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Chemical characterization of mint (*Mentha* spp.) germplasm from Central Java, Indonesia

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Abstract. *Lianah L, Kusumarini N, Hafshah M, Krisantini K, Kurniawati A, Ahmad MA. 2023. Chemical characterization of mint (*Mentha *spp.) germplasm from Central Java, Indonesia. Biodiversitas 24: 4307-4313.* The main objective of this work was to evaluate and compare the leaf production and oil content of the four mint genotypes (*Mentha* spp.) from Central Java, Indonesia. Four genotypes were collected from Rejosari Village, Kudus District, Central Java, Indonesia, namely spearmint (*Mentha spicata*), peppermint (*Mentha x piperita*), orange mint (*Mentha x piperita* 'Orange') and chocolate mint (*Mentha x piperita* 'Chocolate'). Essential oil extraction was carried out by steam distillation. Identification of the chemical compounds of the oil and their relative percentage were analyzed using GC-MS. Leaves productivity ranged between 500-1000 kg/ha, and essential oil content from 0.4 to 1.36%. The major essential oil constituents detected were 1,8-cineole, carvone, limonene, linalool, linalyl acetate, menthol, menthone, menthyl acetate, and piperitenone oxide. Chocolate mint had the highest leaf production and the highest content of essential oil (4.17%) and menthone yield (28%) compared to the other genotypes.

Keywords: Chemical characterization, essential oil, genetic resources, *Mentha*

INTRODUCTION

Essential oil is a vegetable oil with a distinctive aroma. It can be extracted from leaves, flowers, seeds, stems, and the roots of plants (Butnariu and Sarac 2018). However, Julianto (2016) stated that essential oils with the highest economic value are derived from flower organs. It has about 160-200 species of aromatic plants that produce essential oils, mostly from the families Compositae, Gramineae, Labiate, Lauraceae, Myrtaceae, and Umbelliferae. Essential oils are used in various fields, such as health, food and beverage, cosmetics, perfumes, and pesticides (De Oliveira et. al. (2018). Currently, 99 types of essential oils are traded on the world market. Indonesia produces 40 types of essential oils, some of them (spatula, citronella, cloves, galanga, turmeric, ginger, nutmeg, pepper, cinnamon, sandalwood, jasmine, vetiver, ylang, eucalyptus, cardamom, fennel, kaffir lime, frangipani, cubeb, and peppermint) are on the world market. Indonesia is ranked the sixth largest exporter of essential oils in the world (Oec world 2021). The primary exported essential oil is a spice-based essential oil (from lemongrass, nutmeg, cinnamon, ginger, cardamom, fennel, and sandalwood) (58.7%), followed by essential oil of citrus (13.2%), and essential oil of mint (5.6%).

Mentha (Lamiaceae) comprises 18 species and 11 named hybrids (Tucker and Naczi 2007). Mint species are famous for their essential oils. The genus *Mentha* is typically perennial and spread through stolons. In the optimal environment, it can thrive and spread rapidly. The stems are usually square-shaped with opposite leaves. Their flowers are generally white but vary with species; they can be purple or pink. Mint has been cultivated and used for centuries; harvesting is typically carried out by snipping the old (but not too-old) stems with leaves and branches close to the ground, leaving several nodes at the base so the plants continue growing (Banik 2015). According to Hocking (2007), mints can grow well in deep, rich soils of friable texture high in organic matter with a pH range of 6.0-7.5. Mints generally require high water, but the medium should have good drainage. The ideal growing temperatures for mint are warm sunny days (25°C) and cool nights (15°C).

The aromatic mint leaves are used both fresh and dry as flavorings or seasoning in various foods and beverages and are also used in traditional ceremonial rituals and as medicine. The essential oil of mint species has also been used in dental and oral products and fragrances (Sarkic and Stappen 2018). The chemical compounds of mint essential oils composition have been reported (Maia 1998). The differences in the essential oil composition in different species are obtained in the number of commercial constituents (menthol, menthone, carvone, limonene, linalool, menthyl acetate, piperitone, and pulegone). Menthol is mostly used in confectionery, perfumery, and candies. Menthone is used in perfume and flavor (Buleandra et al. 2016). Carvone is a perfumery and oral hygiene product flavor (Bauer 1997). Limonene is an antioxidant and can be used as a solvent, wetting, and dispersing agent. Linalool is used in perfumery, as it has a similar odor to bergamot or French lavender (Pereira et al. 2018). Menthyl acetate is used in perfumery and toilet waters for a lavender odor. Piperitone is used in masking fragrances in dentifrices. Pulegone has a pleasant aroma, midway between peppermint and camphor, but it can be toxic (Windholz 1983). Some of the crucial metabolites in the essential oils of *Mentha* spp. are 1,2-epoxyneomenthyl acetate, 1,8-cineole, 3-octanol, 3-octanone, 3-octyl acetate, beta-caryophyllene, carvone, caryophyllene oxide, cis-dihydrocarvone, cis-sabinene hydrate, decyl acetate, elemol, geraniol, germacrene D, isomenthone, limonene, linalool, menthol, and menthone (Eftekhari et al. 2021).

The National Clonal Germplasm Repository (NCGR) maintains a mint gene bank worldwide. It preserves mint germplasm, including the mint wild relatives (Vining et al. 2020). Our preliminary study (unpublished) has identified four mint genotypes in Central Java, Indonesia, i.e.: spearmint (*Mentha spicata*), peppermint (*Mentha* x *piperita*), orange mint (*Mentha* x piperita 'Orange'), and chocolate mint (*Mentha* x *piperita* 'Chocolate'). The primary goal of this work is to determine the leaf production of the four genotypes and to characterize their volatile oil constituents.

MATERIALS AND METHODS

Study area

The research was conducted from June to December 2022 in a mint grower in Rejosari Village, Kudus District, Central Java, Indonesia, 90 m above sea level. Kudus is located 11.036'-110.50' E and 6.51'-7.16' S. The area's temperature ranges from 22.8°C to 31.6°C with a relative humidity of 86.3% to 89.2% (Research data)

Procedures

Taxonomic identification

The scientific names of the four mint genotypes were based on plant identification, according to Tucker and Naczi (2007). Mint was propagated by stem cuttings, then rooted cuttings with 5-6 nodes were planted in pots (0.75 L) with a pasteurized mixture of cocopeat, compost, and roasted rice husk (v/v, 1:2:1). Each genotype consisted of 20 individual plants. Plants were grown in the open field. Leaf productivity was measured at 16 weeks after planting. Taxonomic identification was conducted in the Biology Structure Laboratory at the Islamic State University of Walisongo, Semarang, Indonesia.

Distillation of mint oil

The distillation process, yield calculation, and oil color determination of 4 mint cultivars were carried out according to Ravindranath et al. (2018) at the CNH Pedurungan Laboratory, Semarang. The colorimeter app (version 1.6.6.6, developed by Research Lab Tools, São Paulo, Brazil) determined oil color. It was installed on an Android smartphone. The app allows online and offline analysis of samples. The interface is easy to use, and color changes can be recorded using the camera in the smartphone (20 megapixels).

GC-MS analysis

GC-MS analysis is used to identify the chemical components in a sample in the GC-MS analysis, the separation of organic compounds was conducted using two methods. Gas-Chromatography (GC) analyses the number of compounds qualitatively, and Mass Spectrometry (MS) analyses the molecular structure of the identified compounds. Mint shoots of each genotype were collected, weighed, placed in a paper bag, and dried in a cabinet drier at 45ºC for 15 hours before oil analysis. The essential oil was extracted by steam distillation in a 1.5 L flask for one hour at 80ºC-90ºC. The oil was analyzed in a Gas Chromatograph (GCMS-QP2010S SHIMADZU) with an HP-5 Phonomenex ZB-5 (30mm x 0.25mm x 0.25mm) capillary column. The oven temperature was programmed from 35ºC to 280ºC at 10ºC/min, and hydrogen was used as carrier gas (2 mL/min). One µL of pure oil was injected into GC. Mass spectra were obtained using an electron impact mode (EIMS) system at 70 eV. The identification was based on the compound's mass spectra by calculating their retention indices (RI) compared to. literature data. (Maryland: 2005) The oil component is calculated based on the relative percentage of dry weight. Other parameters studied were acid number, ester number, and chemical profile of the compound in each genotype of mint. The acid number and ester number were analyzed by titration method.

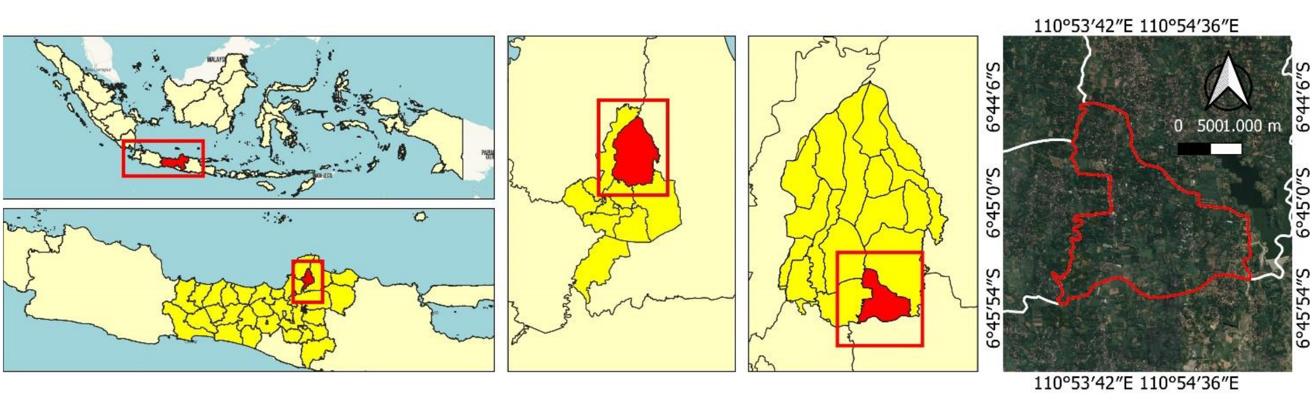

Figure 1. Mint sampling site at Rejosari Village, Kudus District, Central Java, Indonesia

Data analysis

Quantitative data were collected on plant height, leaf number, stem diameter, number of branches, and leaf fresh weight at 16 weeks after planting. Data were analyzed using Excel by calculating the means.

RESULTS AND DISCUSSION

Leaf productivity

Chocolate mint had the highest leaf productivity at 16 weeks after planting, demonstrated by the highest leaf number and shoot fresh weight (Table 1). Chocolate mint is relatively small (14-15 cm in height) after 16 weeks, particularly compared to spearmint, with more than 20 cm in height (Table 1).

Physical and chemical properties of mint oil

The essential oil content of the four mint genotypes is presented in Table 2. Chocolate mint has the highest yield (1.36%) compared to the other 3 genotypes, which have <1% (Table 2). The oil's color and aroma also vary; orange mint oil has gold orange, whereas the other genotypes are brown. It is the first report on *Mentha* sp. Volatile oils cultivated in Central Java, Indonesia, and identifies the genotypes based on their chemical properties (Table 3-6).

Table 3-6 presents each genotype's volatile oil constituent. The major compound of the volatile oil of the mint genotypes is menthol, followed by menthone, except for the orange mint. The major compound of the volatile oil of orange mint is linalyl acetate (Table 5).

Based on the results of the GC-MS analysis, there are similarities in the profiles of orange mint and spearmint oils. The main component is >60%, which appears at a retention time of 19-21 minutes, and the second largest component is 10-14% at a retention time of 16-18 minutes. The chocolate mint oil has more varied compounds, with the highest abundance of 29% at a retention time of 19 minutes (Tables 6 and 7).

Peppermint had the highest acid group (9%), while chocolate mint had 4%, but chocolate mint had the highest percentage of tannin (11.5%) (Table 8). Peppermint had the highest saponification number, whereas chocolate mint had the lowest (Table 8). Spearmint and peppermint had higher flavonoids than chocolate and orange mint (Table 8).

Table 1. Growth and productivity of four mint genotypes from Central Java at 16 weeks

Genotypes	Fresh weight (gram)	Plant height (cm)	Leaf number	Stem diameter (mm)	Branch no
Spearmint (M1)	49.87	20-22	14-21	1	1
Peppermint (M2)	58.05	14-18	16-20	3	3
Orange mint (M3)	49.28	17-18	8-20	3	3
Chocolate mint (M4)	50.17	14-15	19-28	2	2

Table 2. Oil properties of mint genotypes from Central Java, Indonesia

Genotypes	Scientific name2	Essential oil content (%)	Oil color	Aroma
Spearmint (M1)	*Mentha spicata*	0.5	Light brown	Strong
Peppermint (M2)	*Mentha* x *piperita*	0.4	Brown	Spicy
Orange mint (M3)	*Mentha* x *piperita* 'Orange'	0.4	Gold orange	Medium spicy
Chocolate mint (M4)	*Mentha* x *piperita* 'Chocolate'	1.36	Brown/red	Spicy

Note: 2Scientific name was based on plant identification according to Tucker and Naczi (2007)

Table 3. Chemical compounds of spearmint (*Mentha spicata*) volatile oil from Central Java, Indonesia

Peak	Area	RT	Component	SI	Percentage
13	154905310	19.63	Menthol	96	63.5%
10	26053052	18.77	Menthone	94	10.7%
19	12045044	21.90	Piperitone oxide	86	4.9%
11	8589330	19.05	Menthone	93	3.5%
22	7958494	22.93	Methyl acetate	91	3.3%
35	5319679	28.49	Germacrene D	91	2.2%
12	3860734	19.20	Menthol	94	1.6%
30	2544348	26.30	trans-Caryophyllene	93	1.0%
34	2436388	28.07	Bicyclo[2.2.2]octane	72	1.0%
18	2197676	21.40	Pulegon	96	0.9%
5	1941412	13.01	3-Octanol	94	0.8%
28	1403257	26.35	1,6-Octadien-4-ol	81	0.6%
14	1189093	19.88	Menthol	91	0.5%
4	1153088	12.73	Beta-myrcene	92	0.5%

Table 4. Chemical compounds of peppermint (*Mentha* x *piperita*) volatile oil from Central Java, Indonesia

Peak	Area	RT	Component	SI	Percentage
8	227053800	19.69	(-)-Menthol	96	66%
5	18694422	18.76	Menthone	94	5%
16	17090919	22.95	Menthyl acetate	89	5%
13	15878985	21.94	Piperitone	95	5%
24	12365715	28.13	1-Chlorobicyclo 2.2.2 octane	73	4%
26	7954831	28.50	Germacrene-D	91	2%
6	5632587	19.04	Menthone	93	2%
7	5238597	19.21	(-)-Menthol	94	2%
17	3453439	23.21	2-Cyclohexen-1-one	90	1%
22	3362385	26.83	trans-Caryophyllene	94	1%
21	2986508	26.35	1,6-Octadien-4-ol	81	1%
18	2418724	24.44	Bicyclo[3.1.1]hept-3-en-2-one	89	1%
11	1980720	21.12	Piperitone	86	1%
12	1958056	21.42	+)-Pulegone	97	1%

Table 5. Chemical compounds of orange mint (*Mentha* x *piperita* ‘Orange’) volatile oil from Central Java, Indonesia

Peak	Area	RT	Components	SI	Percentage
12	239138885	21.81	Linalyl acetate	97	64%
4	54469495	16.85	Linalool	94	15%
22	18096642	30.22	Cyclohexanemethanol	91	5%
2	11943879	14.48	beta.-trans-Ocimene	95	3%
18	7352895	26.84	trans-Caryophyllene	94	2%
24	6635187	31.48	Veridiflorol	90	2%
15	5091883	25.43	Geranyl acetate	95	1%
3	4055682	14.85	1,3,7-octatriene	95	1%
5	3161973	17.00	Oct-1-en-3-acetyl-acetate	94	1%
1	2997352	12.73	Myrcene	91	1%
14	2647777	24.87	Geranyl acetate	93	1%
9	2429705	20.02	alpha.-terpineol	95	1%
8	2366183	19.45	(-)-Menthol	93	1%

Table 6. Chemical compounds of chocolate mint (*Mentha* x *piperita* ‘Chocolate’) volatile oil from Central Java, Indonesia

Peak	Area	RT	Component	SI	Percentage
13	104436600	19.62	(-)-Menthol	95	30%
10	97705965	18.86	Menthone	94	28%
11	51047780	19.06	Benzofuran	94	15%
20	31526692	22.98	Menthyl acetate	93	9%
8	14228511	14.38	Eucalyptol	93	4%
12	11064250	19.24	(-)-Menthol	94	30%
25	4521981	28.50	Germacrene-	91	1%
3	4273133	12.30	beta.-Pinene	96	1%
22	3939033	26.84	trans-Caryophyllene	94	1%
9	3699311	15.75	trans-4-Thujanol	86	1%
7	3564460	14.25	Limonene	95	1%
1	3140918	10.58	alpha.-pinene	96	1%
16	2542403	21.42	(+)-pulegone	97	1%
19	1995263	22.41	Menthyl acetate	93	1%
2	1807893	12.11	Sabinene	95	1%

Discussion

Four *Mentha* genotypes have chemical variability among mint genotypes (Tables 2-6). The four accessions from Central Java had at least 13 constituents (Tables 3-6), which are dominated by menthol and menthone (Labdelli et al. 2022). It has been shown that genotype has a quantitative effect on volatile organic compounds (VOCs) related to scent. According to Passa et al. (2023), measuring VOCs provides valuable information on the differences in aroma between various strawberry genotypes, enabling the selection of genotypes based on their unique VOC content and volatile stability during the harvest season. Stasiak and

Latocha (2020) have identified 120 compounds from 15 Actinidia genotypes. However, the number of identified compounds differed across the various species. Each species exhibited its own set of primary or volatile compounds, distinguishing their respective flowers. Badr et al. (2021) reported that analysis of genome profiles and essential oil components revealed differences among *Achillea fragrantissima* accessions from different populations. Badr et al. (2021), who studied the genetic variations between wild and regenerated genotypes, confirmed that the in vitro conditions increased genetic variation; however, it was associated with a decrease in the quantity and diversity of essential oil components.

Table 3-6 shows that the highest number of secondary metabolites is chocolate mint (15 compounds), followed by peppermint (14 compounds), spearmint (14 compounds), and orange mint (13 compounds). Two secondary metabolites were found in all four mint variants: menthol and trans-caryophyllene. Menthol is the main constituent in mint (Chang 1948; Verma et al. 2010; Gade and More 2017; Batool et al. 2018; Kowalczyk et al. 2021). Menthol has been widely utilized to treat digestive and respiratory problems. Additionally, menthol exhibits antibacterial and antifungal activities, relieving headaches (Best 2022). Caryophyllene is a volatile compound in the terpenoid group, with a carbon framework consisting of 15 atoms, or sesquiterpenes. Caryophyllene was present in 21 mint genotypes (including four mint variants: spearmint, peppermint, chocolate mint, and orange mint) out of 25 mint genotypes, with varying concentrations (Lu et al. 2022). Caryophyllene has biological activity as an anticancer agent (Fidyt et al. 2016) and treats plaque in the mouth (Pieri et al. 2014, 2016).

Menthol content varied among variants. Menthol is a natural organic compound that provides a refreshing and cooling sensation when inhaled or applied to the skin. The highest menthol content is in peppermint (66.1%), followed by spearmint (63.5%), chocolate mint (29.8%), and orange mint (1%). This finding is unique because orange mint only contained 0.6% menthol, and the main components are linalyl acetate (64.4%) and linalool (14.7%). These components are exclusively found in the orange mint. Linalyl acetate and linalool are volatile components in oranges (Elsharif et al. 2015; Okla et al. 2019; Oulebsir et al. 2022). Linalyl acetate and linalool have a distinct refreshing citrus aroma and calming effect (Deterre et al. 2012). Linalool has the most intense citrus aroma among compounds in its group (Elsharif et al. 2015) related to the -OH group attached to the C-3 structure of linalool. There is a close relationship between distinct aroma, biological activity, and the content of volatile compounds in a natural substance. (Shaaban et al. 2012). Therefore, linalool and linalyl acetate influence orange mint's distinctive scent, distinguishing it from other mint variants.

The GC-MS analysis results also indicated that spearmint, peppermint, and chocolate mint variants have the same eight secondary metabolites, namely menthone, menthol, pulegone, piperitone, methyl acetate, 1,6-octadien-4-ol, and germacrene D, with varying amounts. It indicates that the orange mint variant is the most distinct based on its volatile component. The terpenoid family of compounds, which includes menthol, pulegone, and piperitone, has ten carbon atoms. These substances are classified as homoterpenes, which are terpenoid groups with 10 carbon atoms, based on the number of carbon atoms and the structure of the chemical. Germacrene D has 15 carbon atoms, classified as a sesquiterpene. Germacrene D is also found in the essential oils of mint plants such as *M. spicata* and *M. suaveolens* (Zekri et al. 2023). Pulegone has been found in the oil and dried leaves of *Mentha piperita* (Siano et al. 2005). Piperitone has also been obtained in three mint variants, namely *Mentha piperita, M. spicata, and M. suaveolens* (Giménez-Santamarina et al. 2022; Zekri et al. 2023).

Peppermint has the highest concentration of menthol (60%), whereas orange mint has the lowest 1% (Table 6). The other common compounds obtained in mint studies include limonene, menthone, and cineol. The presence of these compounds contributes to mint oil flavor and aroma. Chocolate mint has the most diverse (15) components (Table 6) and has the highest percentage of menthone (28%, Table 6) compared to the other mint genotypes, which only have 5-10%. In addition, chocolate mint has a unique component of benzofuran (15%) and eucalyptol (4%, Table 6), which was not detected in the other mint genotypes.

Table 7. The three highest compounds in the essential oil of four mint genotypes from Central Java, Indonesia

Mint cultivars	Rt	The order of dominant compound	Abundance (%)
Orange mint	21.28	1	64.45
	16.85	2	14.68
	30.22	3	4.88
Chocolate mint	19.61	1	29.27
	18.86	2	27.85
	19.06	3	14.55
Peppermint	19.69	1	66.11
	18.76	2	5.44
	22.94	3	4.98
Spearmint	19.63	1	63.47
	18.76	2	10.68
	21.9	3	4.94

Table 8. Acid, saponification, and ester number of the mint genotypes

Mint cultivars	Acid number	Saponification number	Esther number	Phenol (%)	Flavonoid (%)	Tannin (%)
Spearmint	7.505	7.334	0.171	0.813	0.005	1.08
Peppermint	9.912	9.228	0.684	4.576	0.004	7.50
Orange mint	8.251	7.334	0.917	1.040	0.002	1.80
Chocolate mint	4.114	3.643	0.471	6.213	0.002	11.5

Eftekhari et al. (2021) stated that several compounds, i.e., 1,2-epoxyneomenthyl acetate, 1,8-cineole, 3-octanol, 3-octanone, 3-octyl acetate, beta-caryophyllene, carvone, caryophyllene oxide, cis-dihydrocarvone, cis-sabinene hydrate, decyl acetate, elemol, geraniol, germacrene D, isomenthone, limonene, linalool, menthol, and menthone, are important metabolites in *Mentha*. These compounds are also present in the four mint genotypes from Central Java. Linalool is a terpene alcohol with a floral aroma as the most potent flavor in orange juice (Bazemore et al. 2013). There are similarities in the profiles of orange mint and spearmint oils (Table 7); i.e., the main component (>60%) appears at a retention time of 19-21 minutes, while the second major component of 10-14% at a retention time of 16-18 minutes. Peppermint has the highest acid group (9%), while chocolate mint has 4% (Table 8). The acid number measures the number of carboxylic acid groups in a chemical compound and indicates the acidic content of the oil. The acid number can determine the quality and stability of oils. The higher the acid number, the inferior the quality of the fat or oil, as it indicates the oil's oxidation, which reflects the oil's freshness and its potential for rancidity. Therefore, the acid number can control the quality of raw materials or products (Idris et al. 2014).

A study by Keshavarz and Sanavy (2018) reported that the leaf and essential oil production of two mint species, *Mentha piperita* and *Mentha arvensis*, increased significantly by applying organic and chemical fertilizers. As a result, the amount of oil components may differ from what we observed in our study, depending on cultural behaviors. The environment also plays an important role, i.e., oil components and their abundance might significantly change in different environments or seasons (Labdeli et al. 2022). In a further study, we plan to determine the optimal growing culture of different mint cultivars to continuously and sustainably produce mint.

This study showed that the best quality oil among the four mint varieties was oil from the chocolate mint. There are similarities in the profiles of orange mint and spearmint oils: the main component is >60%, which appears at a retention time of 19-21 minutes, while the second major component is 10-14% at a retention time of 16-18 minutes. The chemical compounds of the chocolate mint oil are more varied, with the highest abundance of 29% at a retention time of 19 minutes.

In conclusion, chocolate mint had the highest leaf productivity at 16 weeks after planting, the highest oil content, and the most variable compounds compared to spearmint, orange mint, and peppermint.

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