

A comparative study on essential oil yield, chemical composition and antimicrobial activity of essential oils from *Ocimum* species cultivated in Vietnam

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ABSTRACT

This study comparatively evaluated the essential oils of *Ocimum tenuiflorum*, *Ocimum gratissimum*, and *Ocimum basilicum* cultivated in Hanoi, Vietnam. The aerial parts were subjected to hydrodistillation, and the resulting oils were analyzed by GC-FID/MS. Essential oil yields varied significantly among species, ranging from 0.84% (*O. tenuiflorum*) to 1.42% (*O. gratissimum*). Chemical profiling revealed distinct chemotypic patterns: *O. tenuiflorum* was dominated by methyl eugenol (56.28%) with eugenol (15.06%); *O. gratissimum* by eugenol (62.07%) and germacrene D (15.17%); and *O. basilicum* by methyl chavicol (51.45%) and linalool (18.41%). Antimicrobial activity assessed by the broth microdilution method demonstrated that *O. gratissimum* exhibited the strongest inhibitory effects, particularly against *Staphylococcus aureus* (MIC 50 µg/mL) and *Candida albicans* (MIC 50 µg/mL), whereas *O. tenuiflorum* and *O. basilicum* showed moderate activity. Overall, the findings confirm that species identity and chemotype strongly influence essential oil yield, chemical composition, and antimicrobial performance under identical cultivation and extraction conditions.

Keywords: Antimicrobial activity, essential oil, *Ocimum basilicum*, *Ocimum gratissimum*, *Ocimum tenuiflorum*,

INTRODUCTION

The genus *Ocimum* (Lamiaceae) comprises a diverse group of aromatic and medicinal plants widely distributed in tropical and subtropical regions and extensively cultivated for culinary, pharmaceutical, and industrial applications (Viyoch *et al.*, 2006; Zahran *et al.*, 2020). Among the most economically and pharmacologically important species are *Ocimum tenuiflorum* L. (holy basil), *Ocimum gratissimum* L. (clove basil), and *Ocimum basilicum* L. (sweet basil). These species are

characterized by the production of essential oils rich in biologically active secondary metabolites, primarily monoterpenes, sesquiterpenes, and phenylpropanoids, which contribute to their distinctive aroma and functional properties (Zheljazkov *et al.*, 2008; Muráriková *et al.*, 2017). The chemical composition of *Ocimum* essential oils are highly variable and strongly influenced by genetic factors, chemotype differentiation, environmental conditions, and agronomic practices, resulting in considerable intra- and interspecific diversity (Kothari *et al.*, 2004).

Chemotypic variation has been extensively documented within *Ocimum* species. *O. gratissimum* is frequently reported as a eugenol-dominant chemotype in several geographical regions, although thymol-dominant and other minor chemotypes have also been described (Nakamura *et al.*, 1999). *O. basilicum* exhibits remarkable chemical polymorphism, with major constituents including linalool, methyl chavicol (estragole), methyl cinnamate, and eugenol, depending on cultivar and environmental conditions (Tran *et al.*, 2018). Similarly, *O. tenuiflorum* may present eugenol- or methyl eugenol-rich profiles, reflecting differential regulation of the phenylpropanoid biosynthetic pathway (Shiwakoti *et al.*, 2017). Such variability highlights the importance of conducting region-specific investigations to accurately characterize essential oil composition and identify dominant chemotypes.

Essential oil yield is another critical parameter determining the economic feasibility and technological potential of aromatic plants. Reported yields for *Ocimum* species typically range from below 1% to above 2% (v/w), depending on harvest stage, drying method, and extraction technique (Anh *et al.*, 2019). Environmental factors, including temperature, soil conditions, and photoperiod, can also significantly influence volatile accumulation. Therefore, comparative studies conducted under standardized cultivation and distillation conditions are essential to obtain reliable data and minimize variability attributable to methodological differences.

Vietnam offers favorable ecological conditions for the cultivation of aromatic plants, including *Ocimum* species. Nevertheless, comprehensive comparative studies examining essential oil yield, chemical composition, and antimicrobial activity of multiple *Ocimum* species grown under identical environmental and analytical conditions remain limited. Such integrated investigations are necessary to clarify interspecific differences, confirm chemotype

identity, and correlate compositional patterns with biological properties. Therefore, the present study aims to comparatively evaluate the essential oils of *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* cultivated in Hanoi, Vietnam, through assessment of essential oil yield, detailed GC–FID/MS chemical characterization, and antimicrobial activity under standardized experimental conditions.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves and tender stems) of *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* were collected in July 2025 from cultivated fields in Hanoi, Northern Vietnam (21°01'N, 105°51'E). The plants were harvested at the full flowering stage between 08:00 and 09:00 a.m. under dry weather conditions to minimize surface moisture and potential loss of volatile constituents. The plant materials were taxonomically identified by a botanist at Hong Duc University, Vietnam. Voucher specimens were prepared, authenticated, and deposited in the University herbarium under accession numbers OT-0725 (*O. tenuiflorum*), OG-0725 (*O. gratissimum*), and OB-0725 (*O. basilicum*). After collection, the samples were cleaned to remove soil and extraneous matter and air-dried at room temperature (25 ± 2 °C) in a well-ventilated, shaded area for 5–7 days until constant weight was achieved (Ussen *et al.*, 2025). The dried materials were then chopped into small pieces and stored in airtight polyethylene bags at 4 °C in the dark until essential oil extraction.

Essential oil extraction

The essential oils of *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* were obtained by hydrodistillation using a standard Clevenger-type apparatus (Borosilicate glass, DWK Life Sciences, Germany), in accordance with the procedures recommended by the Vietnamese Pharmacopoeia V for volatile oil

determination (Ministry of Health, 2009), with appropriate laboratory-scale adaptation. Briefly, 100 g of air-dried plant material were placed in a 2 L round-bottom flask (Borosilicate glass, Pyrex, USA), and distilled water was added in a sufficient quantity to completely immerse the material (approximately 1.0 L; plant material-to-water ratio of about 1:10, w/v). The mixture was subjected to hydrodistillation for 3 h, calculated from the onset of boiling. The collected essential oils were separated, dried over anhydrous sodium sulfate (Na_2SO_4 , $\geq 99\%$, Aladdin Reagent, China) to remove residual moisture, filtered, and transferred into amber glass vials (Nabila *et al.*, 2022). The samples were tightly sealed and stored at 4 °C in the dark until further chemical and biological analyses (Nabila *et al.*, 2022; Castro *et al.*, 2025).

The essential oil yield was calculated on a dry weight basis and expressed as percentage (v/w) using the following formula:

$$\text{Yield (\%, v/w)} = \frac{\text{Volume of essential oil (mL)}}{\text{Dry weight of plant material (g)}} \times 100$$

Essential oil analysis

The volatile profiles of the essential oils extracted from *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* were characterized using gas chromatography with flame ionization detection (GC–FID) in combination with gas chromatography–mass spectrometry (GC–MS) (Granados *et al.*, 2024). Analyses were performed using a gas chromatograph (Model HP 7890A, Agilent Technologies, USA) equipped with a flame ionization detector (FID) and an HP-5MS capillary column (30 m \times 0.25 mm i.d., film thickness 0.25 μm ; Agilent J&W Scientific, USA), coupled to a mass selective detector (Model HP 5973 MSD, Agilent Technologies, USA). Compound identification was achieved by comparing experimental retention times and calculated retention indices (RIs), obtained relative to a homologous series of n-alkanes (C_8 – C_{30} ;

$\geq 99\%$, Sigma-Aldrich, USA) analyzed under identical conditions, with reference data. Mass spectral patterns were matched against the NIST 2018 library and corroborated using published literature values (Adams, 2007; NIST, 2018).

Antimicrobial assay

The antimicrobial potential of essential oils from *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* was assessed against a set of standard microbial strains comprising Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and the yeast *Candida albicans* ATCC 10231, obtained from the American Type Culture Collection (ATCC, USA). Minimum inhibitory concentrations (MICs) were determined using broth microdilution technique in 96-well microplates (Granados *et al.*, 2024). All assays were performed under standardized conditions of inoculum density, culture media, and incubation parameters in accordance with CLSI guidelines. The MIC was defined as the lowest concentration of essential oil that completely inhibited visible microbial growth.

Statistical analysis

All experimental measurements were conducted in triplicate, and data are expressed as mean values accompanied by standard deviations (mean \pm SD). Statistical processing was performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). Differences among the three *Ocimum* species were evaluated by one-way analysis of variance (ANOVA). When significant effects were detected, Tukey's honestly significant difference (HSD) post hoc test was applied to compare group means. Statistical significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

Essential oil yield

Under identical hydrodistillation conditions, essential oil productivity differed significantly among the three *Ocimum* species cultivated in Hanoi, Vietnam ($p < 0.05$). *O. gratissimum* (1.42%, v/w) and *O. basilicum* (1.40%, v/w) produced comparably high oil yields and did not differ significantly from each other, whereas *O. tenuiflorum* yielded a markedly lower oil content (0.84%, v/w), which was significantly different from the other two species (Figure 1). The yield of *O. gratissimum* obtained in this study is consistent with previously reported values ranging from approximately 1.0–2.0%, depending on ecological conditions and drying treatments (Anh *et al.*, 2019). Similarly, the yield recorded for *O. basilicum* falls within the commonly reported range (0.5–1.5%) for basil cultivated under tropical conditions (Yaldiz *et al.*, 2015). In contrast, *O. tenuiflorum* is frequently characterized by moderate oil accumulation, with reported yields often below 1% under field conditions, as documented for specimens cultivated in the United States (Shiwakoti *et al.*, 2017), which is consistent with the present findings. The higher oil yield in *O. gratissimum* and *O. basilicum* may be associated with a greater density of glandular trichomes and species-specific regulation of monoterpene and phenylpropanoid biosynthesis (Martínez-Natarén *et al.*, 2018). In addition, harvesting at the full flowering stage, as applied in this study, is known to influence oil content due to peak metabolic activity in aerial tissues. Differences in moisture content prior to distillation and slight variations in leaf-to-stem ratio among species may also have contributed to the observed yield variation. Overall, the yield ranking (*O. gratissimum* > *O. basilicum* > *O. tenuiflorum*) highlights clear interspecific variation under identical cultivation and extraction conditions. These findings are consistent with published literature and confirm that oil productivity in *Ocimum* species is strongly dependent on

species identity and associated physiological traits (Padalia *et al.*, 2014; Muráriková *et al.*, 2017).

Chemical composition of the essential oils

Comparative profiling of *Ocimum* essential oils is essential because this genus is well known for pronounced chemotypic plasticity, whereby the same species may exhibit shifts in dominant volatiles depending on genotype, ecological conditions, and harvest or post-harvest practices. GC–FID/MS analysis of the three oils from Hanoi demonstrated high chromatographic coverage (96.23–97.59% total identified constituents) and revealed three distinct dominant compositional patterns, supporting species-level differentiation under identical distillation and analytical conditions (Table 1). *O. tenuiflorum* displayed a methyl eugenol-rich profile (methyl eugenol 56.28%), with eugenol (15.06%) and β -caryophyllene (8.73%) as additional major constituents, indicating a phenylpropanoid-oriented metabolic pattern (Table 1 and Figure 2). This composition is consistent with previously reported methyl eugenol-dominant chemotypes of *O. tenuiflorum* and aligns mechanistically with the phenylpropanoid pathway, in which eugenol is O-methylated to methyl eugenol under chemotype-dependent regulation (Kothari *et al.*, 2004; Renu *et al.*, 2014). Regional studies in Vietnam have likewise reported phenylpropanoid-driven profiles in *O. tenuiflorum*, often with eugenol dominance depending on population and environmental conditions (Le *et al.*, 2024), underscoring the substantial intra-specific variability of this species.

In contrast, *O. gratissimum* was clearly characterized as a eugenol chemotype (eugenol 62.07%), accompanied by a substantial sesquiterpene fraction dominated by germacrene D (15.17%), (*Z*)- β -ocimene (7.26%), and β -caryophyllene (5.62%) (Table 1 and Figure 2). This profile closely aligns with reports from Vietnam and other regions, where eugenol frequently constitutes

approximately 60% or more of the oil composition (Dharsono *et al.*, 2022; Hanh *et al.*, 2022; Hao and Quoc, 2024). Variations in accompanying terpene constituents are commonly attributed to environmental factors such as temperature, irradiance, soil nutrition, phenological stage, and post-harvest drying, all of which may modulate terpene synthase expression and influence carbon flux between mono- and sesquiterpene pathways (Dharsono *et al.*, 2022; Chu *et al.*, 2022).

O. basilicum exhibited a distinct methyl chavicol (estragole)/linalool pattern (methyl chavicol 51.45%; linalool 18.41%), with notable levels of 1,8-cineole (9.82%) and limonene (5.96%), and a comparatively higher proportion of oxygenated monoterpenes than the other two species (Table 1 and Figure 2). This composition fits within established basil chemotype classifications, including methyl chavicol/linalool groupings, and is consistent with extensive literature demonstrating that basil oils may shift among linalool-, estragole-, methyl eugenol-, and methyl cinnamate-dominant chemotypes depending on cultivar and cultivation environment (Muráriková *et al.*, 2017; Chu *et al.*, 2022). Basil is particularly responsive to environmental variables such as light intensity and quality, which can influence methyl chavicol production and the accumulation of oxygenated monoterpenes, providing a plausible explanation for the balanced estragole–linalool profile observed in the present study (Chu *et al.*, 2022; Slougui *et al.*, 2022).

Overall, the three oils demonstrate species-associated chemotype differentiation under identical harvest timing and hydrodistillation conditions. Although phenylpropanoids were abundant in all cases, *O. tenuiflorum* was biased toward methyl eugenol, *O. gratissimum* toward eugenol with higher sesquiterpene contribution, and *O. basilicum* toward methyl chavicol supported by oxygenated monoterpenes. Variations reported across studies are most plausibly

explained by differences in genotype or chemotype structure, phenological stage at harvest, ecological context (climate and soil), and post-harvest or extraction variables that influence volatile retention and relative peak areas (Kothari *et al.*, 2004; Salles Trevisan *et al.*, 2006; Chu *et al.*, 2022).

Antimicrobial activity of the essential oils

The antimicrobial activity of *Ocimum* essential oils is commonly evaluated through MIC values, which provide a quantitative basis for comparing species and chemotypes under standardized conditions. In the present study, the three oils exhibited species-dependent inhibitory profiles, with overall greater susceptibility observed in Gram-positive bacteria and yeast than in Gram-negative bacteria.

As shown in Table 2, *O. gratissimum* oil demonstrated the strongest activity among the tested samples, with MIC values of 100 µg/mL against *E. faecalis*, 50 µg/mL against *S. aureus*, 200 µg/mL against *E. coli*, and 50 µg/mL against *C. albicans*. In comparison, *O. tenuiflorum* showed moderate activity (MIC 200 µg/mL for *E. faecalis*, 100 µg/mL for *S. aureus*, and 100 µg/mL for *C. albicans*), while *O. basilicum* exhibited comparatively weaker activity (MIC 200 µg/mL for *E. faecalis* and *C. albicans*; 100 µg/mL for *S. aureus*). Neither *O. tenuiflorum* nor *O. basilicum* inhibited *E. coli* within the tested concentration range. Notably, none of the three oils inhibited *P. aeruginosa* under the experimental conditions.

The lack of activity against *P. aeruginosa* is consistent with the well-documented intrinsic tolerance of this species to many hydrophobic natural products, attributable to low outer membrane permeability, active efflux systems, and biofilm-associated resistance mechanisms (Nakamura *et al.*, 1999). The superior performance of *O. gratissimum* oil is coherent with its eugenol-dominant profile, as eugenol-rich oils are frequently associated with strong inhibitory effects against Gram-positive bacteria and yeasts.

In contrast, the comparatively higher MIC values observed for *O. basilicum* may be related to its chemotype composition and potential interactions among constituents. Although linalool contributes to antifungal activity, reported MIC values for isolated linalool against *C. albicans* often range from several hundred to over 1000 µg/mL depending on strain and methodology (Cardoso et al., 2016). Moreover, *Ocimum* oils commonly exhibit substantial variability in antifungal performance across studies employing broth microdilution assays (Vieira et al., 2014).

Overall, the MIC ranking observed in this study (*O. gratissimum* > *O. tenuiflorum* > *O. basilicum*) supports the concept that species identity and chemotype composition are primary determinants of antimicrobial potency. The absence of activity against *P. aeruginosa* further highlights the importance of microorganism-specific structural and physiological barriers when interpreting essential oil MIC data.

CONCLUSIONS

This study demonstrated clear interspecific differences among *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* cultivated in Hanoi, Vietnam, in terms of essential oil yield, chemical composition, and antimicrobial activity. *O. gratissimum* and *O. basilicum* showed higher oil yields, while GC–FID/MS analyses revealed distinct chemotypes dominated by methyl eugenol, eugenol, and methyl chavicol/linalool, respectively. These differences were reflected in biological activity, with *O. gratissimum* exhibiting the strongest antimicrobial effects, particularly against Gram-positive bacteria and *C. albicans*, whereas limited activity was observed against *P. aeruginosa*. Overall, species identity and chemotype were identified as key factors influencing essential oil productivity and antimicrobial potential.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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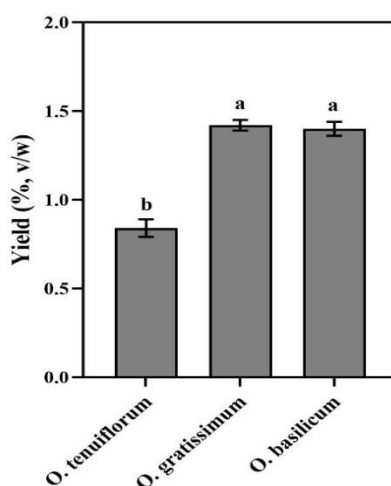


Figure 1: Percentage yield (v/w) of essential oils extracted from three *Ocimum* species cultivated in Vietnam. Results are expressed as the average of three independent replicates with corresponding standard deviations (n = 3). Values marked with different superscript letters differ significantly at the 5% probability level (p < 0.05).

Table 1: Chemical constituents of essential oils from three *Ocimum* species cultivated in Vietnam

Compound ^a	RI _{Exp} ^b	RI _{Lit} ^c	Concentration (%)		
			<i>Ocimum tenuiflorum</i>	<i>Ocimum gratissimum</i>	<i>Ocimum basilicum</i>
α -Pinene	930	932	–	–	0.26 \pm 0.02
Sabinene	971	969	0.47 \pm 0.02	0.28 \pm 0.01	0.34 \pm 0.02
β -Pinene	980	974	0.24 \pm 0.02	–	0.43 \pm 0.02
Myrcene	990	988	–	0.41 \pm 0.03	0.80 \pm 0.03
Limonene	1027	1024	0.39 \pm 0.01	–	5.96 \pm 0.24
1,8-Cineole	1029	1026	–	–	9.82 \pm 0.48
(Z)- β -Ocimene	1030	1032	–	7.26 \pm 0.54	–
(E)- β -Ocimene	1048	1044	–	0.79 \pm 0.06	0.74 \pm 0.03
Terpinolene	1094	1086	1.46 \pm 0.02	0.22 \pm 0.01	–
Linalool	1097	1095	–	0.19 \pm 0.01	18.41 \pm 0.76
Camphor	1144	1141	–	–	0.27 \pm 0.01
Borneol	1164	1165	2.31 \pm 0.03	–	–
Terpinen-4-ol	1178	1174	–	0.59 \pm 0.04	1.30 \pm 0.04
α -Terpineol	1188	1186	0.64 \pm 0.03	–	–
Methyl chavicol	1201	1195	0.83 \pm 0.02	–	51.45 \pm 1.47
Geraniol	1252	1249	0.25 \pm 0.01	0.31 \pm 0.02	0.20 \pm 0.01
Geranial	1260	1264	–	–	0.51 \pm 0.03
δ -Elemene	1333	1335	–	–	0.29 \pm 0.02
α -Cubebene	1345	1348	0.28 \pm 0.01	–	–
Eugenol	1352	1356	15.06 \pm 1.09	62.07 \pm 1.35	–
(E)-Methyl cinnamate	1372	1376	2.27 \pm 0.18	–	–
β -Bourbonene	1384	1387	–	–	–
β -Elemene	1389	1389	3.29 \pm 0.22	1.26 \pm 0.75	0.21 \pm 0.01
Methyl eugenol	1405	1403	56.28 \pm 1.24	–	–
β -Caryophyllene	1420	1417	8.73 \pm 0.57	5.62 \pm 0.34	2.18 \pm 0.27
α -Guaiene	1439	1437	–	–	–
α -Humulene	1448	1452	1.15 \pm 0.03	0.84 \pm 0.05	–
<i>allo</i> -Aromadendrene	1462	1458	–	0.29 \pm 0.02	–
Germacrene D	1486	1484	–	15.17 \pm 0.66	0.89 \pm 0.05
β -Selinene	1491	1489	0.75 \pm 0.02	–	0.64 \pm 0.06
Bicyclogermacrene	1497	1500	–	0.41 \pm 0.03	0.25 \pm 0.01
α -Bulnesene	1506	1509	–	–	0.34 \pm 0.02
γ -Cadinene	1511	1513	1.83 \pm 0.04	–	0.41 \pm 0.02
β -Sesquiphellandrene	1519	1521	–	–	1.26 \pm 0.04
δ -Cadinene	1521	1522	–	0.28 \pm 0.02	–
α -Elemol	1548	1549	0.71 \pm 0.04	–	–
(E)-Nerolidol	1564	1561	0.32 \pm 0.02	–	0.63 \pm 0.03
Longipinanol	1567	1569	0.21 \pm 0.02	–	–
Spathulenol	1580	1577	–	0.24 \pm 0.01	–
Total identified (%)			97.47	96.23	97.59
Monoterpene hydrocarbons			2.56	8.96	18.35
Oxygenated monoterpenes			3.20	1.09	20.69
Sesquiterpene hydrocarbons			16.03	23.87	6.47
Oxygenated sesquiterpenes			1.24	0.24	0.63
Phenylpropanoids			74.44	62.07	51.45

^a Elution sequence determined on an HP-5MS capillary column.^b Experimentally calculated retention indices (RI) on the HP-5MS column.^c Literature retention index values used for comparison.

(–) Indicates unidentified constituents.

All quantitative data represent the mean of three replicates \pm standard deviation (n = 3).

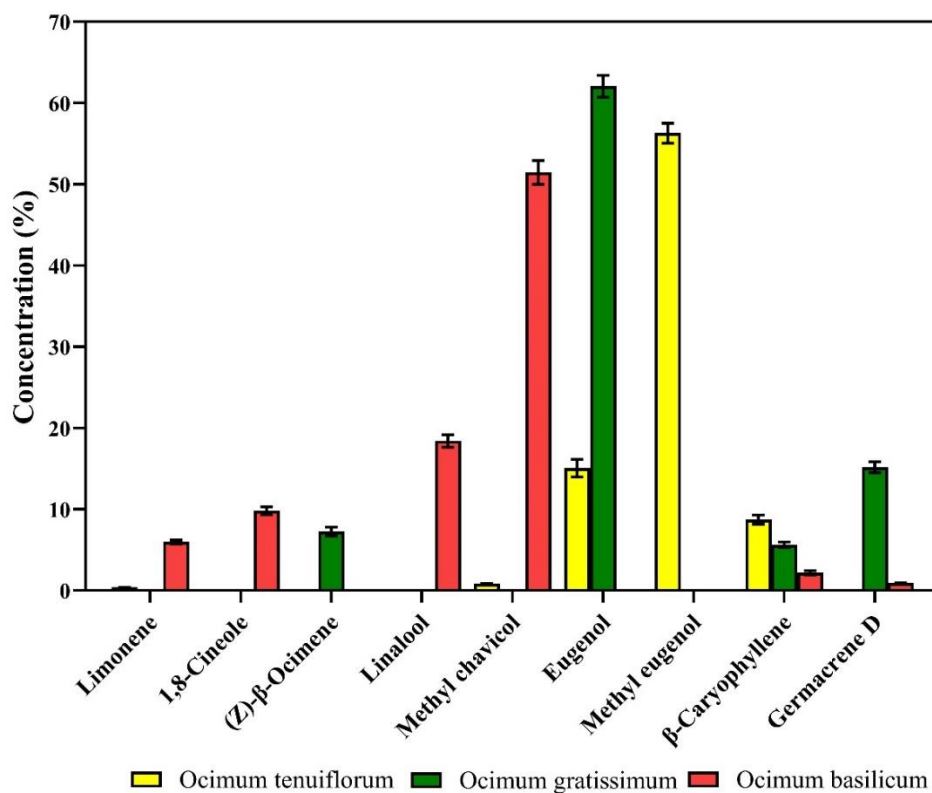


Figure 2. Comparative profile of major constituents (>5%) in essential oils from three *Ocimum* species cultivated in Vietnam

Table 2: Antimicrobial activity (MIC, $\mu\text{g/mL}$) of essential oils from three *Ocimum* species cultivated in Vietnam

Microorganisms	Essential oil		
	<i>Ocimum tenuiflorum</i>	<i>Ocimum gratissimum</i>	<i>Ocimum basilicum</i>
<i>Enterococcus faecalis</i> ATCC 29212	200	100	200
<i>Staphylococcus aureus</i> ATCC 25923	100	50	100
<i>Escherichia coli</i> ATCC 25922	–	200	–
<i>Pseudomonas aeruginosa</i> ATCC 27853	–	–	–
<i>Candida albicans</i> ATCC 10231	100	50	200