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ORIGINAL RESEARCH PAPER

Impact of environmental and geographical position on the chemometric classification of ethanol extracts from Isotoma longiflora leaves

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ABSTRACT

BACKGROUND AND OBJECTIVES: *Isotoma longiflora L* is commonly used as a medicinal plant by the local community in Indonesia, and its geographical position determines its bioactive compounds and hence its efficacy. Ethanol extracts of *Isotoma longiflora* leaves from various locations in Aceh Province were analyzed using a simple infrared spectroscopy technique combined with chemometrics to determine the effect of geographical location and conditions by classification and authentication.

METHODS: *Isotoma longiflora* leaf samples were collected from Aceh Besar (a geothermal manifestation of Ie Suum), Banda Aceh, Aceh Jaya, Bireun, and Central Aceh. Principal component analysis was used to categorize the ethanol extract of *Isotoma longiflora* leaves, and a linear discriminant analysis was used for authentication.

FINDINGS: The principal component analysis score plot indicated 89 percent of total data variance and that the samples formed three distinct groups: group I consisting of Aceh Tengah and Bener Meriah samples; group II of Aceh Besar and Banda Aceh samples; and group III of Aceh Selatan, Aceh Barat Daya, Aceh Jaya, and Bireun. A linear discriminant analysis was then used to validate these results, and the linear discriminant analysis model derived from the cross-validation predicted the origin of *Isotoma longiflora* samples with 100 percent accuracy rate.

CONCLUSION: The *Isotoma longiflora* leaf extracts were successfully classified using Fourier-transform infrared spectroscopy data processed through chemometric calculations (namely, principal component analysis). Based on the cross-validation using linear discriminant analysis showed that the prediction model had a 100 percent accuracy. The present study thus revealed the effect of geographical location on the composition of bioactive compounds in Isotoma longiflora, suggesting the potential of chemometric techniques for quality control and assurance in traditional medicine.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
45	6	5

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INTRODUCTION

Medicines derived from natural sources are among the most common alternative treatments used in communities in Indonesia. According to the World Health Organization, approximately 80 percent (%) of the world's population uses natural medicines (Gutierrez *et al.*, 2022). Moreover, approximately 80% of the world's plant species can be found in Indonesia, a country with a rich biodiversity of between 25,000 and 30,000 plant species. Natural remedies have been used for thousands of years in Indonesia, before the discovery of modern medicine, and this includes the *Isotoma longiflora* plant. The compounds found in *Isotoma longiflora* leaves contain antioxidants that can neutralize free radicals (Imelda *et al.*, 2022). These antioxidants have multiple applications, including the inhibition of the progression of cataracts or cataractogenesis process (Arrigoni-Blank *et al.*, 2002). Phytochemical studies have revealed that *Isotoma longiflora* leaves contain numerous active compounds, specifically phenolic compounds and flavonoids, which confer antiproliferative, antioxidant, and antibacterial properties (Imelda *et al.*, 2022; Onuigbo *et al.*, 2023). The *Isotoma longiflora* plant can readily grow in multiple locations with sufficient water content, and its leaves can be found in almost every location in Aceh Province, Indonesia. The chemical composition of *Isotoma longiflora* leaves originating from various locations in Aceh Province probably differ in terms of the types and amounts of chemical compounds. As the geographical conditions of Aceh Province are quite diverse, these differences must be analyzed. A previous research on differences in chemical content and geographical origin indicated that secondary metabolites are the result of interactions between plants and their environment, and that the correlation between plants and their environment has a more significant impact on the content of secondary metabolites than on that of primary metabolites (Liu *et al.*, 2008). Both biotic and abiotic environmental factors influence the biosynthesis of secondary metabolites, and abiotic stress factors play a role in decreasing the production of secondary metabolites in medicinal plants (Li *et al.*, 2020). As the secondary metabolites profile is altered due to the influence of environmental factors, a change in bioactivity of the medicinal plant should also be expected. Other reports have stated that plants interact with their surrounding abiotic environments during growth and development, including water, soil, light, temperature, and chemicals (Verma and Shukla, 2015). Certain abiotic conditions such as drought, flooding, extreme temperatures, and the presence of toxic chemical elements in the soil will cause stress to medicinal plants and variations in the biosynthesis process (Li *et al.*, 2020). Fourier-transform infrared spectroscopy (FTIR) may be useful for identifying plants from different environments as the infrared (IR) spectrum is a fingerprint pattern characteristic of the plant's chemical composition (Sanchez *et al.*, 2018). However, a multivariate statistical approach is necessary for extracting the necessary information from the FTIR spectrum data. Previous studies have reported the results of a combination of FTIR and chemometric methods to identify plants or their geographical origins, including carobs (Christou *et al.*, 2018), *Chomolaena odorata* L (Abubakar *et al.*, 2021), among others (Zulkifli *et al.*, 2023). In addition to analyzing geographical origin, chemometric techniques have also been used for taxonomic discrimination of closely related plants, such as *Panax ginseng* varieties (Liu *et al.*, 2008). Linear discriminant analysis (LDA) has been used for the authentication of red ginger plants (Purwakusumah *et al.*, 2014), horse milk (Arifah *et al.*, 2022), vegetable oils (Jamwal *et al.*, 2021), and beef meatballs (Lestari *et al.*, 2022). Despite the numerous studies conducted to date, there has been no research on the classification and authentication of *Isotoma longiflora* plants originating from various geographical locations. Considering this context, the identification and authentication of *Isotoma longiflora* plants from multiple locations in Aceh Province was performed as a reference for determining the development and quality control of this plant species, which has the potential to be used as a medicinal plant. Therefore, the aim of this study was to construct a chemometric model for the classification of *Isotoma longiflora* collected from different geographical locations. This study was conducted in Banda Aceh, Indonesia, between 2022 and 2023.

MATERIALS AND METHODS

Study design

Isotoma longiflora plant extract samples from the Aceh Province regencies, including Aceh Besar (AB),

Tabel 1: Comparison of previously used chemometric methods based on infrared spectra

Sample	Determination	Chemometrics	Sources
Green tea	Geographical origins and processing duration	PCA, PLS-DA, SVM	Liu *et al.*, 2022
Wine	Geographical origins	PCA, SIMCA, DA	Hu *et al*., 2019
Walnut	Geographical origins	PCA, MSC	Arndt *et al.*, 2020
Tea	Quality control	SIMCA	Zhu *et al.*, 2019
Sea bass	Geographical origins and quality control	PCA, PLS-DA	Ghidini *et al*., 2019
Chestnut	Geographical origins	PLS-DA, SIMCA	Nardecchia *et al.*, 2020

Note: Discriminant analysis (DA); multiplicative scatter correction (MSC); partial least squares (PLS); soft independent modelling by class analogy (SIMCA); support vector machine (SVM).

Table 2: Sampling locations of *Isotoma longiflora* leaves

No.	Regency	Sample code	Coordinate point	
			Latitude	Longitude
		AB1	5.553216	95.538757
1	Aceh Besar	AB2	5.553216	95.538757
		AB3	5.553216	95.538757
		AJ1	4.878547	95.400946
2	Aceh Jaya	AJ2	4.878547	95.400946
		AJ3	4.878547	95.400946
		B1	5.200639	96.589414
3	Bireun	B2	5.200639	96.589414
		B3	5.200639	96.589414
		AT1	4.684952	96.738941
4	Aceh Tengah	AT2	4.684952	96.738941
		AT3	4.684952	96.738941
		BA1	5.545702	95.351652
5	Banda Aceh	BA2	5.545702	95.351652
		BA3	5.545702	95.351652
		BM1	4.788848	96.736809
6	Bener Meriah	BM2	4.788848	96.736809
		BM3	4.788848	96.736809
		ABD1	3.703605	96.851830
7	Aceh Barat Daya	ABD2	3.703605	96.851830
		ABD3	3.703605	96.851830
		S1	3.061044	97.325568
8	Aceh Selatan	S2	3.061044	97.325568
		S3	3.061044	97.325568

Aceh Jaya (AJ), Biereun (B), Aceh Tengah (AT), Banda Aceh (BA), and Bener Meriah (BM) were obtained. An FTIR instrument was used on each extract to acquire its infrared spectrum at a wavelength of 4000/centimeter (cm) – 400/cm. The spectrum obtained was then analyzed chemometrically using the Unscrambler 10.4 application software (Omar *et al.*, 2019). A comparison of previously employed chemometric methods for analyzing infrared spectra is presented in Table 1.

Extract preparation

Isotoma longiflora leaf samples were obtained from five locations in Aceh Province, and three 1-kilogram samples were collected from each geographical location. Details on sampling locations are presented in Table 2. The *Isotoma longiflora* leaf samples were washed and air-dried for 14 d. The samples were stored for less than 1 week from collection to treatment. After cleaning, the samples were ground into a fine powder using the maceration method for 48 hours with 70% ethanol. The sample was filtered after 48 hours to obtain the filtrate, was then diluted using a rotary evaporator to obtain a thick extract of *Isotoma longiflora* leaves.

FTIR spectrum acquisition

The FTIR spectrometer was calibrated using

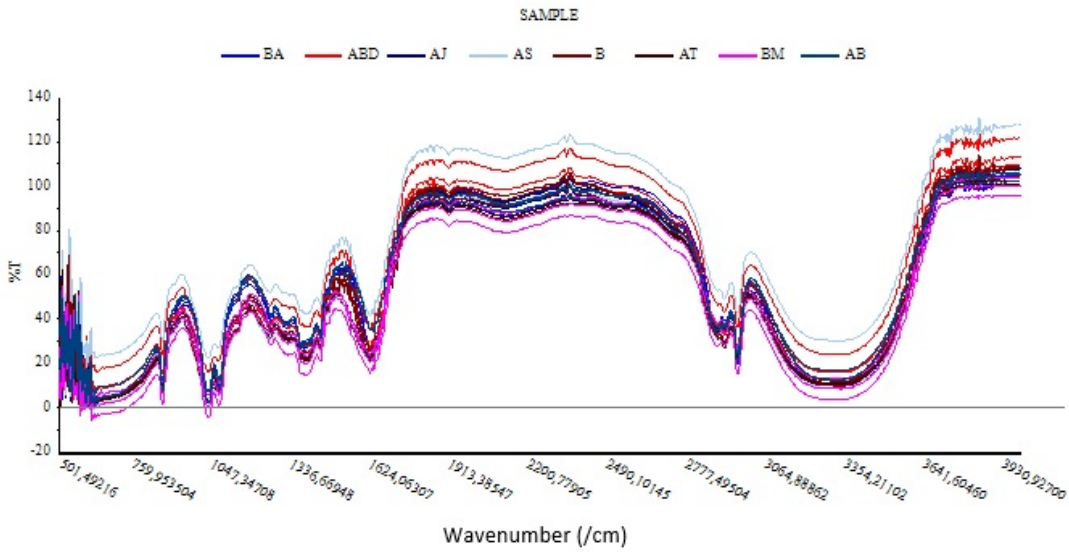

Fig. 1: FTIR spectra of ethanol extracts from *Isotoma longiflora* leaves

a polystyrene film, and the spectral data were compared with those from the National Institute of Standards and Technology. The *Isotoma longiflora* leaf powder sample was analyzed at a 4000–400/cm wavelength, yielding a total of 18 FTIR spectra. The obtained spectral data were saved in Excel (xlsx) for analysis (Cebi *et al.*, 2021; Khanban *et al*., 2022; Sufriadi *et al*., 2023; Tan *et al*., 2021).

Chemometric analysis

Each FTIR spectrum underwent data preprocessing using the first derivative method to develop a discriminant model to identify the origin of the sample using wavelengths of 500–4000/cm. Unscrambler Hierarchical cluster analysis (HCA), principal component analysis (PCA), and LDA 10.4 were performed (Adi *et al*., 2020; Akbar *et al*., 2021; Arifah *et al*., 2022; Nadia *et al*., 2019). Data validation was performed using cross-validation in the LDA model. The equations used in this analysis follow those of a previous study (Chauhan *et al.*, 2021).

RESULTS AND DISCUSSION

FTIR spectrum of the Isotoma longiflora leaf extract

Identification by infrared spectroscopy is based on the specific vibrational frequencies of certain molecular functional groups (Mellado-Mojica *et al*., 2016). A previous study acquired the infrared spectrum (wavelength of 4000–500/cm) from ethanol extracts of *Isotoma longiflora* leaves from multiple locations (Christou *et al*., 2018). Fig. 1 shows the FTIR spectrum of the ethanol extract of *Isotoma longiflora* leaves obtained in the present study.

Fig. 1 shows that the leaves from various locations exhibited the same spectral absorbance pattern, indicating that all samples had identical functional groups. The absorbance at wavelengths of 3000–3700/cm indicates the presence of hydroxyl (O-H) and amine (N-H) functional groups from polysaccharides and proteins (Christou *et al.,* 2018). The bands at 2927/cm and 2935/cm indicate asymmetrical or symmetrical strain to the methylene (CH_2) functional group. The alkene (C=C) functional group was observed at a wavelength of 1680–1600/cm, whereas the C=C aromatic functional group was observed at wavelength of 1600–1475/cm, the methyl (CH_3) functional group was observed at wavelength of 1400-1325/cm, and the alkoxy (C-O) functional group was observed at wavelength of 1100–1000/cm (Dogan *et al*., 2007; Rohman *et al*., 2011; Rohman and Man, 2010). The global functional categories are listed in Table 3. The absorption peaks from the FTIR spectra of leaves did not vary among locations. Fig. 1 shows that the difference between the samples was

Table 3: Typical peaks in the *isotoma longiflora* leaf FTIR spectrum

Spectral wavelength (/cm)								Functional Group/ Assignment
AB	BA	AJ	B	AT	BM	ABD	AS	
3361	3342	3331	3330	3338	3342	3331	3330	Bonded O-H Stretching / N-H stretching (Suresh *et al*., 2016)
2978	2978	2976	2975	2972	2978	2978	2976	CH_3 symmetric stretching; lipid, protein (Suresh *et al.,* 2016)
2897	2902	2922	2922	2931	2922	2922	2931	CH_2 asymmetric stretching; mainly lipid and protein (Suresh *et al*., 2016)
1658	1643	1639	1638	1643	1639	1638	1643	Stretching carbonyl (C=O) (Smith *et al*., 2019)
1633	1627	1633	1634	1622	1633	1634	1622	Assigned to tannins (Smith *et al*., 2019) C=C aromatic of condensed tannins (Smith *et al*., 2019) C=O stretching in flavones (Hands *et al*., 2016) C=O stretching in flavonoids (Smith *et al*., 2019) C=C stretching of the aromatic ring (Smith *et al*., 2019) N-H bending/C-N stretching (Topală *et al*., 2017)
1456	1456	1456	1456	1448	1456	1456	1448	Amide II of proteins, N–H, alkylamine (C–N) (Thummajitsakul *et al*., 2020)
1379	1379	1406	1402	1406	1379	1379	1406	Primary or secondary O–H bending (inplane), and phenol or tertiary alcohol (O–H bend) (Thummajitsakul *et al*., 2020)
1332	1332	1334	1336	1340	1332	1334	1336	CH_3 bending (Thummajitsakul *et al*., 2020)
1086	1056	1083	1076	1080	1056	1083	1076	C–O stretching vibrations in acid or ester (Thummajitsakul *et al*., 2020)

negligible. The absorption intensity of the functional groups differentiated the FTIR spectra of *Isotoma longiflora* leaf samples.

Chemometric analysis

Preprocessing was performed on the FTIR spectrum data of the ethanol extract of *Isotoma longiflora* leaves using the Derivative First method. Fig. 3 illustrates the original and preprocessed FTIR spectra using the Savitzky-Golay transform. This first-derivative method can be used to clarify the peaks and valleys of the FTIR/NIR spectra, separate the overlapping peaks, and eliminate the effects of spectral variations. Moreover, the first derivative can be used to remove the background, increase spectral resolution, and eliminate noise caused by differences in particle size in the extract (Akbar *et al.*, 2021; Cen and He, 2007).

Principal component analysis

The PCA was used to classify the *Isotoma longiflora* leaf samples from various locations. It is an unsupervised pattern recognition technique that can be used to reduce data and examine information to identify factors that can explain trends in a dataset (Gad *et al*., 2013). As PCA can be used to construct discriminant models for closely related plants, it was herein used with the Unscrambler 10.4 application to classify and differentiate the leaf extracts from plants grown in locations with different geographical conditions. Fig. 2 shows that the total variance of PC-1 and PC-2 was 89%. The decomposition of spectral data using PCA, where the Y axis displays the number of principal components (PC) and the x-axis displays the proportion of variance explained by the PC, showed that PC-1 explained 79% of the data variance and PC-2 explained 10% of the data

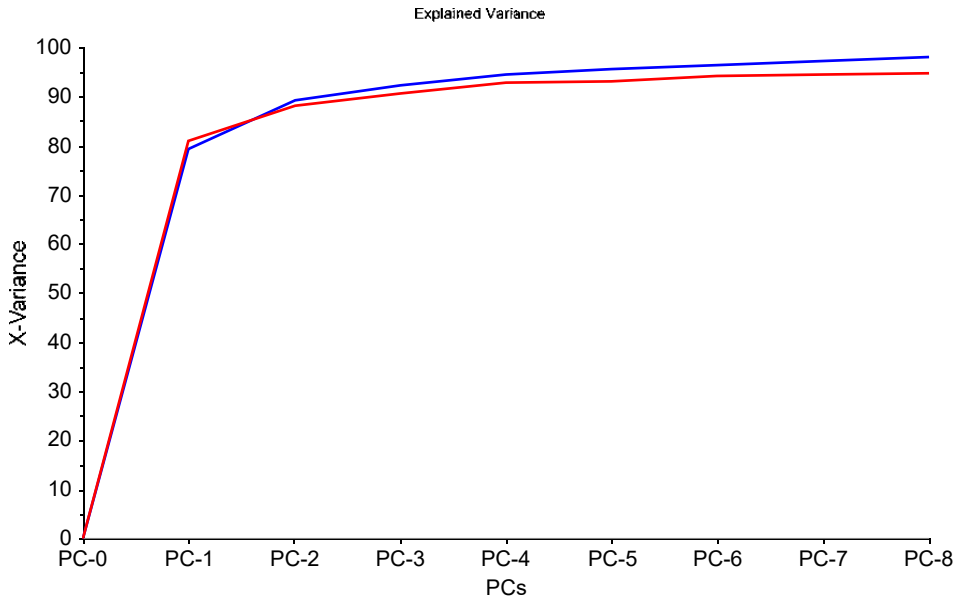

Fig. 2: Results of data variance that PCA can explain

variance. The total percentage of data variance was determined by accumulating the total percentage of variance specified by the previous PC-n and PC (dos Santos Grasel *et al*., 2016).

Fig. 3 shows the PCA score plot, which revealed the formation of three groups: group I with the AT and BM samples, group II with AB and BA samples, and group III with AS, ABD, AJ, and B samples. These results indicated that the various locations where the *Isotoma longiflora* plants grew corresponded to distinct leaf samples. The samples in group I were from the highlands (mountains), and the pattern of chemical contents in leaves from AT and BM were similar. In contrast, group II (AB and BA) comprised samples from locations far from the coast, whereas group III comprised samples from coastal regions. These results indicate that *Isotoma longiflora* leaf characteristics are influenced by the plants' collection location. This can occur because environmental factors strongly influence the secondary metabolite content of a plant, and the synthesis of secondary plant metabolites is affected by the plant's response to the environment (Gargallo-Garriga *et al*., 2014; Sampaio *et al*., 2016). Moreover, abiotic stresses such as the influence of light, temperature, humidity, water availability, nutrients, and salinity in the soil have been shown to significantly affect the content of secondary metabolites in plants (Bernstein *et al*., 2010; Khan *et al*., 2016; Sampaio *et al*., 2016). The FTIR-chemometric combination has been previously used to identify and authenticate samples. For instance, a study that successfully identified differences in coffee leaves obtained from various locations with different climatic conditions using PCA was based on differences in the FTIR spectra (Sanchez *et al*., 2018). The results demonstrated that FTIR spectrum analysis with PCA successfully distinguished coffee leaves taken from locations with different climatic conditions. In another study, FTIR spectra were analyzed using the PCA multivariate statistical method, and cluster analysis can be used to distinguish Fagus sylvatica L plants from different growth habitats (Rana et al., 2008). Another study stated that FTIR spectroscopy combined with chemometric methods was suitable for distinguishing among different geographical origins (Zhao *et al*., 2015). Fig. 4 depicts the loading factor, which is essential because it provides information regarding the factors that influence the formation of the PCA score plots (dos Santos Grasel *et al*., 2016). The loading factor indicated that PC1 had two types of loadings: positive and negative coefficient

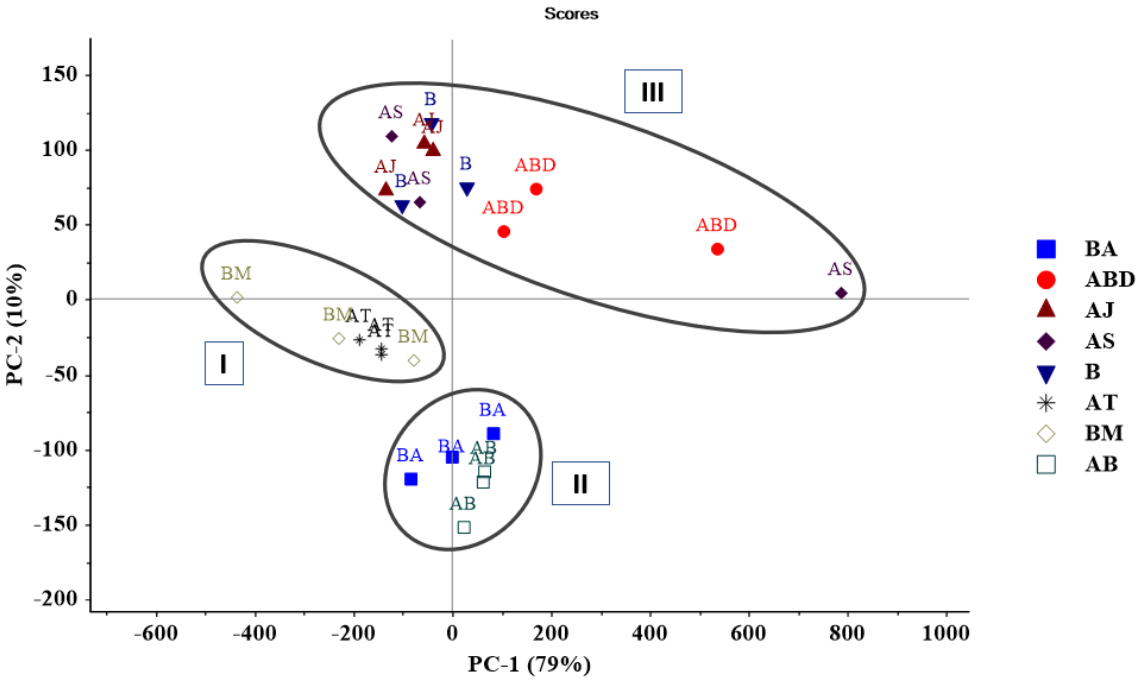

Fig. 3: PCA score plots for the FTIR spectra generated by the ethanolic extract *Isotoma longiflora* leaves

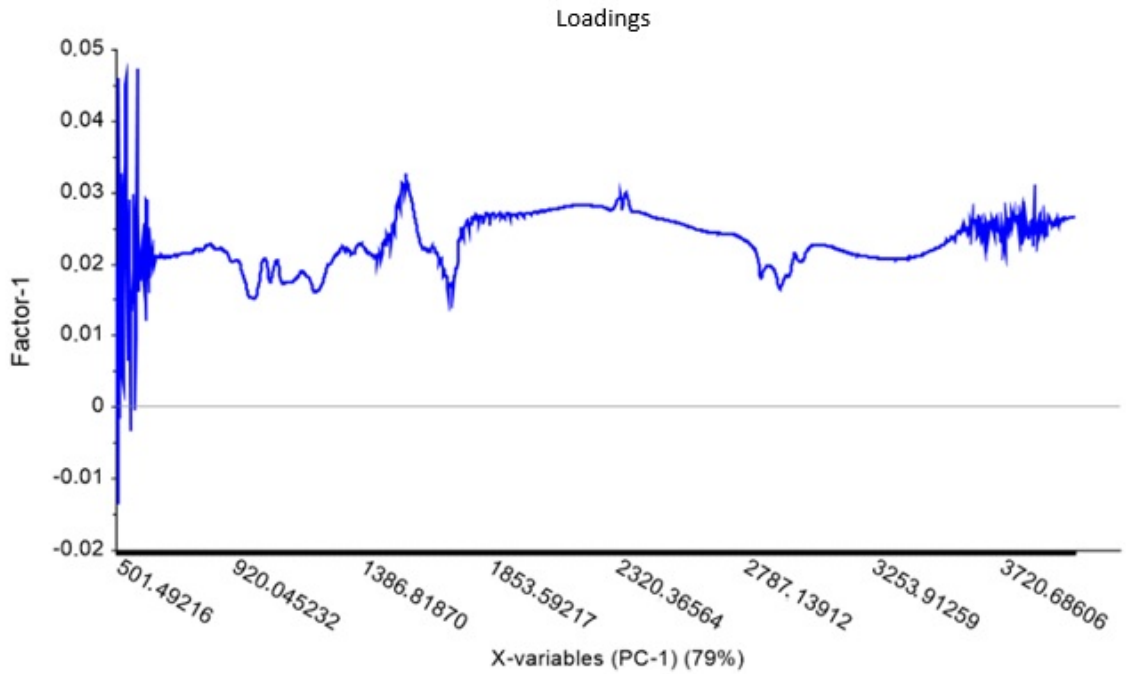

Fig. 4: Loading factor plots PC-1 and PC-2

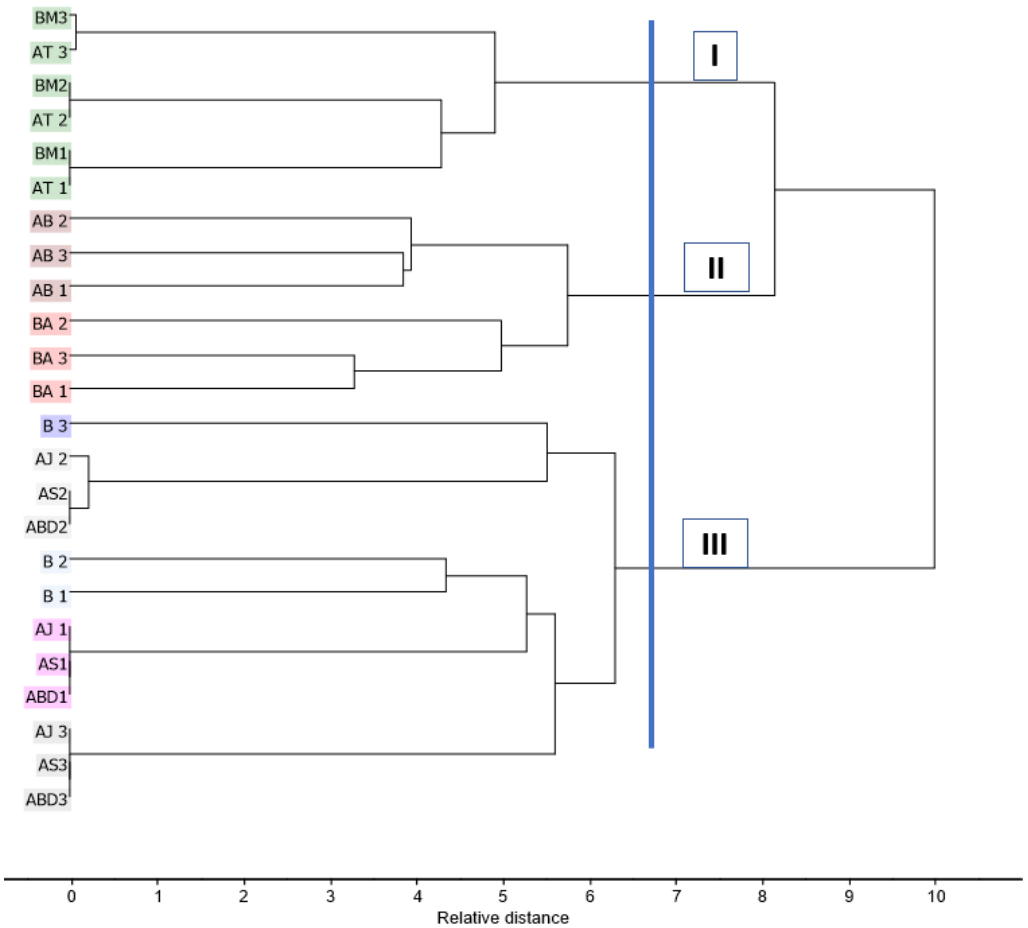

Fig. 5: Dendrogram of FTIR data of the ethanol extract of *Isotoma longiflora* leaves from several locations

values. The spectral band absorptions determined the position of the samples in the PCA score plot. For the PC-1 loading (positive coefficient), several positive absorption bands were dominant, namely at wavelengths 1103/cm, 2997/cm, 2910/cm, 2835/cm, 1573/cm, and 1692/cm. Several functional groups were identified, including C-O, CH_3, CH_2, C=O, and C=C. In contrast, loading with a negative coefficient value produced absorption that was dominant at wavelengths below 600/cm, which is in the fingerprint region, thus hindering the identification of functional groups. The loading factor provides information on the wavelengths of the samples. These absorption bands appeared on the loading factor that determined the position of the sample in the PCA plot, indicating that the classification of the ethanol extract of *Isotoma longiflora* leaf samples produced in the PCA plot was influenced by the similarity in some absorptions of the infrared spectra.

Hierarchical cluster analysis

HCA was used to confirm the results obtained from the PCA and provide a clearer picture of the similarities and differences among the *Isotoma longiflora* leaf extracts. The HCA results are shown in Fig. 5. Three groups were formed: group I with the AT and BM samples; group II with the AB and BA samples; and group III with the AS, ABD, AJ, and B samples.

Linear discriminant analysis

LDA authentication was performed to determine the characteristics of the groups formed in the

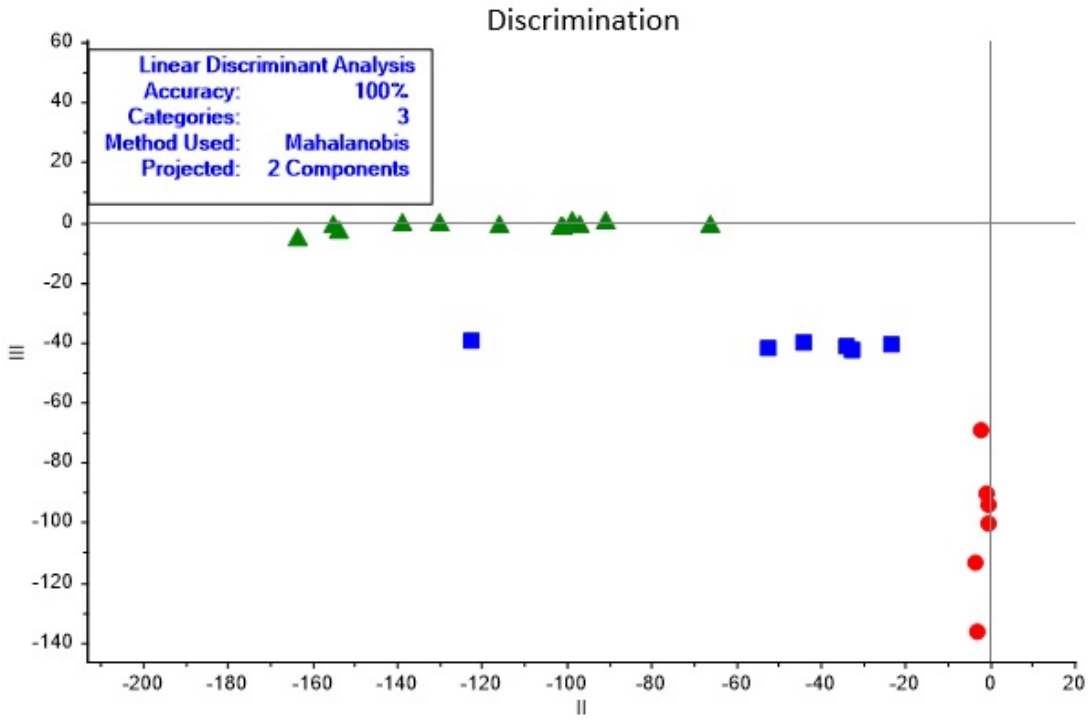

Fig. 6: LDA plot scores

Table 4: Confusion matrix

	Actual	II	III	I
Predicted		1	2	3
II	1	6	0	0
III	2	0	12	0
I	3	0	0	6

PCA (Fig. 6). The LDA prediction model had a 100% accuracy rate, and the LDA model based on the cross-validation results correctly predicted the origin group of *Isotoma longiflora* leaf samples. The confusion matrix for the LDA score plot is in Table 4.

The absence of incorrectly predicted groups indicates that the authentication using LDA was accurate, as supported by the confusion matrix table. The LDA model also demonstrates a reasonable degree of precision. The LDA calculations that influenced the confusion matrix are presented in Table 5. However, the precise components of the extract were not identified, as retrieving this information would require more complex procedures and many resources. Nevertheless, the chemometric approach might provide an alternative for observing the components of the extract, and hence, its potential use in quality control.

Table 5: Prediction results of the LDA model

Sample	LDA Model			Predicted
	I	II	III	
BA 1	-1183.44	-0.62295	-90.6374	II
BA 2	-2363.75	-3.28124	-113.658	II
BA 3	-379.578	-2.22572	-69.1505	II
ABD1	-3861.08	-66.0575	-1.70126	III
ABD2	-7280.79	-100.863	-2.44604	III
ABD3	-7013.67	-90.5694	-0.31428	III
AJ 1	-3876.14	-115.715	-1.61352	III
AJ 2	-7350.3	-138.922	-1.13064	III
AJ 3	-7031.2	-129.93	-0.75488	III
AS1	-3870.99	-96.9194	-1.47946	III
AS2	-7278.33	-163.261	-5.94169	III
AS3	-7039.81	-154.827	-1.27146	III
B 1	-3523.77	-101.374	-2.11552	III
B 2	-5689.31	-98.4065	-0.04601	III
B 3	-9061.34	-153.558	-3.18524	III
AT 1	-1.02229	-44.0626	-39.7827	I
AT 2	-1.78893	-32.4606	-42.6708	I
AT 3	-0.2388	-33.7401	-41.1731	I
BM1	-1.86294	-23.3225	-40.9473	I
BM2	-1.45359	-52.1477	-41.8311	I
BM3	-3.63345	-122.439	-39.1942	I
AB 1	-1562.17	-0.54158	-100.637	II
AB 2	-1274.18	-0.41189	-94.1179	II
AB 3	-3510.66	-2.91662	-136.738	II

CONCLUSION

The extracts from *Isotoma longiflora* leaves were successfully classified based on their FTIR spectra using a chemometric approach. The PCA score plot accounted for 89% of the total data variance, and showed the formation of three groups: group I included samples from Central Aceh and Bener Meriah; group II included samples from Aceh Besar and Banda Aceh; and group III included samples from South Aceh, West Aceh Daya, Aceh Jaya, and Bireuen. The differences among the extract samples were confirmed by the HCA results, providing a clearer picture of the similarities and differences in the observed *Isotoma longiflora* leaf extracts. Authentication was performed using LDA based on the results of the PCA classification with cross-validation of the training and validation sets. The LDA results revealed that the LDA model derived from the cross-validation predicted the origin of the *Isotoma longiflora* samples with 100% accuracy. These findings successfully demonstrate that the geographical conditions of origin affect the composition of the bioactive compounds in *Isotoma longiflora*. The findings of this study suggest the

potential use of FTIR and chemometric spectroscopy to determine the origin of plants, which can be beneficial for the pharmaceutical industry. The chemometric-assisted classification technique could be significant for quality control and assurance, allowing the quality of drug materials (especially from traditional medicine) to be authenticated. This technique is also useful for differentiating among products produced at certain locations, which affects supply chain management, especially when determining the source of the raw material. Further research is required to explore the impact of factors such as climate and soil conditions on the bioactive compounds in *Isotoma longiflora* leaves and other plants. Employing a greater variety of geographical locations can help generate a more comprehensive dataset. These findings can also provide insights into environmental management, to select locations that are suitable for medicinal plant cultivation.

AUTHOR CONTRIBUTIONS

E. Imelda contributed in the conceptualization, experiment, and original-draft writing. K. Khairan acted as a supervisor and contributed in reviewing the final version of the manuscript. R.R. Lubis acted as a supervisor and contributed in reviewing the final version of the manuscript. P. Kemala performed the scientific, results validation, and reviewing the final version of the manuscript. U. Zulfiani performed the scientific, results validation, and reviewing the final version of the manuscript. U. Zulfiani assisted the experiment and performed formal analisis. T. Karma assisted the experiment and performed formal analisis. S. Rahayu assisted the experiment and performed formal analisis. G.M. Idroes performed the scientific, results validation, and reviewing the final version of the manuscript. S.M. Adev performed the scientific, results validation, and reviewing the final version of the manuscript. N.B. Maulydia performed the scientific, results validation, and reviewing the final version of the manuscript. R. Idroes acted as a supervisor and contributed in reviewing the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this manuscript. In addition, ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, were observed by the authors.

ABBREVIATIONS

%	Percent
AB	Aceh Besar
AJ	Aceh Jaya
AT	Aceh Tengah
B	Biereun
BA	Banda Aceh
BM	Bener Meriah
C=C	Alkene
CH_2	Methylene
CH_3	Methyl

cm	Centimeter
C-N	Alkylamine
C-O	Alkoxy
C=O	Carbonyl
DA	Discriminant analysis
IR	Infrared
Fig.	Figure
FTIR	Fourier-transform infrared spectroscopy
HCA	Hierarchical cluster analysis
LDA	Linear discriminant analysis
MSC	<ultiplicative scatter correction
N-H	Amine
O-H	Hydroxyl
PC	Principal component
PCA	Principal component analysis
PLS	Partial least squares
SIMCA	Soft independent modelling by class analogy
SVM	Support vector machine

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