Production and Phytochemical Variation of *Kaempferia rotunda* L. Rhizomes in Relation to Soil Nutrient Conditions on Madura Island

Variasi Produksi dan Fitokimia Rimpang *Kaempferia rotunda* L. pada Berbagai Kondisi Hara Tanah di Pulau Madura

Diana N. Sholehah[1]*, Risqiyah[1], Suci Aulia Putri[1], Choirul Umam[1], Fadlilaturrahmah[2], Sinar Suryawati[1]

[1]Agroechotechnology Study Program, Faculty of Agriculture, University of Trunodjoyo Madura, Bangkalan, East Java 69162, Indonesia

[2]Departement of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjar Baru, South Kalimantan 70714, Indonesia

ABSTRACT

Kaempferia rotunda L. is a traditional medicinal plant in Madura. This study aimed to evaluate the production and phytochemical variation of *K. rotunda* rhizomes from Madura Island and to determine the soil chemical factors associated with these traits. Plant materials and soil samples were collected from ex situ cultivation areas in Bangkalan, Sampang, Pamekasan, and Sumenep. Rhizome production, simplicia yield, extract yield, total phenolics, total tannins, total steroids, and soil nutrient contents were analyzed. The results showed substantial variation among accessions in both production and phytochemical contents. Primary rhizome weight was positively correlated with the number of secondary rhizomes, whereas secondary rhizome simplicia yield was positively correlated with total rhizome simplicia yield. Soil nutrients showed low-to-moderate correlations with production traits but strong correlations with phytochemical contents. Suitable accession selection combined with balanced macro- and micronutrient availability may improve rhizome yield and quality.

ABSTRAK

Kaempferia rotunda L. merupakan tanaman obat tradisional yang digunakan di Madura. Penelitian ini bertujuan mengevaluasi variasi produksi dan fitokimia rimpang *K. rotunda* dari Pulau Madura serta menentukan faktor kimia tanah yang berasosiasi dengan karakter tersebut. Bahan tanam dan sampel tanah dikumpulkan dari area budidaya *ex situ* di Bangkalan, Sampang, Pamekasan, dan Sumenep. Analisis dilakukan terhadap produksi rimpang, rendemen simplisia, rendemen ekstrak, total fenolik, total tanin, total steroid rimpang dan kandungan hara tanah. Hasil penelitian menunjukkan adanya variasi yang nyata antaraksesi pada produksi dan kandungan fitokimia. Bobot rimpang primer berkorelasi positif dengan jumlah rimpang sekunder, sedangkan rendemen simplisia rimpang sekunder berkorelasi positif dengan rendemen simplisia total rimpang. Hara tanah menunjukkan korelasi rendah hingga sedang terhadap atribut produksi, tetapi berkorelasi kuat terhadap kandungan fitokimia. Pemilihan aksesi yang sesuai dan keseimbangan makro- serta mikronutrien berpotensi meningkatkan hasil dan mutu rimpang.

Corresponding author:
Diana N. Sholehah (dianans@trunojoyo.ac.id)

1. INTRODUCTION

Kaempferia rotunda L. is s one of the plants used in traditional herbal medicine in Madura. The species is distributed in tropical and subtropical regions of Asia and Africa and commonly grows wild in wet, humid forests and other shaded areas. *K. rotunda* is known as *kunci pepet* in Indonesia, Bhumichampaka in Hindi, and Blackhorm or Indian Crocus in English (Joy et al., 2006; Kumar et al., 2015). Traditionally, this plant has been used to treat stomach pain, fever, indigestion, diarrhea, colic, inflammation caused by ruptures or injuries, and to accelerate wound healing, and it also functions as a mucolytic (Athira et al., 2020). Previous studies have shown that extracts and isolated compounds of *K. rotunda* exhibit various pharmacological activities, including antibacterial, anticancer, antihyperglycemic, antiandrogenic, and wound-healing effects (Aryantini et al., 2022; Athira et al., 2020). These bioactivities are attributed to its phytochemical constituents, indicating that this species has potential for development into pharmacological and health-promoting products (Lim, 2016). Several bioactive phytochemicals have been identified in *K. rotunda*, including phenolic compounds such as flavonoids, chalcones, quercetin, flavonols, and syringic acid; sterols such as β-sitosterol and stigmasterol; tannin-related compounds such as protocatechuic acid; and hydrocarbons such as crotepoxide (Athira et al., 2020).

The rhizome is the main part of the plant used as a raw material in traditional medicine. However, information on the raw material quality of *K. rotunda* remains limited. Raw material quality can be assessed through exploration across different locations. The selection of rhizome accessions is generally based on key rhizome characteristics related to production performance, including shape, weight, size, and the number of rhizome parts (Nurcholis et al., 2019). Phytochemical content is also an important indicator of medicinal plant quality. It is derived from plant secondary metabolism and is responsible for medicinal properties, thereby contributing to the production of various traditional medicinal products (Borges et al., 2017).

Several factors may influence rhizome production, including plant genotype, cultivation techniques, and site of origin (Sontsa-Donhoung et al., 2021). Plant growth and production depend on the soil's capacity to supply and retain sufficient nutrients in the soil solution. Although soil contains various nutrients required for plant growth, these nutrients are not always available in sufficient amounts for optimal nutrition (Burneo et al., 2021). Previous studies have demonstrated that plant species growing under different soil nutrient conditions may exhibit substantial variation in production traits, including both physical and chemical characteristics.

Madura Island is located in the northeastern part of Java Island and is separated from East Java by the Madura Strait. The island is characterized by climate types D and E, and its agriculture largely depends on rainfall (Hariri et al., 2021; Supriyadi, 2009). In line with the findings of Sholehah et al. (2016), the rhizosphere environment, including soil chemical properties, is an extrinsic factor that significantly influences plant production. Soil levels of N, P, and organic C have been reported to correlate with the dry weight and curcumin content of turmeric rhizomes. Srinivasan et al. (2016) also reported a positive correlation between the availability of organic matter, organic carbon, N, P, K, Zn, and Fe in the soil and nutrient uptake, as well as their roles in the biosynthesis of volatile oil, curcumin, and oleoresin in turmeric. Therefore, this study aimed to identify variation in the production and phytochemical characteristics of *K. rotunda* rhizomes from Madura Island and to determine the soil chemical factors influencing these traits.

2. METHODS

2.1. Study Period and Area

The study was carried out from August to December 2024. The research sites included *K. rotunda* planting areas in four districts on Madura Island: Bangkalan (Latitude -7.02475040, Longitude 112.74919060), Sampang (Latitude -7.19131290, Longitude 113.25322670), Pamekasan (Latitude -7.161367, Longitude 113.482498), and Sumenep (Latitude -7.00172100, Longitude 113.86421700). Environmental conditions at the time of sampling were temperature of 30.0–36.6 °C, relative humidity of 45–65%, and light intensity ranging from 4,200 to 50,000 lux.

2.2. Collection of Plant Material

Plant materials were collected from ex situ cultivation areas, with each location consisting of 10 rhizome clumps. Sampling was conducted during the dry season. The sampling locations, shown in **Figure 1a**, were determined based on interviews with key informants, including medicinal plant farmers, traditional healers, traditional medicine traders, and local community leaders. The accession codes and their origins are presented in **Figure 1b**.

2.3. Collection of Soil Samples

Composite soil samples were collected from a depth of 0–30 cm at each site. Soil samples were analyzed for N, P, K, organic carbon, organic matter, Zn, and Cu. Soil organic carbon and organic matter were determined by wet digestion, while total nitrogen was determined using the Kjeldahl method. Total phosphorus (P_2O_5) was determined using the molybdate blue colorimetric method with a spectrophotometer. Total potassium (K_2O) was determined using atomic absorption spectrophotometry (AAS), whereas Zn and Cu were determined by DTPA extraction (Ojikpong & Undie, 2019; Sulaeman et al., 2005). Nutrient levels were categorized according to Hardjowigeno (2015) and Reddy et al. (2017).

2.4. Evaluation of Rhizome Production and Chemical Attributes

Rhizome morphology of *K. rotunda* is illustrated in **Figure 2**. Production attributes included the number of secondary rhizomes, primary rhizome weight, secondary rhizome weight, simplicia yield, and extract yield. Primary and secondary rhizomes were

counted per clump, with each clump containing 1–3 primary rhizomes.

Simplicia and extract yields were determined according to Widiyastuti et al. (2024). Simplicia was prepared by slicing the rhizomes and drying them in an oven at 60 °C until the moisture content was below 10%. Simplicia yield was calculated as:

$$\text{Simplicia yield (\%)} = \frac{\text{Simplicia weight (g)}}{\text{Fresh rhizome weight (g)}} \times 100\%$$

Extracts were obtained by maceration using 96% ethanol. The filtrate was concentrated using a rotary evaporator. Extract yield was calculated as:

$$\text{Extract yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Simplicia weight (g)}} \times 100\%$$

Phytochemical analysis included total phenolics, total tannins, and total steroids from dried rhizome powder. Total phenolics and tannins were analyzed according to the method of Panzella et al. (2019), while total steroids were analyzed according to Noormazlinah et al. (2019).

2.5. Data Analysis

Statistical analysis was performed using analysis of variance (ANOVA) based on a randomized block design. Significant differences among accessions were further tested using the Scott–Knott test. Similarities and differences among accessions were analyzed using multivariate methods, including hierarchical clustering with heatmaps, principal component analysis (PCA), and Pearson correlation. All statistical analyses were conducted using RStudio software (version 4.4.3).
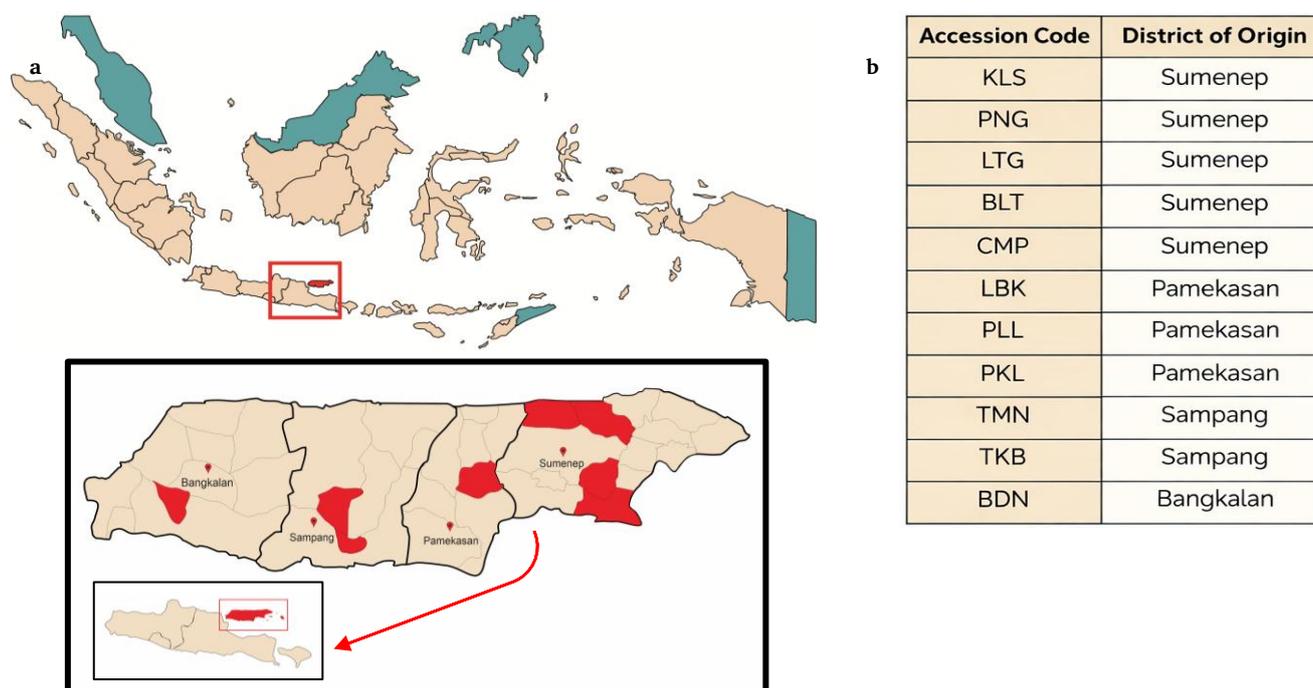

Accession Code	District of Origin
KLS	Sumenep
PNG	Sumenep
LTG	Sumenep
BLT	Sumenep
CMP	Sumenep
LBK	Pamekasan
PLL	Pamekasan
PKL	Pamekasan
TMN	Sampang
TKB	Sampang
BDN	Bangkalan

Figure 1. Location of sampling sites and accession origin of *K. rotunda* from Madura Island. (a) Map of Indonesia showing the location of Madura Island and the sampling districts (Bangkalan, Sampang, Pamekasan, and Sumenep). (b) Accession codes and their districts of origin.

Figure 2. Rhizomes of *K. rotunda*: (a) primary rhizome; (b) secondary rhizome.

3. RESULTS AND DISCUSSION

3.1. Rhizome Production Attributes of *K. rotunda* Accessions

The cultivation of *K. rotunda* in Madura is commonly practiced in home gardens, where irrigation primarily depends on rainfall. This study was conducted during the dry season, when only the rhizomes remained in the soil and the aboveground plant parts had dried out. Leaf emergence typically occurs at the onset of the rainy season. Rhizome production attributes determine yield potential, including simplicia yield. Simplicia refers to the dried form of medicinal plant material that has been sliced or powdered and can be used directly as traditional medicine or as raw material for further herbal processing (Kissinger et al., 2024). Therefore, simplicia yield represents an important parameter in the production of herbal raw materials.

Among the evaluated accessions, BLT showed the highest number of secondary rhizomes and the highest primary rhizome weight. In contrast, PNG exhibited the highest secondary rhizome weight. In terms of simplicia yield, BLT, TKB, and BDN recorded the highest primary rhizome simplicia yield, while LTG showed the highest secondary rhizome and total simplicia yields (**Table 1**). These results indicate variability in rhizome production among accessions collected from different locations on Madura Island.

Table 1. Production attributes of *K. rotunda* accessions from Madura Island

Accession	NSR	PRW (g)	SRW (g)	PRSY (%)	SRSY (%)	TRSY (%)
KLS	22.40 b	15.27 c	1.80 c	40.08 b	20.19 b	27.53 c
PNG	17.00 c	18.32 b	2.24 a	39.26 b	22.30 b	27.34 c
LTG	11.20 d	14.63 c	1.64 d	41.63 b	28.52 a	35.00 a
BLT	43.90 a	26.28 a	1.92 c	47.12 a	22.75 b	31.24 b
CMP	7.90 d	19.70 b	1.78 c	41.09 b	22.64 b	29.69 b
LBK	19.90 c	17.25 b	1.95 c	37.31 c	13.17 c	26.01 c
PLL	17.10 c	15.85 c	1.73 c	41.63 c	12.70 c	21.12 d
PKL	18.00 c	19.01 b	2.03 b	37.96 b	12.85 c	25.74 c
TMN	18.00 c	17.11 b	1.84 c	37.88 c	15.56 c	26.14 c
TKB	19.56 c	20.08 b	1.89 c	47.07 a	20.61 b	30.67 b
BDN	24.10 b	15.26 c	1.88 c	44.29 a	20.08 b	27.11 c

Note: Values followed by the same letter within a column are not significantly different at the 5% level according to the Scott–Knott test. NSR = number of secondary rhizomes; PRW = primary rhizome weight; SRW = secondary rhizome weight; PRSY = primary rhizome simplicia yield; SRSY = secondary rhizome simplicia yield; TRSY = total rhizome simplicia yield. The origin of accession codes is presented in **Figure 1**.

3.2. Phytochemical Variation among Accessions

Phenolic compounds, tannins, and steroids are among the major phytochemicals associated with the therapeutic properties of *K. rotunda* (Azizah et al., 2023; Elshamy et al., 2019). The chemical attributes presented in **Table 2** indicate substantial variation in phytochemical contents among the evaluated accessions. Accession KLS exhibited the highest levels of total phenolics, tannins, and steroids, followed by LBK, TKB, and BDN.

Variability in phytochemical content among accessions may be influenced by both genetic differences and environmental factors. Such variation has been widely reported in medicinal plants, where the accumulation of secondary metabolites is affected by ecological conditions and plant genotype (Borges et al., 2017; Li et al., 2020). The observed differences among *K. rotunda* accessions therefore suggest the presence of diversity in phytochemical production potential.

Table 2. Phytochemical attributes of *K. rotunda* accessions

Accession	Phenolics (mg GAE/kg dry weight)	Tannins (mg/kg dry weight)	Steroids (mg/kg dry weight)
KLS	8736.18 a	1832.97 a	208.40 a
PNG	6962.65 d	1325.51 f	124.73 c
LTG	7094.49 d	1369.73 e	133.50 c
BLT	5968.84 e	1070.94 h	95.97 d
CMP	6715.43 d	1262.42 g	121.86 c
LBK	8141.51 b	1682.92 b	169.84 b
PLL	7054.27 d	1359.11 e	131.73 c
PKL	7084.16 d	1360.31 e	133.86 c
TMN	7233.19 c	1400.05 d	136.04 c
TKB	8133.39 b	1639.30 c	166.13 b
BDN	8033.02 b	1624.95 c	164.57 b

Note: Values followed by the same letter within a column are not significantly different at the 5% level according to the Scott–Knott test. The origin of accession codes is presented in **Figure 1**.

3.3. Multivariate Analysis of Production and Phytochemical Traits

The PCA biplot in **Figure 3** illustrates the relationship between primary rhizome weight and the number of secondary rhizomes. The primary rhizome plays a central role in plant development because it supports aboveground growth and the formation of adventitious roots, while also serving as a storage organ for nutrients. The physiological connection between the primary rhizome and secondary rhizomes may facilitate nutrient translocation and contribute to the development of secondary rhizomes (Lubbe et al., 2023).

Simplicia yield is calculated based on dry matter accumulation, which reflects the metabolic products stored in rhizome tissues (Widiyastuti et al., 2024). In this study, the simplicia yield of secondary rhizomes was lower than that of primary rhizomes, which may be related to the relatively higher water content of secondary rhizomes. Nevertheless, secondary rhizomes contribute substantially to the total simplicia yield.
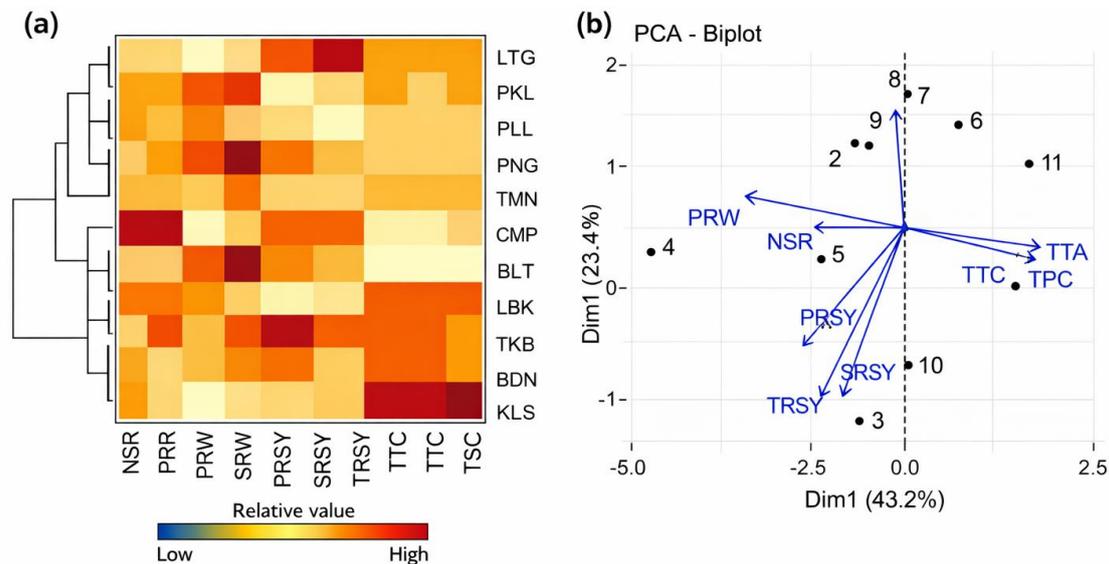
Hierarchical clustering and principal component analysis (**Figure 3**) further revealed grouping patterns among the accessions. Cluster 1 consisted of KLS, LBK, TKB, and BDN, which were characterized by high phenolic, tannin, and steroid contents. Cluster 2 included PNG, PLL, PKL, and TMN, which were characterized by relatively high secondary rhizome weight. Clusters 3, 4, and 5 each contained a single accession. LTG was associated with high secondary and total simplicia yields, CMP was characterized by high primary rhizome simplicia yield, and BLT was associated with high primary rhizome weight and number of secondary rhizomes.

The multivariate analysis indicates the presence of variability among the 11 evaluated accessions. Such variation may reflect differences in genetic background as well as environmental conditions at the sites of origin, including soil nutrient availability. These findings provide preliminary insight into the diversity of *K. rotunda* germplasm from Madura Island.

Figure 3. Multivariate analysis of production and phytochemical attributes of *K. rotunda* accessions. (a) Heatmap with hierarchical clustering showing the relative variation of production and phytochemical traits among accessions. (b) Principal component analysis (PCA) biplot illustrating the relationships among production variables and phytochemical contents. NSR = number of secondary rhizomes; PRW = primary rhizome weight; SRW = secondary rhizome weight; PRSY = primary rhizome simplicia yield; SRSY = secondary rhizome simplicia yield; TRSY = total rhizome simplicia yield; TPC = total phenolic content; TTC = total tannin content; TSC = total steroid content. The origin of accession codes is presented in **Figure 1**.

3.4. Extract Yield and Plant Material Quality

The extract yield of *K. rotunda* rhizomes is presented in **Figure 4**. Accessions that exhibited relatively high production attributes also tended to show higher phytochemical content, particularly TKB, BDN, and PNG. Although this relationship is not necessarily causal, it suggests that plant material with favorable production characteristics may also possess promising chemical properties.

The selection of appropriate plant material is an important step in obtaining satisfactory extract yield. Plant tissues represent complex matrices composed of numerous metabolites (González-Hernández et al., 2024). The quality of plant material therefore plays a key role in determining extract yield, phytochemical composition, and potential biological activity. These characteristics are influenced by several factors, including genetic variation, geographical origin, and environmental conditions (Krakowska-Sieprawska et al., 2022; Ogwu et al., 2025).
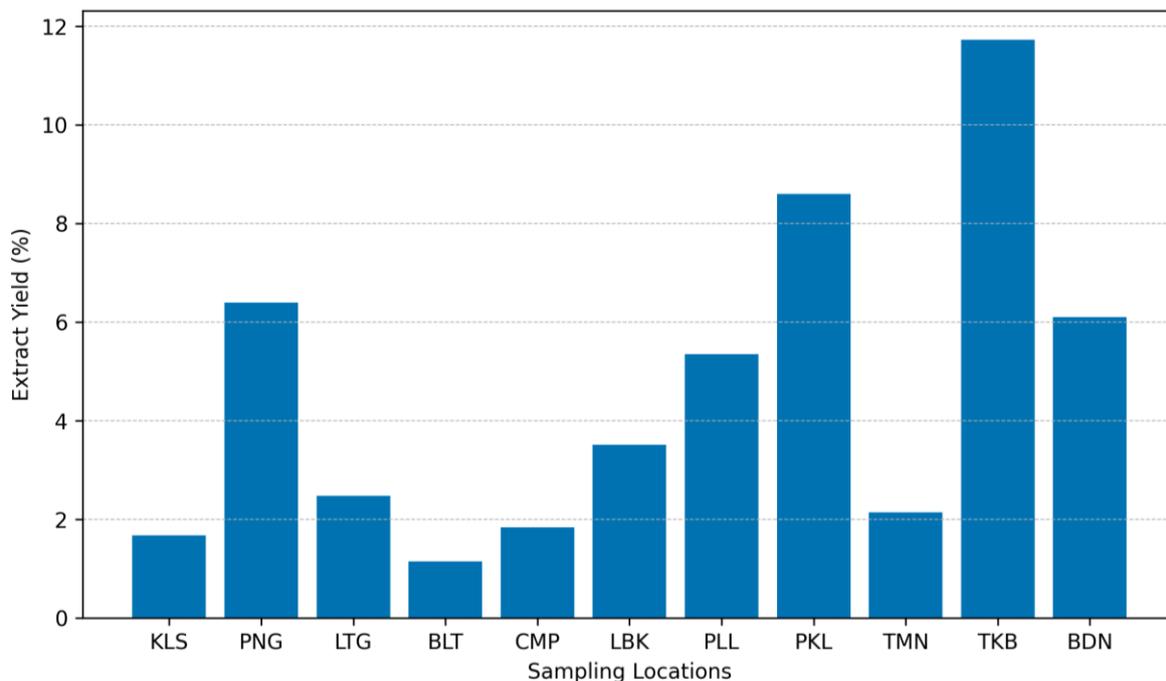
Figure 4. Extract yield (%) of *K. rotunda* collected from different sampling locations. The origin of accession codes is presented in **Figure 1**.

3.5. Soil Nutrient Characteristics of the Cultivation Sites

The soil macro- and micronutrient contents of the *K. rotunda* cultivation areas are presented in **Table 3**. Soil organic carbon levels were classified as moderate to high, while the C/N ratio ranged from low to moderate. Organic matter content was categorized as high to very high. The levels of total N, P, and K were generally moderate to high across the study sites.

The micronutrient contents of Cu and Zn were mostly classified as deficient, except for Cu at the KLS site, which was categorized as sufficient. In Madura, *K. rotunda* is typically cultivated under tree stands where plant litter accumulates. This litter may contribute to maintaining soil organic matter and nutrient availability.

Table 3. Soil nutrient content of *K. rotunda* cultivation areas

Accession	Organic C (%)	Organic Matter (%)	C/N Ratio	Total N (%)	P_2O_5 (mg/100 g)	K_2O (mg/100 g)	Cu (mg/1000 g)	Zn (mg/1000 g)
KLS	6.077	10.477	10.627	0.572	29.547	43.943	0.517	1.062
PNG	4.917	8.478	14.179	0.347	23.454	37.916	0.38	0.945
LTG	4.72	7.29	12.196	0.387	19.724	34.055	0.298	0.868
BLT	5.123	8.833	10.125	0.506	24.5	38.961	0.403	0.966
CMP	4.641	8.003	13.386	0.347	21.994	36.377	0.348	0.914
LBK	5.429	9.361	10.202	0.532	26.166	40.651	0.441	0.999
PLL	4.315	7.44	12.173	0.354	20.127	34.541	0.306	0.879
PKL	4.744	8.179	13.174	0.36	22.563	36.945	0.361	0.925
TMN	4.981	8.588	14.542	0.343	23.698	37.99	0.387	0.944
TKB	5.502	9.486	10.103	0.545	26.487	40.887	0.449	1.002
BDN	5.557	9.581	10.427	0.533	26.802	41.323	0.455	1.012

Note: The origin of accession codes is presented in **Figure 1**.

3.6. Relationship between Soil Nutrients, Production, and Phytochemicals

Adequate nutrient availability during critical growth stages can support rhizome development. Crops within the Zingiberaceae family, including ginger and turmeric, are known to require relatively high nutrient inputs for optimal rhizome formation (Jabborova et al., 2021, 2023). The correlation analysis presented in **Figure 5** indicates that nitrogen shows a moderate relationship with primary rhizome simplicia yield, suggesting that nitrogen may play an important role in rhizome formation and growth.

Secondary rhizome formation showed moderate correlations with several soil nutrients, including N, P, K, Zn, Cu, and organic matter. Increased rhizome dry matter may enhance nutrient uptake in both primary and secondary rhizomes (Ojikpong & Undie, 2019). Nitrogen is considered a key nutrient for rhizome growth (Sontsa-Donhoung et al., 2021), while phosphorus and potassium contribute to starch synthesis and energy metabolism in

rhizome tissues. Micronutrients such as Zn and Cu may also influence the formation of secondary rhizomes by regulating the allocation of photosynthates to storage organs (Liao et al., 2025).

However, rhizome production under dryland cultivation systems is strongly influenced by rainfall during the growing period (Li et al., 2020; Sainju & Liptzin, 2022). The predominance of rain-fed cultivation systems on Madura Island may therefore explain the moderate correlations observed between soil nutrients and rhizome production.

In contrast, soil nutrients showed stronger associations with rhizome phytochemical contents. The correlation analysis indicates that soil macro- and micronutrient levels are associated with the accumulation of total phenolics, tannins, and steroids (**Figure 5**). The rhizosphere environment contains various chemical compounds that interact with plant enzymes and influence biochemical processes (Burneo et al., 2021). Previous studies have also reported that variations in soil nutrients can affect the composition of secondary metabolites in medicinal plants such as turmeric, *Tithonia diversifolia*, *Siparuna muricata*, and *Ornithogalum sintenisii* (Borges et al., 2017; Jabborova et al., 2021; Mammadov et al., 2024; Sampaio et al., 2016). Consistent with these observations, accessions with relatively high phenolic, tannin, and steroid contents in this study were generally associated with locations exhibiting higher soil nutrient levels, particularly KLS, TKB, BDN, and LBK.
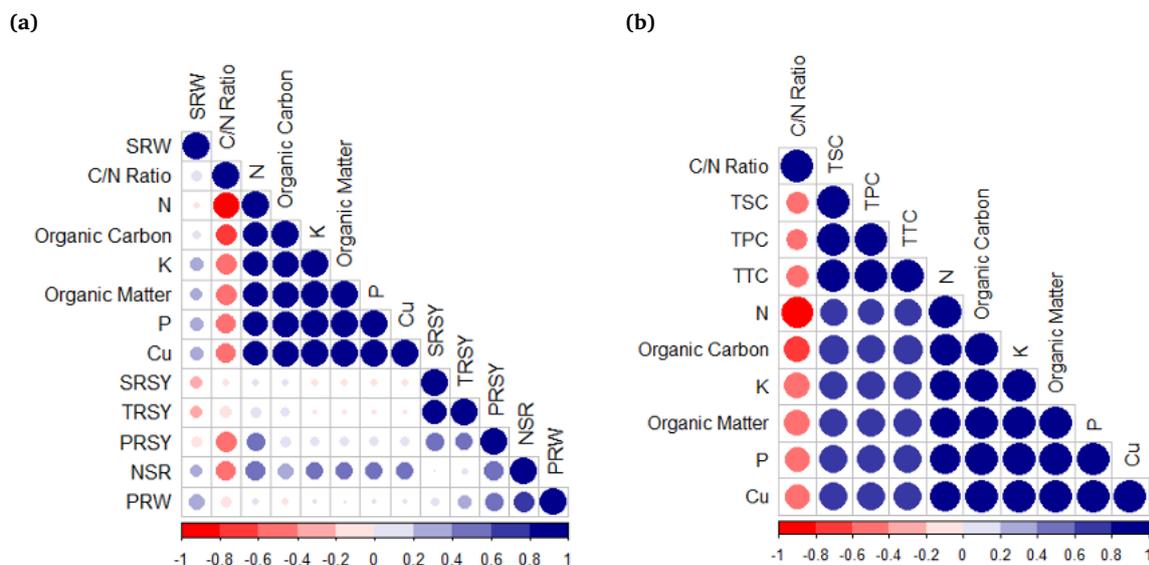
(a)   **(b)**

Figure 5. Pearson correlation matrix of soil chemical properties with rhizome production and phytochemical contents of *K. rotunda*. (a) Correlation between soil chemical properties and rhizome production traits. (b) Correlation between soil chemical properties and rhizome phytochemical contents. Description = NSR: Number of Secondary Rhizome, PRW: Primary rhizome weight, SRW: Secondary Rhizome weight, PRSY: Primary Rhizome Simplicia Yield, SRSY: Secondary Rhizome Simplicia Yield, TRSY: Total Rhizome Simplicia Yield, TPC: Total phenolic content, TSC: Total steroid content, TTC: Total tanin content. Blue circles indicate positive correlations; red circles indicate negative correlations. Circle size represents the strength of correlation ($|r|$).

4. CONCLUSION

This study shows that *K. rotunda* accessions from Madura Island differ in rhizome production and phytochemical content. Variations were observed in rhizome weight, simplicia yield, extract yield, and total phenolic, tannin, and steroid contents among accessions. Primary rhizome weight was positively correlated with the number of secondary rhizomes, and secondary rhizome simplicia yield was positively correlated with total simplicia yield. Soil nutrient levels were generally weakly to moderately correlated with production traits but showed stronger associations with phytochemical contents, indicating that soil chemical conditions may be more closely related to secondary metabolite accumulation than to biomass production under the studied rain-fed conditions. These findings suggest that accession selection and nutrient management are relevant considerations in the cultivation of *K. rotunda*. Further studies under controlled nutrient conditions and with additional characterization of

accession diversity are needed to better understand the relative influence of genetic and environmental factors on rhizome yield and phytochemical variability.

AUTHOR CONTRIBUTIONS

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INFORMED CONSENT STATEMENT

Not applicable.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

The authors declare that no generative AI or AI-assisted technologies were used in the preparation or writing of this manuscript. All contents were produced entirely by the authors without any automated assistance.

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