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Molecular Identification Using DNA Barcoding and Phytochemical Profiling in Four Basil (*Ocimum* spp.) from Different Locations in Bali

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ABSTRACT

Basil (*Ocimum* spp.) is a prominent aromatic plant belonging to the Lamiaceae family, widely recognised for its essential oils. In Bali, various basil varieties are cultivated; however, their specific species have yet to be conclusively identified, leaving their potential bioactive compounds underexplored. This study aims to identify the species of four basil types found in Bali—Kecarum, Selasih, Tulasi, and Ruku-ruku—through genotypic analysis and to investigate their phytochemical profiles. Species identification was performed using DNA barcoding with the Maturase K (*matK*) gene as the molecular marker, followed by DNA sequencing and phylogenetic analysis. Phytochemical composition was examined using gas chromatography-mass spectrometry (GC-MS). The genotypic analysis revealed that all four basil types shared identical genotypes and originated from a common ancestor, as indicated by their positions in the phylogenetic tree and the clades they formed. Specifically, Kecarum and Selasih were genetically identical to *Ocimum americanum* with a genetic distance of 0.000, while Tulasi and Ruku-ruku corresponded to *Ocimum campechianum*, exhibiting a genetic distance of 0.011. GC-MS analysis identified a diverse range of bioactive compounds, including alkaloids, phenolics, saponins, terpenoids, steroids, and tannins. Notable constituents included actinobolin, cyclotrisiloxane hexamethyl-, and methyleugenol. These compounds demonstrated a wide array of biological activities, including antioxidant, antibiotic, antihyperglycemic, antitumor, antidepressant, anti-inflammatory, anaesthetic, analgesic, biosurfactant, antibiofilm, anti-arrhythmic, and sympathomimetic properties.

Keywords: Attractants, Deoxyribose Nucleic Acid (DNA) Barcoding, Maturase K (*matK*), Methyleugenol, *Ocimum* spp., Phytochemicals.

Introduction

Ocimum spp. grows abundantly in various regions of Indonesia and holds significant potential for development by exploring its bioactive compounds and essential oils. Basil essential oil offers numerous benefits for human life and possesses high economic value, especially for export as crude oil. *Ocimum* spp. contains numerous secondary metabolites, including alkaloids, terpenoids, organic acids, tannins, flavonoids, coumarins, quinones, polyphenols, saponins, and their derivatives.[1] The species *Ocimum basilicum* L. exhibits a range of activities, including antioxidant, antibiotic, antimicrobial, antiviral, anticarcinogenic, cytoprotective, anticonvulsant, antihyperglycemic, hypolipidemic, hepatoprotective, renoprotective, neuroprotective, spermicidal, dermatological, and insecticidal activities.[2]

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In the agricultural sector, bioactive compounds found in basil, such as methyl eugenol, serve as attractants, particularly for fruit flies.[3] Methyl eugenol is recognised for inducing pronounced olfactory responses in multiple species of fruit flies, such as the Mediterranean fruit fly (*Ceratitis capitata*) and the oriental fruit fly (*Bactrocera dorsalis*). Methyl eugenol has demonstrated efficacy in attracting these pests, hence improving monitoring and management tactics in agricultural environments. Basil's presence in agricultural settings attracts fruit flies and aids in pest management by enabling the deployment of traps that utilise this olfactory response.[4,5] Several types of basil grow in Bali, including *Kecarum, Selasih, Tulasi*, and *Ruku-ruku*. However, few reports have been made on the phytochemical compounds, species, and kinship of these basil varieties. This study aims to identify the species of four Ocimum varieties found in Bali (*Kecarum, Selasih, Tulasi*, and *Ruku-ruku*) through DNA barcoding using the maturase K (matK) gene as a molecular marker and to analyse the phytochemical composition of their extracts using gas chromatography-mass spectrometry (GC-MS). This study addresses the limited knowledge of the genetic identity, phylogenetic relationships, and phytochemical profiles of four basil types native to Bali—*Kecarum, Selasih, Tulasi*, and *Ruku-ruku*. By employing DNA barcoding with the maturase K (*matK*) gene as a molecular marker, this research provides precise species identification and elucidates the evolutionary relationships of these basil varieties in comparison with other *Ocimum* species registered in GenBank. Furthermore, the use of GC-MS analysis to comprehensively characterise the phytochemical compounds in the ethanol extracts of these basil types uncovers their bioactive potential. This dual approach not only enhances understanding of the genetic and chemical diversity

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of *Ocimum* spp. in Bali but also highlights their potential applications in medicine, agriculture, and essential oil production, paving the way for sustainable utilisation and value-added innovations in Indonesia's aromatic plant resources.

Materials and Methods

This research is a descriptive observational study conducted at the Laboratory of Genetic Resources and Molecular Biology, Udayana University.

Collection of Plant Samples

Basil samples were obtained from three growing locations in Tabanan, Bali in July 2024: Kecarum and Selasih from Senganan Village (8°21'59.8 "S 115°09'18.1" E) at an altitude of 1,032 meters above sea level (masl); Tulasi from Wongaya Gede Village (8°23'31.1 "S 115°06'42.5"E) at an altitude of 835 masl; and Ruku-ruku from Marga Village (8°28'47.5"S 115°10'13.5"E) at an altitude of 450 masl (Figure 1). These four types of basil were then qualitatively characterised and directly observed in the field for the morphology of their crowns, leaves, stems, fruits, and flowers. Samples were stored at the Genetic Resources and Molecular Biology Laboratory, Udayana University (voucher no. SDGBM/78/24/038, SDGBM/78/24/039, voucher no. SDGBM/78/24/040, and SDGBM/78/24/041).

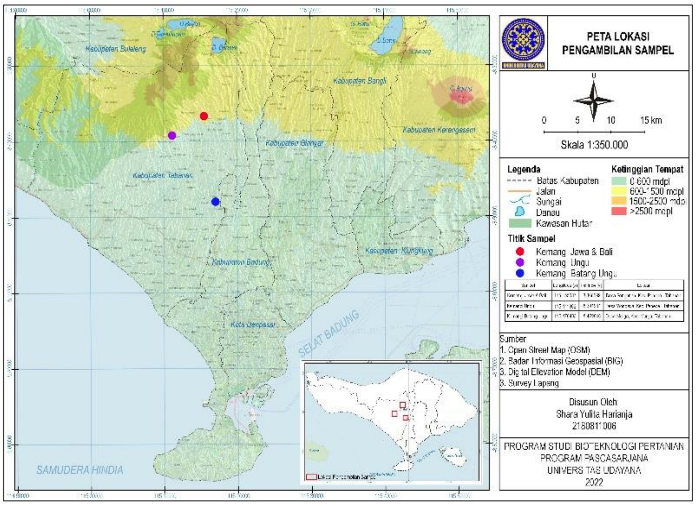

Figure 1: Map of sampling locations for four types of basil in Tabanan Regency, Bali, Indonesia

Isolation and Amplification of matK Gene by Polymerase Chain Reaction (PCR)

Basil DNA was isolated, and total DNA was extracted using Quick DNA Plant/Seed (Zymo Research, D6020). The extraction aimed to break down the chloroplast cells and obtain the DNA from the chloroplast cells in the leaves. The isolated DNA was amplified using the KOD FX NEO PCR method (Toyobo, KFX-201) with the primer pair *matK* forward 3F-R (5'-CGT ACA GTA CTT TTG TGT TTA CGA G – 3') and reverse *matK*-IR-F (5'ACC CAG TCC ATC TGG AAA TCT TGG TTC -3'), producing a fragment of 850 bp. The total volume of the sample analysed was 50 µL, with Polymerase Chain Reaction (PCR) conditions as follows: pre-denaturation at 95°C for 2 minutes for one cycle, denaturation at 94°C for 1 minute, annealing at 58°C for 45 seconds, extension at 72°C for 45 seconds, and post-extension at 72°C for 5 minutes for 35 cycles. The resulting amplicon was visualised by electrophoresis on 1% TBE agarose gel with a 100 bp DNA ladder. Electrophoresis (Mini-Sub Cell GT Horizontal Electrophoresis System dan UView Mini Transilluminator, Bio-Rad Laboratories, Inc, USA) was carried out at 90 V for 30 minutes. The DNA bands formed were observed using a UV-transilluminator. The purified PCR products were then sequenced using the bi-directional sequencing technique.

Phylogenetic Construction

The nucleotide sequences from the sequencing results were compared with the database available at NCBI (www.ncbi.nlm.nih.gov) to find sequences homologous to the basil sequences from Bali by performing nucleotide Basic Local Alignment Search Tool (BLAST) analysis. Accessions with the highest similarity or identity percentage were selected, and sequence alignment was performed using BioEdit and ClustalW programs. A phylogenetic tree was constructed in Molecular Evolutionary Genetic Analysis (MEGA) software (MEGA Software Development Team version 11) using the Maximum Likelihood method and the Tamura 3-Parameter substitution model. The accuracy of the tree was evaluated using a 1000x bootstrap analysis. Phylogenetic construction aimed to determine the kinship level of the basil types and observe their evolutionary rates.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Dried leaf samples were extracted using the maceration method with 96% ethanol for three days. The filtrate obtained was evaporated using a vacuum rotary evaporator at 40℃ to produce crude extract for GC-MS Analysis. GC-MS analysis was performed using an Agilent 7890B MSD 5977B with a Wakosil ODS/5C18-200 silica column, sized 4.6 x 200 mm. Samples were injected at a volume of 1 µL into the GC-MS column at an injection temperature of 290°C for 27 minutes. Phytochemical compounds were identified using Willey database version 7.0 by comparing the mass spectrum and fragmentation patterns of reference compounds stored in Willey's library.

Results and Discussion

The morphology of the plant samples was examined and characterised. Characterisation of the crown (botany), stem (caulis), leaf (folium), flower (flos), and fruit (fructus) revealed that Kecarum and Selasih samples have similar morphological characteristics, as do Tulasi and Ruku-ruku. However, there were differences in plant height, colour (anthocyanin pigment), crown shape, leaf shape, leaf tip, leaf base, and leaf arrangement (Figure 2).

Figure 2: Morphology of a) Selasih; b) Kecarum; c) Tulsi; d) Ruku-ruku

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Samples of the four types of basil obtained from three different areas of Tabanan Regency, Bali, were identified morphologically, referring to Tjitrosoepomo's Plant Morphology book[6] and Indriyanto's Dendrology book.[7] The four types of basil exhibit varied morphology, influenced by environmental and genetic factors. The morphology of Kecarum and Selasih resembles *Ocimum americanum* L.[8], while Tulasi and Ruku-ruku are similar to *Ocimum sanctum* var. Rama (red holy basil)[9]; and *Ocimum campechianum* from Argentina.[10] Differences in anthocyanin pigments, primarily found in young lateral branches and inflorescences, contribute to the distinct colours of the four Basil types.

Phenotypic plasticity allows plants to adapt their morphology in response to varying environmental stimuli, including light availability, soil composition, water supply, and biotic interactions. Altitude significantly influences the morphological characteristics of plants, affecting their growth, structure, and overall adaptability. The relationship between altitude and plant morphology is a critical area of plant ecology and physiology study. As altitude increases, plants encounter a range of environmental stresses, including lower temperatures, reduced atmospheric pressure, and increased UV radiation. These factors can lead to notable changes in morphological traits, which are essential for survival and reproduction in challenging conditions. A study showed that plants along an altitudinal gradient in the Kashmir Himalaya exhibited changes in growth dynamics, with higher altitudes resulting in shorter stature and altered inflorescence characteristics due to the stress of lower temperatures.[11] Similarly, a study on *Nothofagus cunninghamii* showed variations in stomatal conductance and photosynthetic rates based on their altitude of origin, indicating a direct relationship between altitude and plant physiological performance.[12]

The results of DNA amplification targeting the *matK* gene yielded amplification products of 859 bp for Kecarum, 860 bp for Selasih, 857 bp for Tulasi, and 862 bp for Ruku-ruku. The visualisation results of the isolated basil DNA bands were clear and thick, with some faint smears visible near the DNA bands (Figure 3). The successful isolation of basil DNA was indicated by clear DNA bands that glowed on the gel when irradiated with ultraviolet light, signifying a large amount of DNA present in the sample.

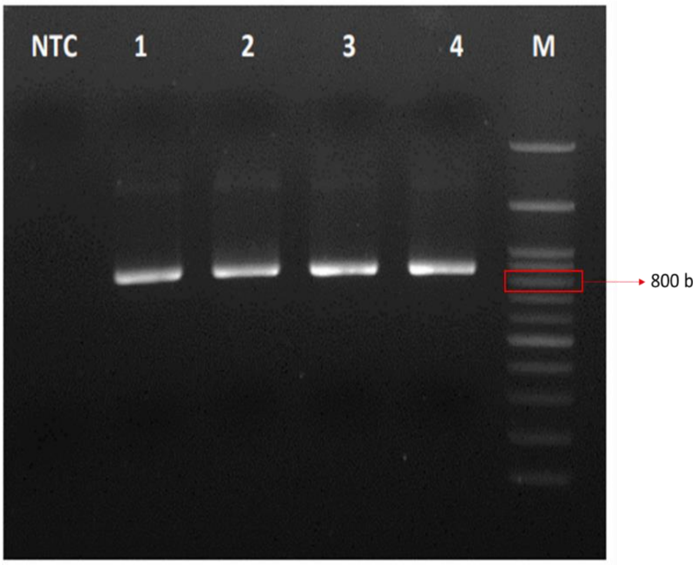

Figure 3: Electrophoresis result of PCR amplification using matK gene

Figure 4 shows a phylogenetic tree combining the sequences of four basil types from Bali with homologous sequences obtained from BLAST. The tree is divided into two main clades. The four basil types are grouped in the same clade, distinct from several other basil accession numbers listed in GenBank. *Orthosipon stamineus* and *Clerodendranthus spicatus*, which also belong to the *Lamiaceae* family, appeared as outgroups in the phylogenetic tree. DNA barcoding has been recognised as a highly effective tool for species identification, offering substantial reliability and accuracy across diverse taxonomic groups. This molecular approach utilises short, standardised DNA sequences to differentiate species, thereby enabling rapid and precise identification, particularly in cases where morphological traits are unclear, or species exhibit cryptic characteristics. The utility of DNA barcoding is especially notable in the identification of medicinal plants, where it achieves high success rates, exceeding 90% accuracy in distinguishing species.[13] Such performance is critical for applications in biodiversity assessments and conservation, as it facilitates efficient cataloguing of species within complex ecosystems.[14] The development of extensive DNA barcode libraries has further enhanced identification accuracy, with success rates nearing 100% in specific taxonomic groups.[15] This is particularly evident in research on cryptic species, where conventional morphological approaches often fail to resolve closely related taxa. In the context of accelerating biodiversity loss and environmental change, DNA barcoding stands out as a reliable and indispensable method for species identification.[16]

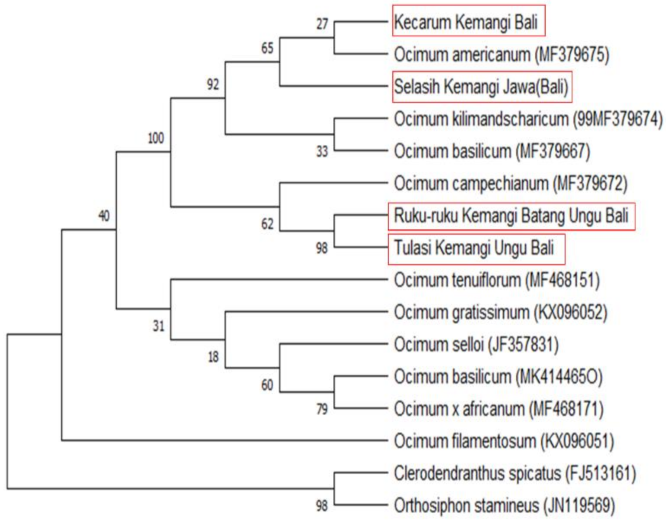

Figure 4: Basil phylogenetic tree from Bali using the Maximum Likelihood method

DNA amplification using the molecular marker *matK* showed good results, marked by a firm and clear DNA band that glows under ultraviolet light (Figure 3). The thick and clumped DNA bands indicate a high concentration and intact total DNA. This study utilised *matK* as a primer in the DNA amplification process of basil. The maturase K gene (*matK*) is a molecular marker recommended by The Consortium for the Barcode of Life (CBOL) Plant Working Group as a locus for plant DNA barcoding.[17] The *matK* gene is ideal for plant identification due to its suitable size, high substitution rate, and significant variations in nucleic acid levels.[18] The matK gene is acknowledged for its exceptional capacity to differentiate specimens, especially in research concerning intraspecies variation. Research demonstrates that matK displays greater variability than other often utilised barcoding areas, such as rbcL and ITS, rendering it especially efficient for differentiating closely related species.[19–21] Based on the smallest genetic distance, highest identity percentage, and position in the *matK* sequence phylogeny tree, Kecarum and Selasih are genotypically identical and closely related to *Ocimum americanum* (MF379675) with a sequence length of 783 bp. The accuracy of the phylogenetic analysis is indicated by a bootstrap value of 100. The *matK* sequences of Tulasi and Ruku-ruku are also genotypically identical and closely related to *Ocimum campechianum* (MF379672) with a sequence length of 782 bp, with a bootstrap value of 100. In the combined phylogeny tree of the four sequences with homologous sequences from BLAST analysis, two main clades are formed, indicating the kinship relationship divided into three clades. The calculation of genetic distance and identity percentage of the four basil types also demonstrates genetic closeness (Table 1). The branch positions on the phylogenetic tree strongly suggest that the four Balinese basil sequences are closely related and originate from a common ancestor.

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Table 1: Pairwise distance and percentage identity of Kecarum, Selasih, Ruku-ruku, and Tulasi sequences with *Ocimum* species in GenBank

Species	Accession No	Kecarum		Selasih		Ruku-ruku		Tulasi	
		PD	ID	PD	ID	PD	ID	PD	ID
Kecarum	-	-	-	0.000	99.80%	0.014	98.40%	0.014	98.50%
Selasih	-	0.000	99.80%	-	-	0.014	98.50%	0.014	98.60%
Ruku-ruku Bali	-	0.014	98.40%	0.014	98.50%	-	-	0.000	99.80%
Tulasi Bali	-	0.014	98.50%	0.014	98.60%	0.000	99.80%	-	-
O. kilimandscharicum	MF379674	0.001	99.70%	0.001	99.80%	0.013	98.60%	0.013	98.80%
O. americanum	MF379675	0.000	99.80%	0.000	100.00%	0.014	98.50%	0.014	98.60%
O. gratissimum	KX096052	0.864	45.80%	0.864	45.80%	0.846	46.30%	0.846	46.20%
O. x africanum	MF468171	0.855	46.00%	0.855	46.10%	0.837	46.50%	0.837	46.50%
O. campechianum	MF379672	0.010	98.90%	0.010	99.00%	0.011	98.80%	0.011	98.90%
O. tenuiflorum	MF468151	0.854	46.00%	0.854	46.10%	0.835	46.50%	0.835	46.50%
O. filamentosum	KX096051	0.871	45.30%	0.871	45.40%	0.852	45.80%	0.852	45.80%
O. selloi	JF357831	0.878	45.50%	0.878	45.60%	0.858	46.00%	0.858	46.00%
O. basilicum	MF379667	0.001	99.70%	0.001	99.80%	0.013	98.60%	0.013	98.80%
Clerodendranthus spicatus	FJ513161	0.863	45.90%	0.863	46.00%	0.844	46.40%	0.844	46.30%
Orthosiphon stamineus	JN119569	0.863	45.90%	0.863	46.00%	0.844	46.40%	0.844	46.30%

PD (Pairwise distance), ID (percentage of identity)

Phytochemical screening based on GC-MS analysis, the four types of basil produced chromatogram peaks indicating the presence of various phytochemical compounds or secondary metabolites at different concentrations. The main constituents of each type of basil were identified by the highest Area Under Curve (AUC) values in the chromatogram (Figure 5). Kecarum contains the following compounds as main constituents: 2-Propanamine, Benzenemethanol, Phenethylamine, Acetic acid, D-alanine, Amphetamine, Benzeneethanamine, and Actinobolin. Additional compounds present include 2-Heptanamine, sec-Butylamine, 2-Hexamine, 4-methyl-, 2-Octanamine, 2-Aminonadecane, Bactobolin, and Ethylamine. Selasih main constituents were Cyclotrisiloxane, hexamethyl-. Other notable compounds include dI-Phenylephrine, N-Desmethyltapentadol, Benzeneethanamine, 3-Methoxyamphetamine, pyrido [3,4-d] imidazole 1,6-discarboxilic acid, Propanamide, Metaraminol, Cyclobutanol, 2-Ethylacridine, Atomoxetine, Fluoxetine, and Tetrasiloxane. While, Tulasi's primary compound was Methyleugenol. Additional compounds identified were Linalool, Isoborneol, Eugenol, Copaene, Cyclohexane, Caryophyllene, 1,4,7-Cycloundecatriene,1,5,9, Humulene, Germacrene D, Naphthalene, Metaraminol, Benzeneethanamine, Tocainide, Propanamide, Di-Phenylephrine, 1,2-Benzisothiazol-3-Amine, Benzo[H]Quinoline, and 2-Ethylacridine. The Ruku-ruku sample also primarily contains Methyleugenol. Other compounds found include Isoborneol, Copaene, Cyclohexane, Caryophyllene, 1,4,7-Cycloundecatriene,1,5,9-, Humulene, Germacrene D, Metaraminol, Tocainide, Propanamide, Phenylephrine, 1,2-Benzisothiazol-3-Amine, Benzo[H]Quinoline, 2-Ethylacridine, Benzenemethanol, and Atomoxetine.

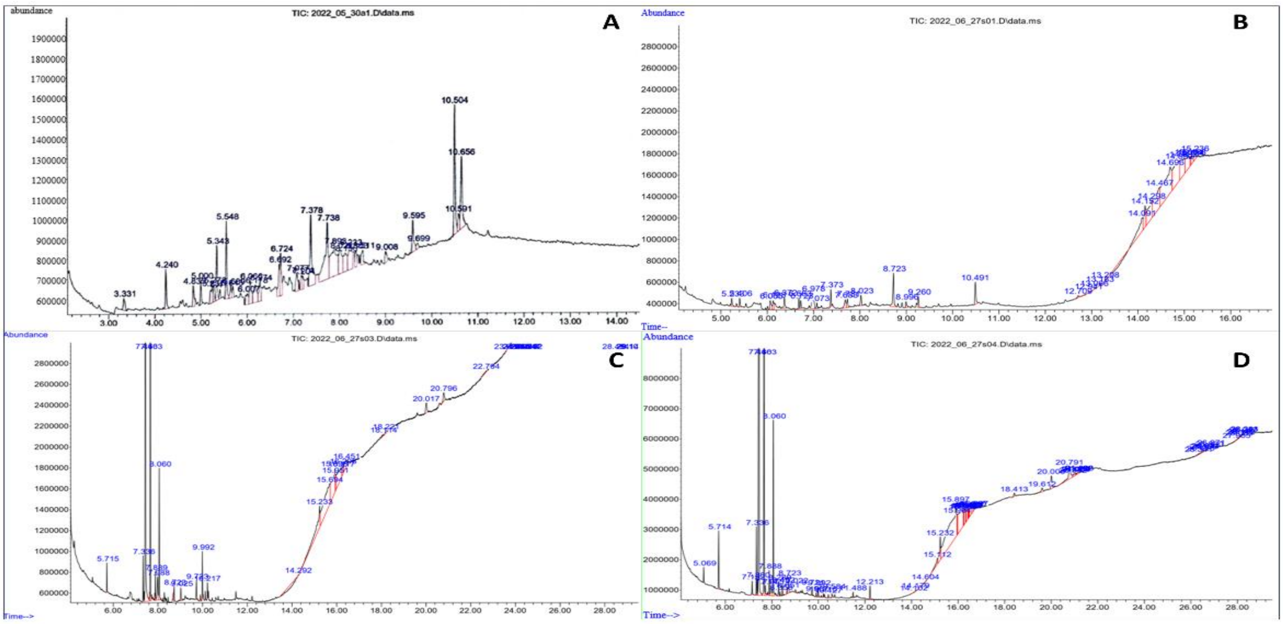

Figure 5: Chromatogram of compounds in ethanol extract of A) Kecarum; B) Selasih; C) Ruku-ruku; D) Tulasi produced on GC-MS analysis

GC-MS analysis of the ethanol extracts of Kecarum, Selasih, Tulasi, and Ruku-ruku revealed numerous secondary metabolites with various biological activities. Some compounds are shared among the four types of basil. Although certain compounds have limited information regarding their functions and activities, further studies and research are needed. Various compounds in basil have been widely reported for their biological activity. The compounds identified in the four basil types are predominantly alkaloids, phenolics, saponins, terpenoids, and steroids. These compounds exhibit several biological and pharmacological activities, as shown in Table 2. The main constituent in Kecarum is

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Actinobolin, with the highest AUC value (10.06) at a retention time of 7.738 minutes. Actinobolin, first described by Haskel Bartz, is an antibiotic compound isolated from gram-positive actinomycetes, namely *Streptomyces griseoviridus* var. *atrophaciens*. This compound exhibits broad-spectrum antimicrobial activity and shows potential as a chemotherapeutic agent in certain neoplastic diseases.[22] Actinobolin has antitumor and anticarcinogenic activity, notably in Leukemia ascites L1210, where it inhibited cancer cells and increased the lifespan of mice implanted with this leukaemia type.[23] Actinobolin also acts as an antiviral and antiherpes agent, preventing herpes virus replication. [24] The main constituent of Selasih is identified as Cyclotrisiloxane, hexamethyl-, with an AUC value of 21.16 at a retention time of 14.469 minutes. Hexamethylcyclotrisiloxane is a cyclic organosilicon molecule that has been recognised as a notable constituent in numerous medicinal plants, demonstrating potential antibacterial characteristics. Zohdi *et al*. (2023) emphasised its occurrence in the ethanolic and aqueous extracts of Malaysian propolis, demonstrating antibacterial efficacy against various pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*.[25] Another study by Mahmud *et al*. (2018) also observed that hexamethylcyclotrisiloxane is linked to the antibacterial capabilities of olive leaf extract, underscoring its significance in phytochemical research. Hexamethylcyclotrisiloxane's presence is also documented in *Syzygium alternifolium,* corroborating its identification in several medicinal plants.[27] The compound's occurrence in various plant species highlights its potential use in creating natural antibacterial medicines, necessitating further exploration of its therapeutic applications. Methyleugenol is the main constituent of Tulasi and Ruku-ruku. This volatile compound is used as a trap or attractant for male fruit flies, particularly *Bactrocera* sp. (Dondo, 2018). Methyleugenol in *Ocimum campechianum* shows larvicidal activity with up to 100% mortality.[28] Setiyanto *et al*. (2023) demonstrated that methyleugenol is more effective than commercial attractants, increasing fruit fly catches by 20% at 50% concentration and up to 145% at 100% concentration.[29] Methyl eugenol is a naturally occurring chemical present in more than 450 plant species, especially within the Myrtaceae family and numerous herbs and spices. Significantly, elevated levels of methyl eugenol have been detected in plants like *Melaleuca linariifolia*, which comprises up to 86.8% of this chemical in its essential oil (Silva et al., 2010). Additional sources comprise basil (*Ocimum basilicum*), sweet bay (*Laurus nobilis*), and nutmeg (*Myristica fragrans*), which are frequently utilised in culinary practices and traditional medicine.[30,31] Methyl eugenol demonstrates substantial applicability chiefly in agriculture and pharmaceuticals. It is acknowledged as an active metabolite due to its bioactive features, which include possible antibacterial and antifungal actions.[32] It also act as an attractant for several kinds of fruit flies, especially Bactrocera genus. Research has shown that traps baited with methyl eugenol efficiently capture substantial quantities of fruit flies, becoming an essential element in integrated pest management systems.[33,34] Methyl eugenol significantly contributes to male annihilation strategies, hence augmenting its effectiveness in diminishing fruit fly populations, which protects crops and boosts agricultural output.[35,36] This study showed the difference in secondary metabolites produced by each basil sample collected in Bali. Molecular identification showed that kecarum and selasih belong to the same species. However, they showed different main constituents identified by GC-MS, which predicted the influence of environmental conditions, especially those related to the different altitudes. Increased exposure to UV radiation at higher altitudes can lead to enhanced production of secondary metabolites, such as phenolic compounds, which serve protective roles in plants. A study on the plants from the Lamiaceae and Asteraceae families showed increased levels of phenolic compounds when growing at higher altitudes, suggesting an adaptive response to elevated UV exposure.[37] This metabolomic variation is crucial for understanding how plants modify their morphology and biochemistry to cope with environmental stressors.

Table 2: Biological activity of phytochemical compounds in the ethanol extract of four types of basil from Bali based on GC-MS analysis

Chemicals	Biological Activities*
Ethylamine	Antibacterial, herbicide[38,39]
Cyclotrisiloxane, hexamethyl-	Antihyperglycemic[40]
N-Desmethyltapentadol	Analgesics (opioids), anesthetics[41]
Atomoxetine	Antidepressants, ADHD treatment[42]
Fluoxetine	Antidepressants, anti-anxiety, anti-inflammatory[43]
Acetic acid	Antihypertensive[44]
D-alanine	Antibacterial, antiviral[45]
Amphetamine	Psychostimulants (ADHD medication)[46]
Actinobolin	Antitumor, anticarcinogen, antiviral, antibiotic[47,48]
2-Heptanamine	Antitubercular, anticarcinogen, antimutagenic, antimicrobial, antioxidant, cardioprotective, Sympathomimetic [49,50]
2-Octanamine	Feromon[51]
2-Aminonadecane	anti-biofilm pathogenic bacteria, biosurfactant [52]
Bactobolin	Antibiotic, antibacterial, antitumor [53]
Linalool	Antimicrobial[54]
Isoborneol	Antiviral, analgesics[55]
Eugenol	Antibacterial, analgesics, antioxidant, antineoplastic, apoptosis inducer, anaesthetics, anti-inflamantory[56]
Copaene	Attractant[57]
Cyclohexane	Anticarcinogenic, antitumor[58]
Methyleugenol	Insecticide, attractant[56]

Chemicals	Biological Activities*
Caryophyllene	Weevils insecticide[59]
2-Propanamine	Herbicide[60]
Humulene	Antitumor[61]
Germacrene D	Weevils insecticide, antimicrobial, antioxidant[62]
Naphthalene	*Repellent,* antimicrobial[63]
Metaraminol	Sympathomimetic, analgesics, anesthetics[64,65]
Benzeneethanamine	antioxidant, anti-inflammatory, antimicrobial[66,67]
Tocainide	Anaesthetics, anti-arrhythmic medication[68]
Propanamide	Anti-inflamantory[69]
dI-Phenylephrine	Sympathomimetic, cardiotonic, repellent[70]
Benzenemethanol	Antibiotic, anti-inflamantory[71,72]
Phenethylamine	Alzheimer's treatment [73]
2-Ethylacridine	Antibacterial, antioxidant[74,75]

* The biological activities listed in this column are based on studies in several published articles

Conclusion

This study found that the four types of basil growing in Bali are morphologically and genetically identical, as evidenced by similar DNA sequences and branching positions in phylogenetic trees. The basil also contains secondary metabolites with diverse biological activities. Notably, Tulasi and Ruku-ruku contain high levels of methyleugenol, a compound proven by many studies to control fruit fly populations effectively.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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