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Genetic Diversity and Chemicals Profile of Ginger (*Zingiber officinale* Roscoe) in Indonesia

Dyah Subositi [a], Harto Widodo [a], Rohmat Mujahid [a], Nuning Rahmawati [a], Fanie Indrian Mustofa [a], Sari Haryanti [a], Ika Yanti Marfuatush Sholikhah [a], Anshary Maruzy [a], Yuli Widiyastuti [a]

[a] *Medicinal Plant and Traditional Medicine Research and Development Center, NIHRD-MoH, Karanganyar, Central Java, Indonesia*
Corresponding author: [*]dyah.subositi@gmail.com

***Abstract*— *Zingiber officinale* is a medicinal plant used to treat various ailments by many ethnic groups in Indonesia. Information on genetic variation and chemical profiling of this plant, especially in the traditional herbal formula in Indonesia, is still limited. The objective of this study was to determine genetic variation and chemical profiling of *Zingiber officinale* accessions to compile database information. Inter-Simple Sequence Repeats (ISSR) were used to evaluate the genetic diversity of *Z. officinale*, and Fourier transforms infrared (FTIR) was used to analyze chemical profiling. Dice index similarity was used to calculate a similarity index between accessions, and Unweighted Pair Group Method Using Arithmetic Mean (UPGMA) was used to construct a dendrogram. The ISSR method for genetic profiling proved that *Z. officinale* from 14 selected ethnic groups were divided into three clusters. The similarity index among *Z. officinale* accessions ranged from 0.567 to 0.971, indicating high genetic diversity. The high degree of genetic variety detected by ISSR markers demonstrated the marker's efficiency in detecting variation in this *Z. offcinale* germplasm collection. The FTIR technique's phytochemical profile of Z. officinale analysis shows slight differences in spectra and can be grouped into three clusters. There was no correlation of clustering of *Z. officinale* accessions between geographical origins based on genetic and chemical profiles. Our findings may be valuable information for breeding, conservation, and utilization of *Z. officinale*.**

***Keywords*— *Zingiber officinale*; genetic diversity; ISSR; FTIR.**

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I. INTRODUCTION

Plants are an important source of traditional medicines and are used to treat various ailments due to their minimum of having side effects; plants are easily accessible and affordable [1], [2]. Indonesia consists of 1,068 ethnic groups and represents a wealth of culture, including local medicinal plant knowledge. The National ethnomedicine survey of local knowledge and medicinal plants in Indonesia was conducted in 2012 and observed 209 ethnic groups in 26 provinces (Ristoja). Ristoja aims to create an ethnomedicine database of local herbal formulas and medicinal plants knowledge. It revealed about 15,733 used herbal formulas and 19,738 medicinal plant information in these herbal formulas (1,740 plant species) [3].

Zingiber officinale (ginger) is one of the medicinal plants used in the herbal formula in Ristoja. This revealed that 169 ethnic groups used *Z. officinale* to treat various ailments such as pre and postnatal care, cough, common cold, stomachache, etc. The most of part plant used was rhizome. Currently, ginger is a significant commercial resource and a commonly used spice and folk remedy throughout the world [4]. Gingerols, shogaols, and paradols are the primary phenolic compounds found in ginger, and they are responsible for the different bioactivities of ginger[5]. In Indonesia, there are three varieties of ginger based on size and color of the rhizome, i.e., *Z. officinale* var. *amarum* (small white ginger known as 'jahe emprit'), *Z. officinale* var. *officinale* (big white ginger or giant ginger known as 'jahe gajah'), and *Z. officinale* var. *rubrum* (small red ginger known as 'jahe merah') [6].

Plants produce many secondary metabolites as a chemical adaptation to their geographic regime. Environmental factors consist of climate, rainfall, temperature, and soil composition. Meanwhile, agricultural factors include irrigation, fertilization, plantation density, and harvest time. These two factors influence the structures and characteristics of phytochemicals of the plants [7]. Albeit *Z. officinale* varieties can be distinguishable by morphological characteristics and organoleptic properties, it becomes complicated to discriminate quality within the same varieties of ginger due to

environmental factors where they grow. The differences of certain compounds in the rhizome can be correlated to their biological activity [8]. In recent years, numerous DNA-based approaches have been successfully employed to characterize medicinal plants and herbal medicines for quality control and standardization [9]. Genetic variations within plant varieties can be asses using polymerase chain reaction (PCR)-based molecular markers such as AFLP, SSR, RAPD, and RFLP [10]. In comparison, Fourier Transform Infrared Spectra (FTIR), a spectroscopy-based assay, is a convenient and acceptable method for identification, screening, and clustering phytochemicals of plant samples [11].

Genetic evaluation and characterization of *Z. officinale* grown in the various location allow for identification and genotype selection that could compose germplasm collections, consequently guaranteeing for conservation of the diversity of the species. Furthermore, identifying and selecting beneficial traits for exploitation in breeding applications [12]. Germplasm is one of the genetic resources for the development of varieties. The utilization and development of genetic resources are influenced by the level of diversity [13]. Germplasm is the foundation of good variety breeding. Breeding efficiency depends on the number of germplasm resources and the genetic diversity of these resources [14]. As mentioned above, various marker techniques are developed to evaluate genetic diversity[13]. Molecular characterization utilizing molecular markers, especially ISSR (Inter-simple sequence repeats), has been used to examine genetic diversity in Zingiberaceae at the inter/intra-specific level during the last two decades [15]. ISSR has several advantages such as no prior knowledge of genome sequence, high degree polymorphism, reliability, high reproducibility, and very useful for the characterization of accessions and cultivars of several species [16], [17], [18]. This study aimed to study genetic diversity by ISSR molecular marker and chemical profiling of *Zingiber officinale* using FTIR used as a medicinal plant by selected ethnic groups in Indonesia.

II. Materials and Methods

Genetic diversity and the chemical profiling of *Z. officinale*, it is necessary to collect samples from selected ethnic groups based on RISTOJA research held in 2012. The schematic of the research stage is shown in Figure 1. Samples were collected in September and October 2014. As many as 24 ethnic groups were selected as sampling locations, *Z. officinale* was found only in 14 ethnics for genetic samples and 13 ethnic groups for phytochemical samples (Figure 2). *Zingiber officinale* samples were divided into two kinds of *Z. officinale* accessions based on rhizome inner color, namely white rhizome and red rhizome (code ZR). Samples for genetic analysis and phytochemical profile were processed as follows:

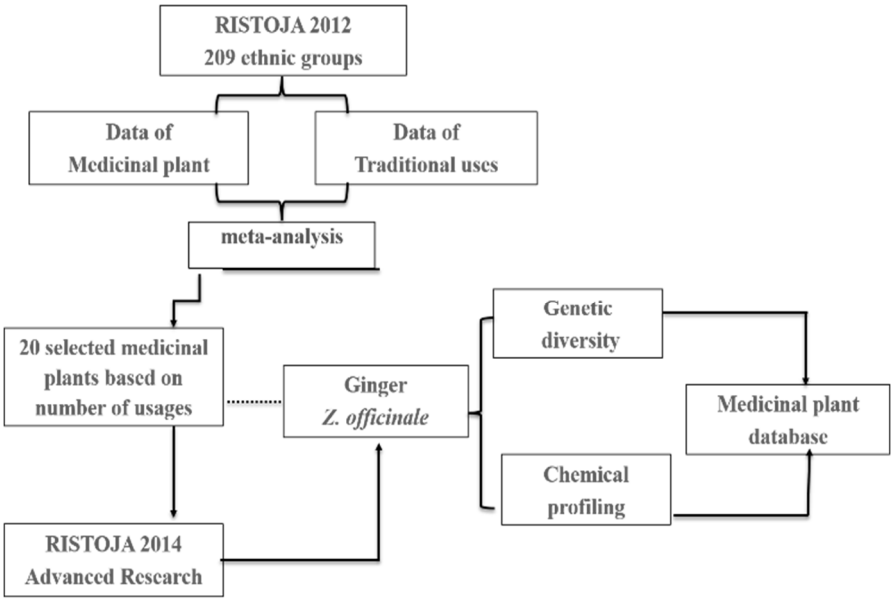

Fig. 1 The schematic of the research stage

A. Genetic Diversity Analysis

1) Genomic DNA Extraction: Total Genomic DNA was extracted from 0.1 gr frozen leaves following the protocol of kit DNA isolation (Sigma GenEluteTM Plant Genomic DNA Miniprep Kit, Catalog Number G2N70). The quality and quantity of extracted genomic DNA were determined using a UV-Vis spectrophotometer at 260/280 nm and electrophoresis using a 0.8% agarose gel. DNA genome considered a good quality proceeded for amplification step.

2) PCR-ISSR Analysis: Eleven ISSR primers were screened out of 26 ISSR primers based on their ability to produce clear DNA bands and high levels of polymorphism (Table 1). PCR reaction mixture in total 25 µL was contained DNA template (25 ng/µL) 2 µL, PCR mix 12.6 µL, primer 1 µL, dan nuclease-free water 9.6 µL. The amplification reaction was performed in a thermal cycler (C-1000 Bio-Rad, USA), programmed for 3 min pre-denaturation step at 95˚C, followed by 39 cycles of 94˚C for 1 min as denaturation step, annealing at 42-52˚C (depending on the primer used) for 60 s, and elongation at 72˚C for 2 min, and a final extension at 72˚C for 8 min. The amplified products were separated on 2%

agarose gels with 1XTBE buffer set at 50 V for 90 minutes. Electrophoresis gel was visualized under UV light and documented using a gel documentation system (Imaging System XR + Bio-Rad, USA).

3) Data Analysis: Only clear and distinct DNA bands were scored as "0" for absence bands and "1" for presence bands from each primer used and in all accessions. Similarity indexes of all accessions were measured using Dice similarity [19]. The Unweighted Pair Group Method generated the dendrogram using Arithmetic Mean (UPGMA) cluster analysis. NTSYS software version 2.0 was used for the entire data analysis in this study.

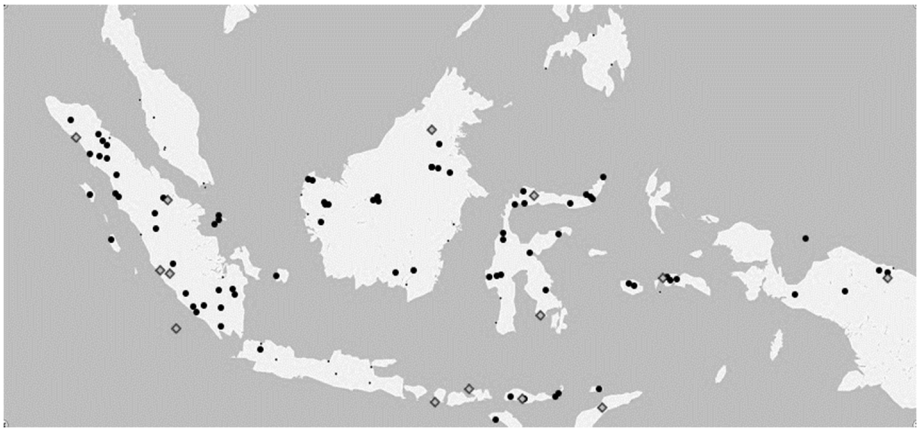

Fig. 2 The location of the study area (RISTOJA) data is processed with MapSource® Garmin® mapping software). • = ethnic groups that use *Zingiber officinale* in concoction; ◊ = location sampling of genetic and phytochemical materials

B. FTIR Analysis

Sample for phytochemical analysis is processed by extracting the rhizome's chemical compound and screening the extract using the FTIR method.

1) Sample Preparation: The cleaned and dried samples were ground with a size of 40 mesh. A gram of sample was extracted in 10 mL of methanol for 15 minutes by sonication. The extract was centrifuged at 10,000 rpm for 5 minutes. The supernatant was used as a test solution

2) FTIR Profile: A volume of test solution 100 µL was dropped in the FTIR Nicolet iS10 compartment, allowed to evaporate, then read at wavelength numbers 400 to 4000. The absorbance was saved as FTIR spectra.

3) Data Analysis: FTIR spectra exported to Ms.excels for Principal Component Analysis (PCA) performed by Unscrambler v9.7 software.

III. Results and Discussion

A. Genetic Diversity

Information on the genetic diversity of medicinal plant species is useful for utilizing and conservation management of germplasm. Molecular markers have been widely used to characterize and assess the genetic diversity of medicinal plant species [20]. Inter-Simple Sequence Repeat (ISSR) molecular markers have several advantages in genetic diversity study due to their abundance, reproducibility, produce high polymorphism level, not being affected by environmental factors, information, effectiveness, and efficiency [21]. Genetic markers can be used to assess the number of distinct genotypes and the extent of clonality, and the total genetic diversity of a population [22]. All *Z. officinale* accessions revealed clear polymorphism and banding profiles from ISSR selected primers used in this study, as shown in Figure 3.

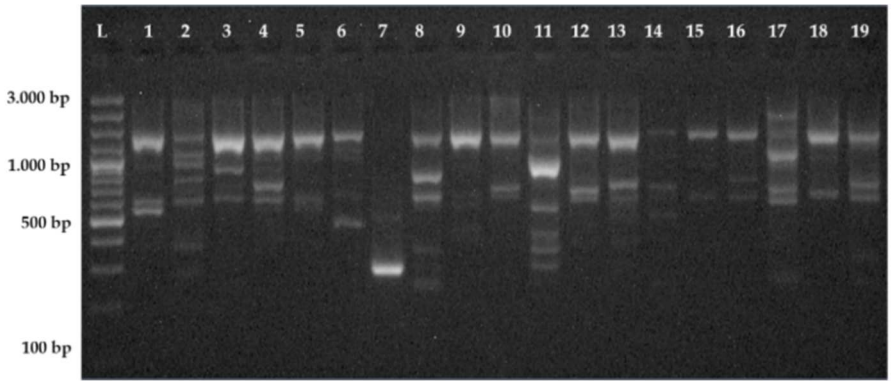

Fig. 3 ISSR profiles of 19 *Zingiber officinale* accessions using primer ISSR (CA)6GT. Lane 1-19 were the samples, L : 100 bp DNA ladder. 1. Aceh_Kluet; 2. Riau_Sakai, 3. North Kalimatan_Agabag, 4. South east Sulawesi_Moronene, 5. West Nusa Tenggara_Sasak, 6. West Nusa Tenggara_Mbojo, 7. East Nusa Tenggara_Belu Tetun, 8. East Nusa Tenggara_Ngada, 9. Maluku_Alune, 10. Papua_Nimboran, 11. Aceh_KluetZR, 12. Riau_SakaiZR, 13. Bengkulu_Muko-MukoZR, 14. Bengkulu_RejangZR, 15. Bengkulu_EngganoZR, 16. Central Sulawesi_BuolZR, 17. West Nusa Tenggara_SasakZR, 18. Maluku_AluneZR, 19. Papua_NimboranZR

A total of 85 DNA bands were produced by 11 selected primers, varied between 5-11 DNA bands in each primer, and DNA bands size ranged from 100 bp to 2,050 bp (Table 1). The average polymorphism percentage of 19 *Z. officinale* accessions was found to be 83.5%.

TABLE I
ISSR PRIMERS USED AND NUMBER OF DNA BANDS OF 19 ACCESSIONS OF ZINGIBER OFFICINALE

Primer Sequences	Total number of bands	Mono-morphic bands	Polymorph-ism percentage (%)	Bands size (bp)
(AC)8C	6	2	66.7	180 – 1,950
(AC)9G	8	0	100	120 – 1,920
(AG)8G	5	3	40	195 – 2,050
(CA)6GT	10	1	90	180 – 1,910
(GA)8YC	6	2	66.7	105 – 1,920
(GT)8A	7	1	85.7	190 – 2,010
(GT)8C	10	0	100	115 – 1,995
(GT)6CC	11	0	100	110 – 1,820
(TG)8C	7	3	57.1	100 – 1,850
(TC)8RG	8	2	75	100 – 1,895
(TC)8G	7	0	100	180 – 2,020
Total	85	14		
Average			83.5	

Primer (AG)8G derived the lowest number of amplified bands (5 bands) and the lowest polymorphism (40%); meanwhile, primer (GT)6CC generated the greatest number of amplified bands (11 DNA bands) and also the highest polymorphism (100%). ISSR primers also used by Das et al. [23] to assess the genetic diversity of 60 accessions of *Z. officinale*; the study was used 9 ISSR primers and produced 75 DNA bands with sizes 180 to 1,000 bp and an average polymorphism percentage of 55%. Seven ISSR primers also found successfully revealed polymorphism in 18 *Z. officinale* cultivars from various Indian regions; the primers generated 81 bands with the size ranged 200 bp to 3,000 bps and showed an average polymorphism of 66.7% [24].

The data from ISSR analyses were used to calculate genetic similarity and continued by dendrogram construction. The dendrogram was performed by the unweighted pair group method with the arithmetic mean (UPGMA) method, and the result is shown in Figure 4. Dendrogram revealed four major clusters with a similarity index of 0.622; three clusters among them have only one member in each cluster (Cluster I, II, and III). The similarity index among *Z. officinale* accessions ranged from 0.567 to 0.971, indicating high genetic diversity among accessions. Cluster IV included 16 accessions and could be divided into two minor clusters IV A and IV B at a similarity index of 0.690; minor cluster IV B consists of *Z.officinale* red rhizome from Bengkulu, Aceh, and Central Sulawesi. Meanwhile, minor cluster IV A could be further divided into sub minor cluster IV A1 included eight accessions, and sub minor cluster V A2 included five accessions based on rhizome color. *Zingiber officinale* accessions from West Nusa Tenggara_Sasak and North Kalimantan_Agabag have the closest similarity at 0.971.

ISSR molecular markers have been widely used to analyze genetic diversity, especially intraspecific variations in species. This study showed high diversity among *Z. officinale* accessions collected from several Indonesia locations by the value of ranging from 0.5 to 0.9 of similarity index. A Similar result of high genetic variation was found in 16 *Z. officinale* cultivars ranging from 0.538 to 0.938 detected using RAPD molecular [25]. Antala et al. [26] also reported high genetic diversity among 31 *Z. officinale* genotypes by ISSR marker varying 0.39-0.95 similarity index. Meanwhile, moderate to low variation (0.76-0.97) among 46 *Z. officinale* accessions by RAPD and ISSR marker [27]. Oktavioni et al. [28] reported several *Z. officinale* accessions collected from 16 provinces in Indonesia showed narrow genetic variation by using SRAP molecular marker.

The clustering results reveal no clear correspondence between accessions grouping pattern and location where the samples were collected. Clustering of *Z. officinale* accessions unrelated to geographical origin was also reported in a previous study, such as clustering 16 cultivars of *Z. officinale* in India [25]. *Zingiber officinale* is a widely used plant by people in Indonesia for spices and medicinal plants; *Z. officinale* is also a trading material between a community in a different area for its use or cultivation materials. This migration may result in some *Z. officinale* accessions sharing similar characters in geographically distinct regions, although *Z. officinale* is vegetatively propagated. According to Cardoso et al. [12], exchange materials between communities or farmers who cultivated *Z. officinale*, followed by maintaining the same genotype over time, affected mutation and natural selection over the years. Genetic variety is not only influenced by their geographical origin, but it is also likely to be influenced by human selection and the distribution of the most useful genotypes in the population [29], [30]. Due to their restricted dispersal capabilities, plants that reproduce vegetatively always exhibit high levels of clonal clustering. Furthermore, clonal plants can be customized to adapt to various environmental circumstances [31]. It has long been recognized that habitats with differing conditions encourage the coexistence of locally adapted genotypes through diversifying selection, resulting in high levels of genetic variation [31].

There was high genetic variability among ginger accessions collected from nearby locations compared to distant places, such as SasakZR and Sasak accessions; those accessions were collected from the same origin region. The genetic variation among accessions in the same area is due to cultivars differences. According to the dendrogram (Figure 4), in general, the clustering pattern of ginger accessions was found to be its morphological difference of rhizome color. Wahyuni et al. [32] reported 22 accessions of *Z. officinale* in Indonesia to have low genetic variability based on AFLP marker. Moreover, the UPGMA dendrogram showed red *Z. officinale* genetically far from big *Z. officinale* but closer to some small ginger accessions. Muda et al. [19] also reported genetic variation of three *Z. officinale* cultivars from Malaysia conducted by RAPD molecular marker; the dendrogram revealed the cultivars were separated into a different group.

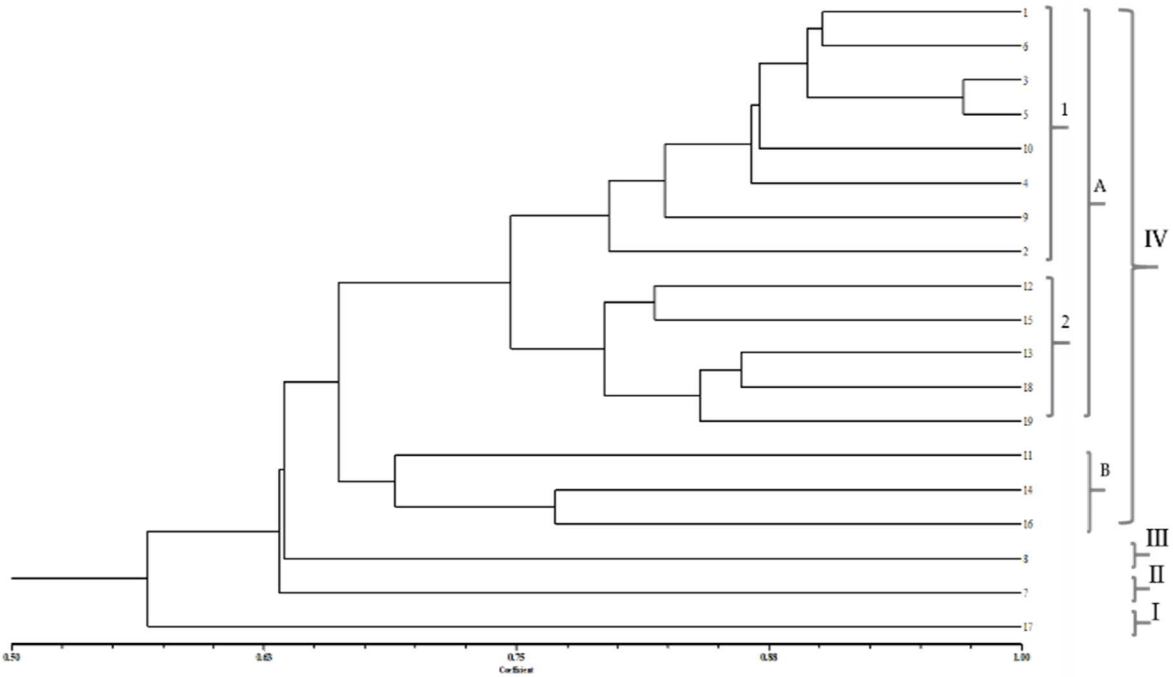

Fig. 4 UPGMA Dendrogram of 19 accessions of *Zingiber officinale* in Indonesia based on ISSR markers. 1. Aceh_Kluet, 2. Riau_Sakai, 3. North Kalimatan_Agabag, 4. South east Sulawesi_Moronene, 5. West Nusa Tenggara_Sasak, 6. West Nusa Tenggara_Mbojo, 7. East Nusa Tenggara_Belu Tetun, 8. East Nusa Tenggara_Ngada, 9. Maluku_Alune, 10. Papua_Nimboran, 11. Aceh_KluetZR, 12. Riau_SakaiZR, 13. Bengkulu_Muko-MukoZR, 14. Bengkulu_RejangZR, 15. Bengkulu_EngganoZR, 16. Central Sulawesi_BuolZR, 17. West Nusa Tenggara_SasakZR, 18. Maluku_AluneZR, 19. Papua_NimboranZR

Besides ISSR molecular marker used to assess genetic diversity, in this study, *Z. officinale* accessions chemical characterization was also performed using Fourier Transform Infrared Spectroscopy (FTIR). Clustering patterns based on both methods were slightly different (Figure 5). The grouping of several medicinal plant accessions using the chemical profile method is generally based on geographical origin, including topography and agroclimatic conditions; moreover, the clustering pattern could be different from clustering using a molecular marker. The incongruence in grouping patterns between ISSR marker and FTIR was reported by Kumar and Roy [33] in *Cassia tora*; however, both techniques revealed the diversity and authenticated the origin of the six *C. tora* population. Genetic characterization and chemical profiling of vegetatively propagated plants are useful to identify bio-geographical patterns between populations for identifying desired plant material [24].

The wide range distribution of *Z. officinale* in Indonesia is one advantage factor in maintaining the diversity by the habitat heterogeneity. According to Ashraf et al. [34], the heterogeneity habitat is important in maintaining diversity by diversifying selection and preventing decreased diversity inhomogeneity habitat by the preferential selection, especially in clonal species. The identification and characterization of *Z. officinale* germplasm are essential in providing data and connecting conservation and utilization of its genetic resources. Based on ethnobotanical data, knowledge of medicinal plant distribution supports baseline information for further research, such as pharmacological studies and genetic diversity. Those data are important in genotype selection, conservation, and preventing unsustainable collection overexploitation [35].

B. FTIR Profiling of Zingiber officinale

Chemo-profiling using FTIR has been more efficient, especially for authentication purposes, conveniently applied for herbal product quality control [33]. All IR spectra of 15 *Z. officinale* accessions collected from different geographic origins of selected ethnic groups measured at mid-infrared regions (4,000–650 cm−1) showed the plant's characteristic bands but no significant difference within the population. A very broad and strong intensity at 3,700 to 3,600 cm^{-1} is attributed to stretching vibration of the O-H bond. A strong intensity with a sharp peak at 2,900 cm^{-1} and medium intensity with a narrow peak at 2,800 cm^{-1} characterize the presence of the group of carboxylic acids [36]. The spectra patterns showed intensive similarity and depicted only a slight divergence profile, which indicated no difference in the functional group present in *Z. officinale* populations.

An FTIR assay and principal component analysis (PCA) exhibited a powerful combination method to differentiate the various components. It efficiently discriminates 10 different populations of five grass species [37] and effectively discriminates Chinese and Korean soybeans [38]. Wang et al. [39] successfully distinguished between different Lingzhi species using that method. Score plot PCA to the IR spectra resulted in three clusters (Figure 5). Cluster II showed a slight difference at IR fingerprinting regions <1,500 cm^{-1} and the existence of a peak at 925 cm^{-1}, otherwise Cluster I and Cluster III closely similar to each other. The spectra peaks among the *Z. officinale* population were diverse in the fingerprint region 1,500 to 6,000 cm^{-1}, since each compound's absorptions pattern was generally very complex. It also included bending vibration of $–CH_3$ dan $–CH_2$ [42] (Table 2).

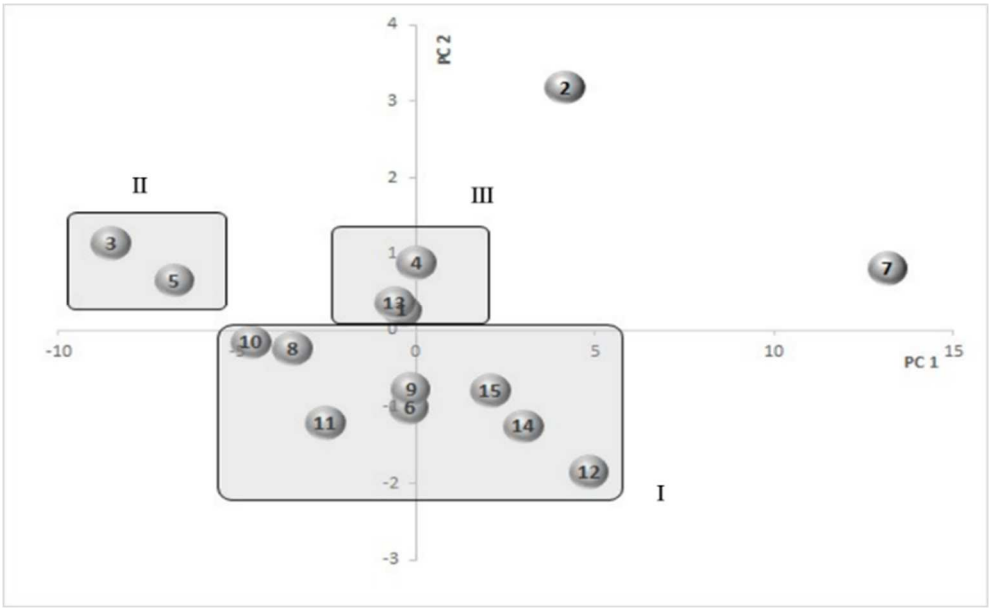

Fig. 5 Score plot PCA FTIR of 15 *Zingiber officinale* accessions from selected ethnic groups at wavenumber 4,000-600 cm^{-1}. 1. Riau_Sakai, 2. North Kalimatan_Agabag, 3. South east Sulawesi_Moronene, 4. West Nusa Tenggara_Sasak, 5. West Nusa Tenggara_Mbojo, 6. East Nusa Tenggara_Belu Tetun, 7. Maluku_Alune, 8. Papua_Nimboran, 9. Central Sulawesi_BuolZR, 10. Aceh_KluetZR, 11. Riau_Sakai, 12. Bengkulu_Muko-MukoZR, 13. Bengkulu_RejangZR, 14. Bengkulu_EngganoZR, 15. Papua_NimboranZR

Zingiber officinale accessions from Maluku_Alune presented out-layer of the cluster more likely due to the absence of a peak at 815 cm^{-1} and the intensity of spectral peak at 1,035.15 cm^{-1} and 3,331.59 cm^{-1} rather than the entire absorbance spectra pattern, which was very much similar to the others. The intensity absorption results were directly proportional to the functional groups' concentration [42]. Whereas *Z. officinale* collected from North Kalimantan_Agabag ethnic group resulted in IR spectra closely to cluster I and cluster III except for the absence of the peak at 815 cm^{-1}.

TABLE II
IR PEAKS OF FUNCTIONAL GROUPS PRESENT IN RHIZOME OF ZINGIBER OFFICINALE FROM SELECTED ETHNIC GROUPS AT WAVENUMBER 4,000-600 CM-1.

Wavenumber (cm^{-1})	Functional group	Vibration Type	Alune	Agabag	Cluster I	Cluster II	Cluster III	Reference
814-816			-	-	+	+	+	
924-926	C-OH	Stretch	+	+	+	-	+	[40]
1034-1036	C-OH	Stretch	+	+	+	+	+	[36]
1122.40	C-C-O	Bend	-	-	-	+	-	[41]
1235-1236	C-O-C	Stretch	+	+	+	+	+	[36]
1268-1270	C-O-C	As. stretch	+	+	+	+	+	[36]
1374-1377	C-H3	Bend	+	+	+	+	+	[36]
1430-1432			-	+	-	-	+	
1451-1455	C=C Aromatic	Stretch	-	+	+	+	+	[36]
1515-1516	C-H2	Bend	+	+	+	+	+	[42]
1601-1605	C=C	Stretch	+	+	+	+	+	[40], [42]
1705-1713	C=O	Stretch	-	+	+	+	+	[40], [42]
2853-2855	C-H	Stretch	+	+	+	+	+	[40]
2924-2926	C-H2	As. Stretch	+	+	+	+	+	[42]
3331-3373	O-H	Stretch	+	+	+	+	+	[36], [40], [42]

Note: As.: asymmetric; the wavenumber conformity to the references are ± 10 cm^{-1}; +: peak presence, - : absence peak

The current study showed different clustering results in the genetic-based and phytochemical-based assay in Z. officinale. ISSR describes the diversity of nucleotides that make up DNA sequences that are more stable than that of metabolites, while the FTIR spectra reveal the chemical content of ginger. Chemical content, especially secondary metabolites, is a consequence of the adaptive physiological responses of plants to the environment. The secondary metabolites synthesizing involves many genes or even the regulation of poly-genes cluster. Epigenetic regulations and modifiers allow altering the secondary metabolite composition of the plants [43].

IV. CONCLUSION

This study's conclusion revealed 19 *Z. officinale* accessions in Indonesia show high genetic variation based on ISSR molecular markers; furthermore, the clustering pattern was not influenced by geographical origin. The FTIR profiles were grouped in three clusters with two accessions were outside the groups. A phytochemical-based clustering using FTIR indicated that *Z. officinale* accessions grouping was similar to genetic variations grouping that was no correlation to geographical location. The findings of this study can be utilized to compile a database of Indonesian medicinal plants.

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