38	Loamy	6.02	3.58	0.23	0.39
23	111.10240	-7.65469	766	31.38	22.46	46.16	Loamy	6.43	3.98	0.40	0.95
24	111.25095	-7.68237	851	15.93	16.09	67.98	Sandy loam	7.00	2.56	0.13	0.34
25	111.24902	-7.68394	865	33.24	18.81	47.96	Loamy	7.02	3.36	0.53	0.29

Samples are taken from 1 = Porong-Sidoarjo (East Java), 2 = Imogiri-Bantul (Yogyakarta), 3 = Banjarsari-Surakarta (Central Java), 4 = Kedungjajang-Lumajang (East Java), 5 = Kemambe-Grobogan (Central Java), 6 = Katekan-Grobogan (Central Java), 7 = Brati-Grobogan (Central Java), 8 = KR Cibinong-Bogor, 9 = Banyudono-Boyolali (Central Java), 10 = Jagoan-Blitar (East Java), 11 = Pojok-Blitar (East Java), 12 = Sidorejo-Blitar (East Java), 13 = Sambeng-Magelang (Central Java), 14 = Kedungan-Magelang, 15 = Borobudur-Magelang (Central Java), 16 = KR Bogor-Bogor (West Java), 17 = Turen-Malang (East Java), 18 = Pasuruan-Pasuruan (East Java), 19 = Purwodadi-Pasuruan (East Java), 20 = Jatipurno-Wonogiri (Central Java), 21 = Gajahrejo-Pasuruan (East Java), 22 = Cangkring-Sleman (Yogyakarta), 23 = Plumbon R. atsiri-Karanganyar (Central Java), 24 = Plaosan-Magetan (East Java), and 25 = Ngerong-Magetan (East Java).

The organic carbon of 25 soil samples ranged from 1.26 to 7.8%, in which samples from Banjarsari-Surakarta exhibited the highest organic C level of 7.8% and Porong-Sidoarjo samples had the lowest values of 1.26%. In this investigation, soil samples were obtained at depths ranging from 0 to 20 cm. Sari *et al.* (2017) reported no significant change in organic C content at a soil depth of 0-30 cm. Soil organic carbon accounts for two-thirds of the carbon in soils, and has a direct impact on soil fertility and structure as an aggregate-forming substance that serves as a binder between soil particles

(Chen *et al.*, 2025; Triadiawarman, 2015). In addition to carbon, nitrogen is an essential element for protein synthesis and plant vegetative growth. Nitrogen is expected to have a significant impact on the nutritional content of purslane plants (Montoya-García *et al.*, 2025). Soil nitrogen levels fluctuate depending on use and management (Triadiawarman, 2015). This study's total N values ranged from 0.2 to 0.92%.

Another environmental element investigated in this study is the heavy metal content of soil samples. Table 2 revealed the contents of several heavy metals

from 25 soil samples where CO grows, including Ba, Zr, Sr, Rb, Pb, Zn, Fe, Mn, Cr, V, Ti, Ca, K, and S, with varied levels for each component. According to their toxicity to organisms, which is determined by their capacity to accumulate in biological systems, chemical reactivity, and interactions with cells and tissues, heavy metal contamination in soils can be classified into three groups, namely highly toxic (e.g., Pb, Cd, and Hg), less toxic (e.g., Ti, As, and Bi), and essential (e.g., Fe, Zn, Co, and Cu) (Imtiaz *et al.*, 2025). These heavy metals have significant environmental concerns due to their persistent and poisonous nature, posing threats to plants and ecosystems. Heavy metals can limit plant growth, reduce fertility, disrupt natural processes, and harm microbial populations (Kamaraj *et al.*, 2025). Highly toxic heavy metals such as Pb, Hg, and Cd, can accumulate in the body, produce hazardous compounds, and interact with essential biological components, resulting in cell damage and reduced bodily function (Banisharif and Amooaghaie, 2025). Pb in the soil enters plants by attaching to the cell wall of the root, primarily via esterified pectin. Heavy metals delay plant growth and promote oxidative stress by converting cysteine into reduced and oxidized glutathione. They also block and change membrane transport mechanisms, which interferes with photosynthesis (Rai *et al.*, 2019; Naz *et al.*, 2025).

The TFC, TPC, and DPPH radical scavenging activities of CO leaf extracts derived from 25 areas are compiled in Table 3. Extract from KR Bogor-Bogor revealed the highest TFC and TPC corresponding to 33.06 $\mu\text{g QE/g}$ and 97.15 mg GAE/g, respectively. Extract from Kedungan-Magelang had the lowest TFC (1.50 $\mu\text{g QE/g}$), whereas extract from Jatipurno-Wonogiri, showed the lowest TPC (23.83 mg GAE/g). Phenolic substances are recognized to have antioxidant abilities by neutralizing free radicals through redox characteristics, allowing them to serve as reducing agents and hydrogen donors. Flavonoids serve as protective agents for plants against various stresses, functioning as unique UV filters, signal molecules, allopatric compounds, phytoalexins, and detoxifying agents (Shukla *et al.*, 2025). Figure 1 exhibited IC_{50} values of CO leaf extracts from 25 different locations (from lowest to highest) for DPPH radical scavenging activity. Among these samples, the extract coming from KR Cibinong-Bogor showed the lowest IC_{50} value of 42.14 $\mu\text{g/mL}$ (Figure 1). Samples with the lowest IC_{50} values indicated the highest DPPH radical scavenging activities and vice versa. According to Jumina *et al.*, (2019) and Setha *et al.*, (2013), a substance is classified as a very powerful antioxidant if the IC_{50} value is less than 50 $\mu\text{g/mL}$, a strong antioxidant if the IC_{50} value is 50-100 $\mu\text{g/mL}$, a moderate antioxidant if the IC_{50} value is 100-150 $\mu\text{g/mL}$, and a weak antioxidant if the IC_{50} value is 151-200 $\mu\text{g/mL}$. Based on the measured IC_{50} values, the KR

Cibinong-Bogor extract has a very powerful significant antioxidant activity with the IC_{50} value less than 50 $\mu\text{g/mL}$. All evaluated samples showed lower antiradical activity than that of positive control of *L*-ascorbic acid having an IC_{50} value of 2.21 $\mu\text{g/mL}$.

In this study, the chemometric approaches were used to categorize the 25 different CO leaf extracts using unsupervised pattern recognition of principal component analysis (PCA). Using the score plot of the first principal component (PC1) and second principal component (PC2) as the output of PCA, it is easier to visualize and analyze multidimensional, heterogeneous, and elaborate datasets. Figure 2 exhibited PC1 and PC2 of the evaluated samples based on the variables of clay, organic C, dust, N-total, pH, sand, P total, altitude, TPC, TFC, and DPPH. PC1 contributed to 23.9% of the variance, while PC2 and PC3 contributed to variances of 22.4% and 16.5%, respectively, therefore the first three PCs accounted for 62.8%. This suggests that the three components can successfully capture the bulk of the variability in the dataset, demonstrating the resilience of the chemometric analysis used to classify the samples. The score values of PC1 and PC2 are also called latent variables in which samples with similar scores of PCs have similar characteristics based on the variables used during PCA analysis (Man *et al.*, 2011). Based on PC1 and PC2, samples of 12 (CO from Sidorejo-Blitar, East Java) and 10 (Jagoan-Blitar, East Java) exhibited close similarity compared to others. This is not surprising since both samples come from the same regions, therefore, both samples have similar soil characteristics and physicochemical properties.

Figure 3 depicts the loading plot of PCA which may be used for investigating the correlation among variables analyzed (clay, organic C, dust, N-total, pH, sand, P total, altitude, TPC, TFC, and DPPH radical scavenging activities). The loading plot of PCA demonstrated how the vectors are pinned from the origin of $\text{PC1} = 0$ and $\text{PC2} = 0$. The loading plot was also interpreted as the weight of each variable on PCs. If two vectors create a tiny angle, then both variables are positively correlated. If variables make an angle of approximately 90° , they are unlikely to be correlated, and when two variables are divergent and form a big angle (about 180°), they imply a negative correlation (Widodo *et al.*, 2019). The results reveal a positive correlation between altitude, P total, TPC, TFC, and CO extracts' DPPH radical scavenging activity. This is consistent with the findings of Widodo *et al.* (2019) and Elkomy *et al.* (2025) which showed that total phenolic compounds have a positive correlation with antioxidant activity. Extracts with higher TFC are often linked with stronger antioxidant activities, whereas those with lower TFC shown lower activity (Liu *et al.*, 2025).

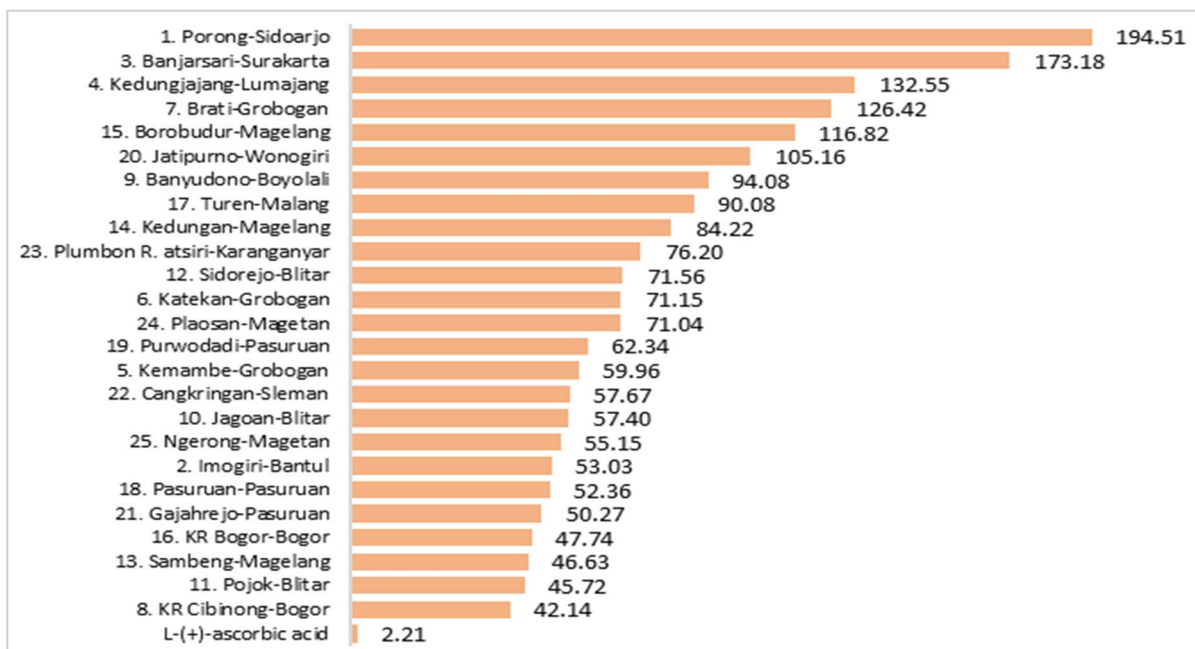
Table 2. XRF analysis results on heavy metal compositions of soil samples (%)

Sample	Ba	Zr	Sr	Rb	Pb	Zn	Fe	Mn	Cr	V	Ti	Ca	K	S
1	488.2	85.9	343.6	21.8	30.8	54.5	43109.1	1576.4	0.0	0.0	2090.3	21632.4	5011.1	0.0
2	256.1	78.0	349.0	22.1	0.0	92.7	29769.3	950.7	0.0	53.6	1628.2	47560.4	5099.6	0.0
3	0.0	122.7	130.2	15.5	19.4	61.4	44568.5	1341.1	0.0	113.7	2715.9	6729.6	2931.0	0.0
4	245.4	102.8	255.1	17.3	0.0	0.0	87842.4	1777.7	0.0	196.4	3865.7	8642.8	3208.6	0.0
5	0.0	95.8	121.0	34.0	22.9	55.2	16631.2	234.8	23.7	0.0	1199.1	35693.7	2635.6	28806.0
6	0.0	32.1	241.2	14.1	0.0	28.9	6222.2	169.9	0.0	0.0	266.2	124195.9	1644.1	0.0
7	0.0	132.6	87.9	34.4	0.0	60.0	18877.2	267.6	44.6	0.0	1293.6	15858.4	2806.6	23984.4
8	0.0	141.2	19.5	0.0	0.0	67.3	52942.3	2093.5	0.0	0.0	2856.7	860.1	0.0	21113.0
9	0.0	85.3	437.0	21.1	0.0	222.8	29889.4	854.3	41.2	0.0	1492.5	17381.2	3032.0	12961.0
10	169.6	34.1	487.6	7.7	0.0	35.4	36802.0	1103.6	21.6	0.0	1283.4	16165.3	2356.6	19437.4
11	196.6	38.7	504.1	10.0	0.0	0.0	35315.9	971.3	0.0	64.2	1374.9	16903.0	2536.7	20731.5
12	198.0	41.3	543.2	14.9	0.0	80.4	47424.0	1187.3	0.0	0.0	1868.5	27288.5	4912.0	0.0
13	161.7	106.7	210.2	19.3	25.1	0.0	75773.0	1518.9	0.0	173.2	3457.8	6804.6	2871.5	0.0
14	0.0	105.4	98.3	5.8	0.0	0.0	77128.6	1944.7	0.0	91.3	3191.7	810.2	541.2	0.0
15	0.0	97.8	81.4	9.4	0.0	0.0	64598.9	1335.7	0.0	138.1	2785.7	2669.5	838.7	28426.4
16	0.0	127.6	58.8	6.3	15.4	0.0	50663.0	1295.3	0.0	102.9	2777.4	515.4	477.2	24861.8
17	150.0	69.9	173.3	0.0	0.0	0.0	71078.5	1593.9	27.1	127.0	2582.1	5014.9	666.2	23042.0
18	157.2	115.5	101.6	12.6	0.0	0.0	84890.3	2035.7	29.0	126.8	3505.5	3067.5	1445.8	14913.6
19	176.9	118.1	95.4	15.0	0.0	0.0	89721.2	1844.9	48.9	219.4	4070.9	2302.3	1428.0	26005.1
20	149.7	75.9	156.8	19.9	0.0	56.5	99991.7	1839.2	45.8	223.5	3478.7	8094.8	1962.2	19264.3
21	81.5	98.8	169.3	13.8	0.0	40.5	61813.4	1375.2	41.5	139.7	2673.5	5081.7	1352.3	27045.8
22	0.0	67.6	412.3	24.5	0.0	47.3	26591.4	753.8	0.0	52.0	1474.9	14947.4	6167.3	0.0
23	0.0	67.1	101.2	12.6	0.0	60.5	17993.1	610.1	43.3	0.0	1031.3	11252.2	1679.1	22750.4
24	0.0	84.5	271.9	19.6	32.1	111.3	26460.5	506.4	40.3	87.4	1488.1	7995.5	2509.5	27655.5
25	0.0	91.6	282.1	20.9	76.4	399.3	40275.6	927.6	0.0	0.0	2235.6	37248.7	3564.8	0.0

See Table 1 for each number's sample location description

Table 3. Total flavonoid content (TFC), total phenol content (TPC), and radical scavenging DPPH activity of *C. odorata* extracts

Sample	Mean±SD		
	TFC (µg QE/g)	TPC (mg GAE/g)	IC ₅₀ of DPPH (µg/mL)
1. Porong-Sidoarjo	5.23±0.74	33.34±0.41	194.51±7.29
2. Imogiri-Bantul	10.35±0.91	65.28±0.45	53.03±0.37
3. Banjarsari-Surakarta	7.00±1.60	33.45±0.17	173.18±3.65
4. Kedungjajang-Lumajang	6.11±0.20	43.26±0.72	132.55±0.80
5. Kemambe-Grobogan	2.54±0.28	37.19±0.60	59.96±0.42
6. Katekan-Grobogan	5.14±0.87	52.04±0.34	71.15±0.90
7. Brati-Grobogan	4.83±0.58	34.54±0.50	126.42±0.75
8. KR Cibinong-Bogor	6.55±0.60	81.32±0.18	42.14±0.12
9. Banyudono-Boyolali	9.99±0.27	53.32±1.30	94.08±2.06
10. Jagoan-Blitar	19.62±1.35	32.13±0.15	57.40±0.37
11. Pojok-Blitar	8.75±0.73	83.33±0.34	45.72±0.54
12. Sidorejo-Blitar	7.94±0.53	64.38±0.29	71.56±0.56
13. Sambeng-Magelang	10.16±0.36	92.46±0.25	46.63±0.51
14. Kedungan-Magelang	1.50±0.27	45.29±0.46	84.22±1.16
15. Borobudur-Magelang	3.93±0.16	46.01±0.41	116.82±0.60
16. KR Bogor-Bogor	33.06±1.61	97.15±1.00	47.74±1.28
17. Turen-Malang	3.63±0.52	34.62±0.38	90.08±0.56
18. Pasuruan-Pasuruan	14.68±0.17	85.55±0.24	52.36±0.58
19. Purwodadi-Pasuruan	21.52±1.76	78.77±0.75	62.34±0.63
20. Jatipurno-Wonogiri	20.35±1.15	23.83±1.21	105.16±0.36
21. Gajahrejo-Pasuruan	10.16±0.36	42.89±0.47	50.27±0.92
22. Cangkringan-Sleman	9.02±0.65	79.93±0.38	57.67±1.62
23. Plumbon R. atsiri-Karanganyar	10.79±0.17	78.64±0.74	76.20±1.24
24. Plaosan-Magetan	21.23±1.31	72.82±0.07	71.04±0.81
25. Ngerong-Magetan	28.97±4.73	67.52±0.57	55.15±0.63

**Figure 1. The IC₅₀ values (µg/mL) for DPPH radical scavenging activity of L-(+)-ascorbic acid and CO leaf extracts from 25 different locations. Samples with the lowest IC₅₀ values indicated the highest DPPH radical scavenging activities and vice versa**

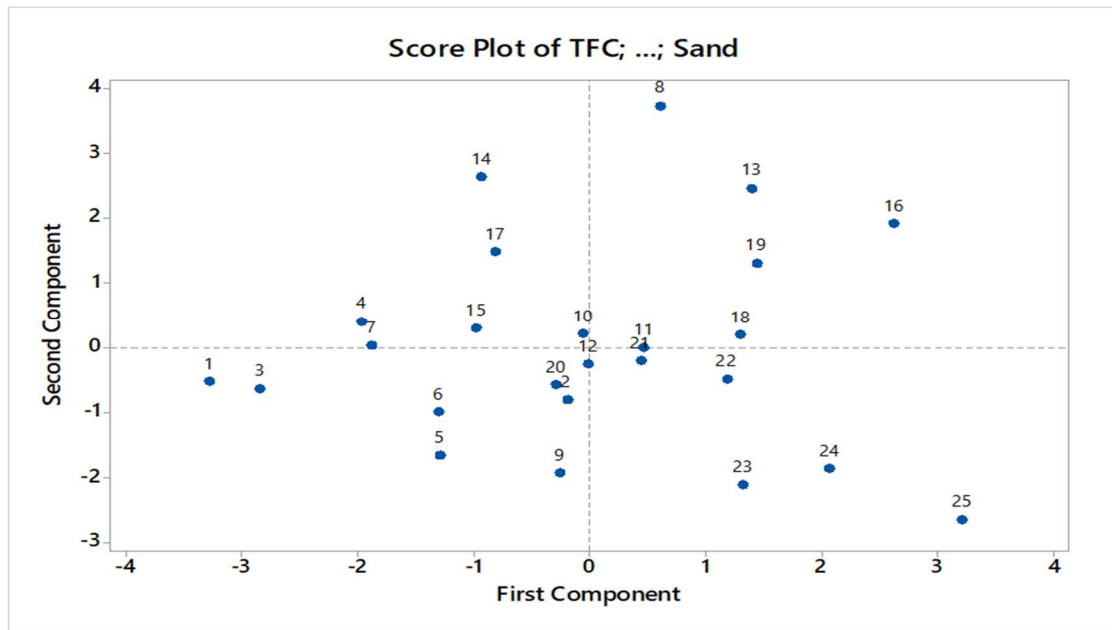


Figure 2. The score plot of PCA for the classification of CO extract samples using variables of clay, dust, sand, organic C, N-total, P total, pH, altitude, TPC, TFC, and DPPH radical scavenging activities (See Table 1 for each number's sample location description)

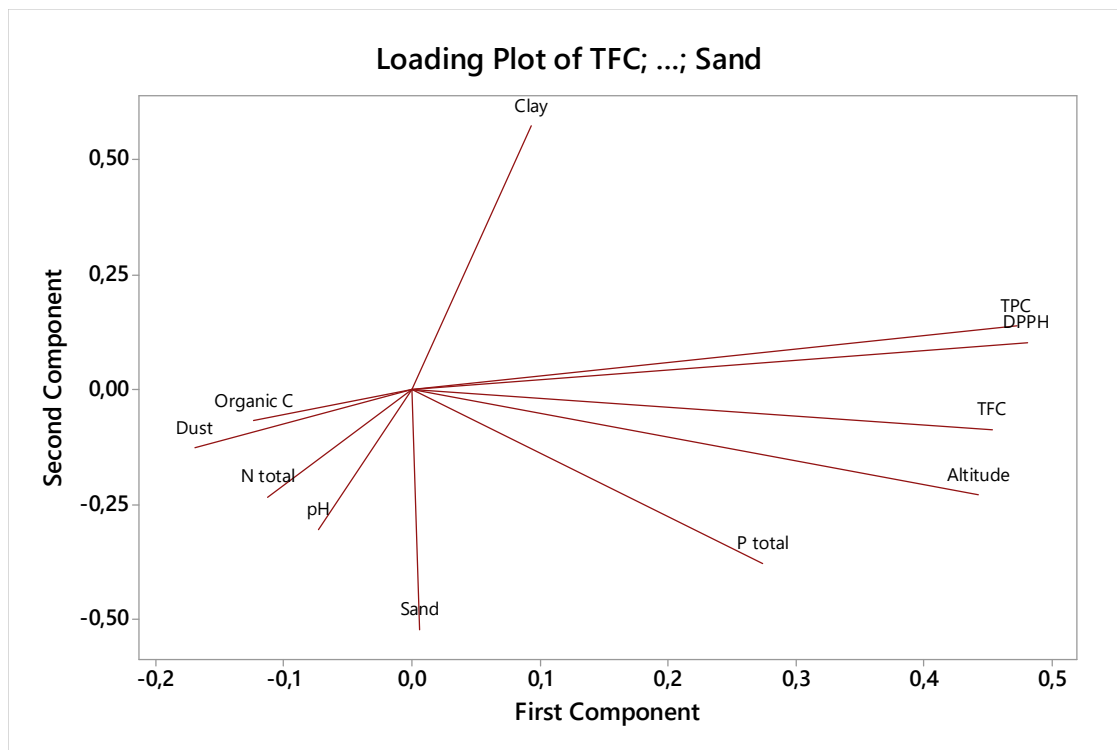


Figure 3. The loading plot of PCA for the correlation among variables namely clay, organic Carbon, dust, Nitrogen total, pH, sand, total P, altitude, TPC, TFC, and DPPH radical scavenging activities of CO extract samples.

Conclusion: Among 25 CO leaf extracts, the KR Cibinong-Bogor ethanolic extract demonstrated the strongest DPPH radical scavenging activity with an IC_{50}

value of 42.14 $\mu\text{g/mL}$. PCA was successfully used to classify 25 different CO extract samples based on the variables clay, organic C, dust, N-total, pH, sand, P total,

altitude, TPC, TFC, and DPPH radical scavenging capabilities. The loading plot of PCA revealed a positive correlation between altitude and total P on TPC, TFC, and DPPH radical scavenging activity. This study discovered that several CO leaf extracts exhibit extraordinary phytochemical capabilities that may be influenced by particular environmental variables, signifying a high bioactive component concentration and potential as new antioxidants. Further research on chemical components, in vivo biological activity, and mechanism of action is required to offer a thorough baseline for future CO leaf extract development and evidence-based public use.

Acknowledgments: We would like to thank the Research Organization of Health, BRIN for financing support under the 'Rumah Program' in 2024 with decree number 6/III.9/HK/2024 and Directorate of Research, Universitas Gadjah Mada for supporting the payment of Article Processing Charge through scheme of *Rekognisi Tugas Akhir* 2025.

Author contributions: All authors made equal contributions to the study's concept and design. All authors reviewed and approved the published version of the manuscript.

Conflict of interest: All of the authors state that there is no conflict of interest in the publishing of this article.

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