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DNA Barcoding Based on *mat*K Gene and Phytochemistry Analyses of Local Balinese Kayu Tangi (*Lagerstroemia* sp.)

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Abstract. Kayu tangi *(Lagerstroemia* sp.) is one of the medicinal plants that is often used traditionally by Balinese people for treating insomnia, diabetes, and dysentery. This study aims to determine the phylogenetic trees and the active compounds in *Lagerstroemia tomentosa* extract and its antioxidant activities. The methods used in molecular identification are DNA isolation, PCR, and DNA sequencing. The DNA sample was BLASTed to see its homology with the sequences in GenBank. These sequences were then used for phylogenetic tree analysis. The phytochemical analysis was done using the GC-MS method and the antioxidant activity test used the DPPH (1,1-diphenyl-2-picrylhydrazyl) after being reacted with the tested extract. The results of molecular identification showed that the samples used were closely related to *Lagerstroemia tomentosa* species (MW044208.1). The antioxidant activity test results showed an IC50 of 2.1 ugml-1 which is included in the very strong category. The phytochemical test results showed that the most dominant compounds contained in the plant stem bark were furfural (AUC 8.54%), beta-sitosterol (AUC 6.00%), and gamma-sitosterol (AUC 3.81%). Based on PubChem data, these compounds have antioxidant and antimicrobial properties as well as insecticide and herbicide. In conclusion, kayu tangi is very close to *Lagerstroemia tomentosa* and it's potentially used as a medicine with strong antioxidant activities.

Keywords: Antimicrobial; Antioxidant; Kayu Tangi; Medicine Plant; Usada Taru Pramana.

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INTRODUCTION

Medicinal plants are often used by the community as an alternative medicine. Balinese people in particular have information on various medicinal plants contained in the Taru Pramana lontar. Lontar Taru Pramana contains 166 names of Balinese plants (Arsana, 2019), one of the medicinal plants contained in Taru Pramana is kayu tangi (*Lagerstroemia* sp). Kayu tangi bark can be used as a cure for insomnia (Adnyana, 2021). In addition, according to Riyanti *et al*. (2021), the bark extract can reduce blood glucose. The plant names contained in the lontar use local languages, kayu tangi, so many plant names are not known nationally or internationally. Likewise, with the efficacy of kayu tangi and its plant parts, there is no scientific evidence yet as medicine. Therefore, further research is needed to obtain this information through molecular and phytochemical analysis to determine the scientific name or the taxonomy and efficacy of the plant bioactive compounds.

Plant species identification can be done by conventional means, namely morphological identification. However, morphological observations alone are not enough to find information on genetic diversity, since morphological traits are limited and very easily influenced by the environment. Currently, a modern way has been developed to assess the genetic diversity of plants, namely by using the DNA barcoding method. According to Pham et al. (2022), plant identification with DNA barcoding methods generally requires standard coding genes as markers to compile phylogenetic trees, one of the genes used as a marker is the matK gene. The matK gene is a coding gene for the sub-unit K maturase enzyme found in plant chloroplasts. The matK gene has a sequence length of 1500 bp and

was located between the chloroplast introns in the matK gene. The substitution rate of the matK gene is three times higher at the nucleotide level and six times higher at the amino acid level when compared to the rbcL gene. Therefore, the matK gene is widely used in taxonomic and phylogenetic studies because it provides higher resolution in comparing plant species. Roslim, et al, 2023, also used metK as a primer in the taxonomy of Cleome gynandra L. (Briq) (maman). DNA Barcoding will produce sequences that become samples in the formation of phylogenetic trees. This sample is used as a comparison with species in the GenBank to determine the relationship between species. In addition, phylogenetic trees are useful for estimating differences that occur from one genetic ancestor to the next (Taib et al., 2023).

Balinese people have been utilizing plants as traditional medicine since ancient times. However, there is still little comprehensive scientific evidence found, namely the determination of secondary metabolites that have potential bioactivity, and quantitative levels of secondary metabolites to bioactivity testing. These tests can be carried out through phytochemical analysis to study methods of analyzing the biochemical contained in plants or animals as a whole or their parts, including how to isolate or separate them (Qomaliyah et al., 2023). In addition, secondary metabolite compounds in each plant vary depending on where the plant grows, so it is possible that each plant has new compounds that have not been identified. Currently, many people suffer from metabolic syndrome diseases. The uncontrolled condition of metabolic syndrome triggers the formation of excess free radicals through the formation of reactive oxygen species (ROS) which further aggravates health conditions (Hasim et al., 2020). Compounds that can inhibit the effects of free radicals in the body are antioxidant compounds. Some plants are able to produce natural antioxidant compounds, one of which is kayu tangi. Based on the study conducted by Musdalifah and Iqbal (2022) *kayu tangi* contains corosolic acid compounds. Corosolic acid is the dominant triterpene compound in bungur plants also called kayu tangi that functions as an antidiabetic (Riyanti et al., 2021) and has been confirmed as a potential antidiabetic drug due to its potential to inhibit α-glucosidase. In addition, this plant also contains flavonoid compounds, alkaloids, polyphenols, tannins, and saponins which are useful as antioxidants.

Molecular identification research on kayu tangi needs to be done to add information to the cultivation of medicinal plants so that the plant breeding process is easier because there is a relationship between species described in the phylogenetic tree.

Thus, the purposes of this study were to obtain scientific evidence that the kayu tangi can be used as a medicinal plant by analyzing the phytochemical compounds and genetically identification of kayu tangi and also to encourage the development of traditional medicine based on scientific standards that will contribute to health independence.

METHODS

This research was conducted during the dry season from July to October 2023. Sample testing was carried out at the Genetic Science Indonesia Laboratory, Udayana University Agricultural Biotechnology Laboratory, and Denpasar-Bali Forensic Lab. The plant samples used were *Lagerstroemia tomentosa* growing in Renon Field, Denpasar Regency, Bali.

Lagerstroemia tomentosa is a street or yard shade tree, this plant can grow well at an altitude of 0-300 m above sea level, with an annual temperature of 18-35 ° C, average rainfall of 2,000-3,500 mm/year, getting full sunlight and moist soil with a pH between 5.5-6.5 (Rahmah et al., 2021). Renon field has geographical conditions that are suitable for the growth of *Lagerstroemia tomentosa* because Renon field is located in South Denpasar which has an altitude of 0-12 m above sea level with an annual temperature of 24-29°C, average rainfall of 1,800-4,800 mm/year. This shows that the renon field has the right qualifications for the growth of Lagerstroemia tomentosa.

Total DNA Isolation

Isolation and purification of kayu tangi leaf DNA using the Quick DNA plant/Seed Miniprep kit (Zymo Research, D6020). A total of 150 mg of leaves were pulverized with a mortar and the flour obtained was loaded into a lysis tube by adding 750 µl of buffer, vortexed for 5 min, and centrifuged at 10,000 rpm for 1 min. In the next step, 400 µl of supernatant was centrifuged for 1 minute at 8,000 rpm, the liquid phase was discarded, and 1,200 genomic lysis buffer was added, and mixed until smooth. 800 µl of liquid was taken for centrifugation at 10,000 rpm for 1 minute, and the liquid phase was discarded. The solid was added 200 µl DNA pre-wash buffer and

centrifuged at 10,000 rpm for 1 minute. The mixture was added 35 µl DNA elution buffer, and centrifuged at 10,000 rpm for 30 seconds to elute the DNA.

DNA Amplification

DNA amplification by PCR using matK primers, namely matK-f primer (5'-ACC CAG TCC ATC TGG AAA TCT TGG TTC-3') and matK-r primer (5'-CGT ACA GTA CTT TTG TGT TTA CGA G-3'). The PCR reaction process used 35 cycles. The stages consist of pre-denaturation of template DNA for 3 minutes at 95°C. Then denaturation at 96°C for 10 seconds, followed by annealing at 53°C for 30 seconds, and finally extension at 72°C for 45 seconds. PCR results are then followed by electrophoresis using 1% agarose gel. The results of PCR were then visualized with a UV transilluminator.

Electrophoresis and DNA Sequencing

Electrophoresis was performed using 1% TBE agarose gel and the visualized DNA was sequenced. The first step was to view the results of the forward and reverse sequences using the Bioedit application. Bioedit displays the arrangement of nucleotides with different symbols and colors, namely A (Adenine) green, G (Guanine) black, C (Cytosine) blue, and T (Thymine) red. The Bioedit app combines the two forward and reverse DNA sequences to become a consensus sequence ready for BLAST analysis.

Phylogenetic Tree

The phylogenetic tree of kayu tangi was made based on matK gene sequences using MEGA 11 application with the Kimura 2-parameter neighbor-joining method and 1000 times bootstrap. The Kimura 2-parameter model was chosen because it has the most complete phylogenetic analysis model, namely the same nucleotide frequency and different substitution rates so that the results can be used to find genetic distance and similarity.

Preparation extract of kayu tangi bark

Extraction was carried out by maceration of samples in 96% ethanol, referring to the method carried out by (Mansouri et al., 2022). Maceration was carried out for 3 x 24 hours by adding 200 ml of solvent per maceration to 50 g of tangi bark simplisia. The macerates obtained were combined and then vacuumed to obtain the crude extract.

Identification of active compounds

The ethanol extract of kayu tangi bark was analyzed by GC-MS method. The column type used was HP-5MS ultra inert with a column length of 30 m, a diameter of 0.25 mm, and a column thickness of 0.25 µm. The vaporized and moving compounds were captured by the detector and displayed in the chromatogram. The temperature in the first minute after the sample is injected is 50°C then for 2 minutes the temperature is held until it reaches 100°C. The temperature was increased by 7°C every minute to 300°C and in the last 3 minutes, the column was heated to 325°C as the final temperature. Identification of phytochemical compounds using Willey database version 7.0 by comparing between mass spectrum patterns and fragmentation patterns of reference compounds stored in the database. To determine the biological activity of the compound, it was traced based on the database in PubChem in 2021.

Antioxidant activity testing

Antioxidant activity testing was conducted according to the methods of Horvat *et al*. (2022) by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical conversion indicator after being reacted with the tested extracts. The absorbance of DPPH was read using UV spectrophotometry at λ 517 nm. The concentration and DPPH absorbance values were regression analyzed to obtain the antioxidant power. The antioxidant power was determined by IC_{50} value, which is the concentration of the extract that can counteract DPPH radicals by 50%. The IC_{50} value was then matched with the antioxidant strength category from Alqahtani *et al*. (2019).

RESULT AND DISCUSSION

Morphology of Kayu Tangi (*Lagerstroemia* sp*)*

The morphological study of kayu tangi was done and the results as presented below. Kayu tangi is a deciduous tree that can grow up to 20-30 m tall. The trunk is quite strong with a rough bark texture. The trunk of the kayu tangi is grayish brown with gray fibrous bark.

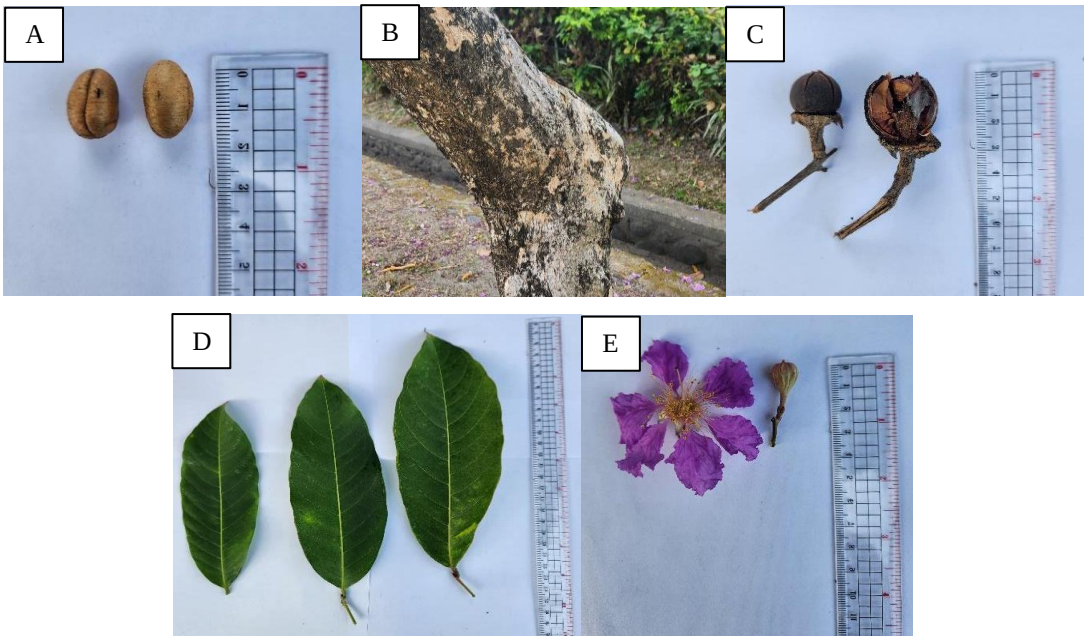


Figure 1. Morphology of Kayu Tangi (Description: A. Seed, B. Stem, C. Fruit, D. Leaf, E. Flower)

The leaves are single leaves with pinnate leaf bones lanceolate or ovate, measuring about 8-18 cm long and 4-6.5 cm wide, petioles measuring about 4-8 mm, and under the leaf surface there is tomentose. The leaf color is light green in the early growth phase and turns dark green when mature (Rahmah et al., 2021). The observed sample tree is 23 m tall with leaves 16 cm long, 5 cm wide, and petiole 6 mm. Compound flowers are pale pink or purple with crinkled petals and borne in panicles measuring about 8-20 m, sepals measuring about 3-4 mm, many stamens clustered in the center, dense ovary, flowers are located from leaf axils or twig tips. According to Waghmare and Tatke (2021), the fruit is round with a length of 1.8- 2.5 cm and a diameter of 1.5-2 cm, and the tip of the fruit is pointed like a needle with a length of 0.3 mm. The fruit is green when it is young and brown when it is old, it takes about 3-4 months to get ripe fruit.

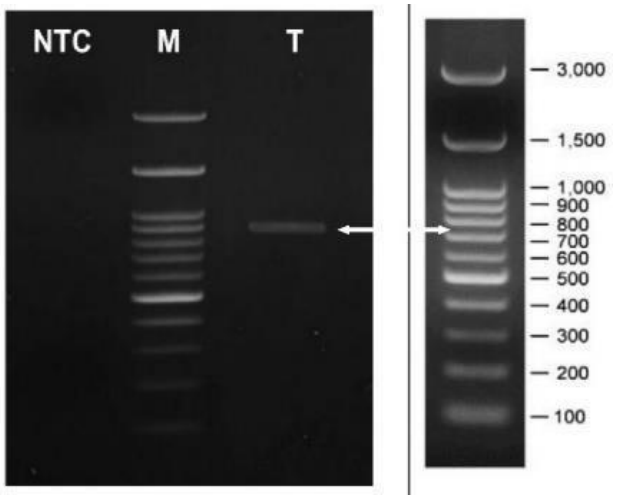
DNA Isolation and amplification

The total DNA of kayu tangi was tested for concentration and purity. The purity value of kayu tangi DNA was measured with NanoDrop and obtained an absorbance ratio of 1.86. This value is included in the good category because it is in the range of 1.8-2.0. This is evidenced by the statement from Dewanata and Mushlih (2021), namely the DNA purity value below 1.8 indicates that the extracted DNA contains contaminants in the form of protein compounds, while according to Fitriyani *et al.* (2022), the DNA purity value above 2.0 indicates that there are still contaminants in the form of RNA. The concentration value of the DNA isolation sample is 100.5 ngµl. This concentration value has met the requirements for the DNA samples used to be successfully amplified. The quality and quantity of DNA isolation will determine the results of DNA band formation during the electrophoresis process.

The concentration value of the DNA isolation sample is 100.5 ng/µl. DNA amplification using PCR with the matK gene obtained DNA with a size of 862 bp. This DNA length meets the requirements for DNA barcoding because the length is not more than 1000 bp (Cahyaningsih *et al.,* 2022). DNA sequences and DNA bands from PCR are presented in Fig. 2. Based on the results of electrophoresis, the DNA band formed is quite thick and there is no smear, indicating that the DNA sample already has high quality (Setyawati and Zubaidah, 2021). This is triggered by the right annealing temperature and the concentration and purity of DNA in accordance with existing procedures

The DNA sequences obtained were then analyzed for species kinship using the BLAST program. The method used was to compare the sample DNA sequences with several species in GenBank or NCBI (Table 1). Based on the BLAST program analysis, kayu tangi showed 100% percent identity, E-value 0.0, and 100% query cover to *Lagerstroemia tomentosa.*

DNA Band PCR result **Nucleotide sequence**



GCCCCCCTTACCCAGTCCCATCTGGAAATCTTGGTTCAAAA
CCTTCGCTACTGGGTGAAGGATGCCTCTTCTTTGCATTTATT
ACGTTTCTTTTTCTACGAGTATTGTAATTGGAATAGTCTTAT
TACTCCCCAAAAACATATTTCCATTTTTTTAAAAGGTAATC
CAAGATTATTCTTGTTCCTATATAATTCTTATGCATGTGAAT
ACGAATCCATCTTCCTTTTTCTCCGTAATCAATCTTCTCATT
TCCGGCCAACATCTTCTGGAGTCTTTTTTGAGCGAATATAT
TTCTATGTAAAAATAGAACTTCCTGTTGAAGTTTTTTTTGAT
AATGATTTTCGGGACATTCGAGCCTTCTTCAAGTATTCTTTC
ATGCATTATGTTAGATATCAAGGAAAATCAATTCTGGCTTC
GAAAGATACACCCTTTCTGATGAATAAATGGAAATATTACC
TTGTCAATTTATGGCAATATCGTTTTTACGCGTGGTCTCAAC
CAGGAAGGATCAATCTAAACCGATTAGGAAAGTATTCTCTT
GACTTTTTGGGCTATTTTTCAAACGTGCAACTAACTTTTTCA
GTGGTACGAAGTAAAATGCTAGAAAATGCATTTCTGATAA
ATACTGCTATGAAGAAGCTCGAAACAACAGTTCCAATTATT
CCTTTGATTGGATCGTTGTCTAAAGCGAAATTTTGTAACGT
ATTAGGACATCCCGTTAGTAAGTCGACCCGGACCGATTCAT
CAGATTCTGATATTATCGACCGATTTGTGCGTATATGCAGA
AATCTTTCTCATTATCACAGCGGATCCTCAAAAAAAAAGAG
TTTATATCGAATAAAATATATACTTCGACTT

Figure. 2. PCR DNA Electrophoresis of Kayu Tangi and Its DNA Sequences
Description: NTC (No Template Control), M (marker), T (Kayu tangi sample)

Table 1. BLAST Results of Kayu Tangi (*Lagerstroemia sp*) Sequences

Name of species	Percentage of Identity (%)	E-value	Query Cover (%)	Accession number
Lagerstroemia tomentosa	100	0.0	100	MW044208.1
Lagerstroemia indica	99.86	0.0	100	MH552355.1
Lagerstroemia ruffordii	99.79	0.0	100	LC258574.1
Lagerstroemia speciosa	99.64	0.0	100	MW355189.1
Punica granatum	97.53	0.0	99	OL690133.1
Lafoensia punicifolia	97.47	0.0	99	MZ493790.1
Heimia salicifolia	96.52	0.0	99	OL537885.1
Woodfordia fruticosa	96.32	0.0	99	KX344547.1
Lafoensia pacari	95.46	0.0	99	KF555401.1
Sonneratia alba	93.52	0.0	99	KM255093.1
Syzgium cumini	89.17	0.0	95	GU135062.1

This indicates that *Lagerstroemia tomentosa* is the same species as the sample studied, while other species are on average at a percent identity value of 89-99% which indicates that the species is still related to the sample studied. The results of this study are also in line with the statement from Shafqat et al. (2020) that the most similar results will be characterized by a query coverage value close to 100%, an e-value close to 0, and a percentage identity close to 100%. The lower the e-value, the more significant the calculation results. Comparison sequences that have percent identity and query cover close to 100% indicate that the sequence has a close relationship with the target sample because the higher the percentage value, the closer the relationship. The percent identity and query cover values also affect the bootstrap value when building a phylogenetic tree. A small bootstrap value causes instability of the branches formed so that the resulting phylogenetic tree is not accurate in identifying the evolutionary process of a species (Qian et al., 2023).

Similarity and Genetic Distance

The similarity value is a percentage used to show the level of similarity between the sample sequences and the sequences in GenBank after being aligned, while the genetic distance is the opposite of similarity because the smaller the genetic distance value, the closer the relationship. The similarity analysis results show that kayu tangi is similar to *Lagerstroemia tomentosa* with accession number MW044208.1, which has a value of 100% (Table 1) and a genetic distance of 0.00 (Table 3). In addition, there are three sequences that have a large percentage of similarity, namely *Lagerstroemia ruffordii* (LC258574.1), *Lagerstroemia indica* (MH552355.1), and *Lagerstroemia speciosa* (MW355189.1) with a percentage value of 99%,

while the genetic distance is only 0.01. Clearer results can be seen in Tables 3 and 4. This large percentage value is obtained because the four sequences come from the same genus, Lagerstroemia. This causes them to have a close kinship relationship as indicated by the high similarity percentage value and short genetic distance (Vessal et al., 2023).

Phylogenetic Tree

Based on the phylogenetic tree formed (Fig. 2), the kayu tangi is close to the *Lagerstroemia tomentosa* species which has accession number MW044208.1. The close kinship between the sample sequences and *Lagerstroemia tomentosa* is characterized by adjacent branches on the

phylogenetic tree the sample sequences are on the same branch as *Lagerstroemia tomentosa* In addition, this result is reinforced by a bootstrap value of 78%. A bootstrap value above 70% indicates that the branch formed is accurate and will not change when reconstructed (Gosselin *et al.*, 2022).

Table 2. Similarity Percentage of Kayu Tangi with Species in GenBank

Number	Accession Number	Similarity Percentage (%)											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Kayu Tangi	ID											
2	GU135062.1	89	ID										
3	MW044208.1	100	89	ID									
4	MH552355.1	99	88	99	ID								
5	LC258574.1	99	88	99,8	99	ID							
6	MW355189.1	99	90	99	99	99	ID						
7	KM255093.1	93	85	93	93	93	92	ID					
8	OL537885.1	96	88	96	95	96	95	92	ID				
9	KX344547.1	96	90	96	96	97	96	93	96	ID			
10	OL690133.1	97	90	96	96	96	96	93	96	98	ID		
11	MZ493790.1	97	90	96	97	97	96	93	96	98	99	ID	
12	KF555401.1	95	90	95	94	95	95	92	95	97	97	98	ID

Description: GU1350621 (Syzgium cumini), MW044208.1 (Lagerstroemia tomentosa), MH552355.1 (Lagerstroemia indica), LC258574.1 (Lagerstroemia ruffordii), MW355189.1 (Lagerstroemia speciosa), KM255093.1 (Soneratia alba), OL537885.1 (Heimia salicifolia), KX344547.1 (Woodfordia fruticosa), OL690133.1 (Punica granatum), MZ493790.1 (Lafoensia punicifolia), dan KF555401.1 (Lafoensia pacari).

Table 3. Genetic Distance of Kayu Tangi with Species in GenBank

Number	Accession Number	Genetic Distance											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Kayu Tangi	ID											
2	GU135062.1	0.11	ID										
3	MW044208.1	0.00	0.11	ID									
4	MH552355.1	0.01	0.12	0.01	ID								
5	LC258574.1	0.01	0.12	0.002	0.01	ID							
6	MW355189.1	0.01	0.10	0.01	0.01	0.01	ID						
7	KM255093.1	0.07	0.15	0.07	0.07	0.07	0.08	ID					
8	OL537885.1	0.04	0.12	0.04	0.05	0.04	0.05	0.08	ID				
9	KX344547.1	0.04	0.10	0.04	0.04	0.03	0.04	0.07	0.04	ID			
10	OL690133.1	0.03	0.10	0.04	0.04	0.04	0.04	0.07	0.04	0.02	ID		
11	MZ493790.1	0.03	0.10	0.04	0.03	0.03	0.04	0.07	0.04	0.02	0.01	ID	
12	KF555401.1	0.05	0.10	0.05	0.06	0.05	0.05	0.08	0.05	0.03	0.03	0.02	ID

Description: GU1350621 (Syzgium cumini), MW044208.1 (Lagerstroemia tomentosa), MH552355.1 (Lagerstroemia indica), LC258574.1 (Lagerstroemia ruffordii), MW355189.1 (Lagerstroemia speciosa), KM255093.1 (Soneratia alba), OL537885.1 (Heimia salicifolia), KX344547.1 (Woodfordia fruticosa), OL690133.1 (Punica granatum), MZ493790.1 (Lafoensia punicifolia), dan KF555401.1 (Lafoensia pacari).

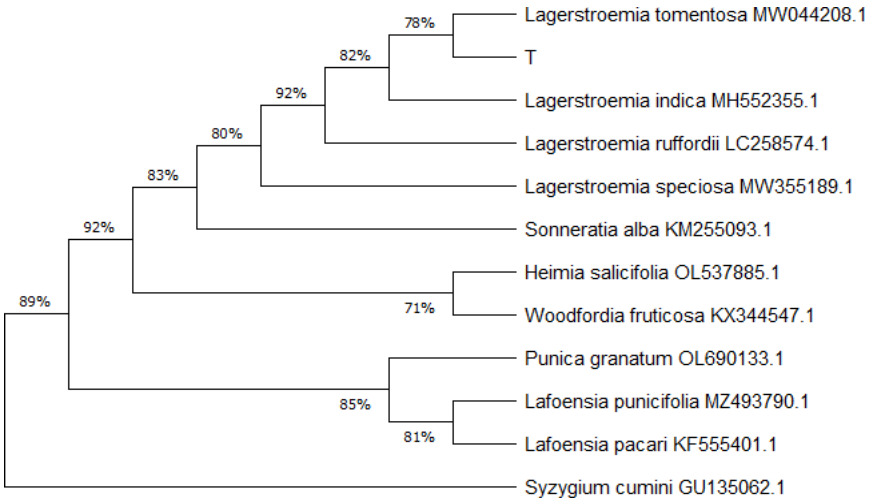


Figure 2. Phylogenetic Tree of Kayu Tangi (T) Based on matK Gene using neighbor-joining method bootstrap 1000 times.

The three samples that showed high similarity values were also on branches adjacent to the sample sequences. This indicates that the four sequences come from the same ancestor, it's just that what distinguishes them is environmental factors, resulting in morphological changes.

Phytochemical Analysis of Kayu Tangi

Analysis of Gas Chromatography-Mass Spectrophotometry (GC-MS) results of ethanol extract of kayu tangi are in the form of chromatograms showing the retention time of detected molecules and peak diagrams showing the quantity proportion of molecules present.

Based on Figure 2, twenty-five compounds were detected that have a quality value of >90 percent as shown in Table 4. The dominant compounds are those with large AUC (Area Under Curve) values.

The three compounds with the dominant proportion were furfural with an AUC value of 8.54% followed by beta-sitosterol (6%) and gamma-sitosterol (3.81%). Furfural is a heterocyclic aldehyde organic compound with five carbon atoms and has major benefits as a solvent in the lubricating oil refining and nitrocellulose industries, as a fungicide, and is used as a raw material for the manufacture of tetrahydrofuran and furfuryl alcohol compounds (Dutt, 2019). In addition, furfural is widely used as an insecticide, herbicide, and fungicide (Table 5). Furfural has the molecular formula $C_5H_4O_2$, a molecular weight of 96.09 gmol-1, boiling point of 162°C.

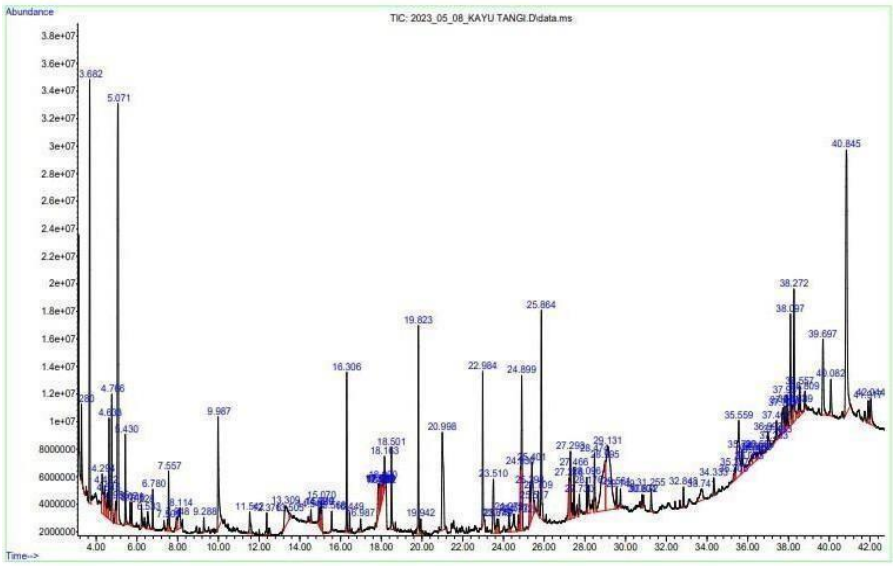


Figure 3. GC-MS Analysis Results of Kayu Tangi Ethanol Extract Chromatograms

Table 4. Compound content of tangi bark extract based on GC-MS analysis

No	Name of compound	Molecular formula	Retention Time (RT)	AUC (Area Under Curve) (%)	Function of compounds
1	Furfural	$C_5H_4O_2$	5.07	8.54	Lubricating oil purifier and nitrocellulose, raw materials for insecticides, herbicides, and fungicides; synthesis of tetrahydrofuran, furfuryl alcohol, and furoic acid
2	2-Furan methanol	$C_5H_6O_2$	5.43	1.03	Solvent for phenolic resins, paints, dyes, and adhesives
3	2-Furan- carboxaldehyde, 5-methyl	$C_6H_6O_2$	7.56	0.87	Antifungal
4	Cyclotetra- siloxane, octamethyl	$C_8H_{24}O_4Si_4$	8.11	0.82	Antioxidant
5	Benzenepropan oic acid, ethyl ester	$C_{11}H_{11}O_2$	15.57	0.22	There is no report yet
6	1-tetradecene	$C_{14}H_{28}$	16.31	1.78	Antioxidant
7	Tetradecene	$C_{14}H_{30}$	16.45	0.22	Antimicrobial
8	Cycloheptasil-oxane, tetradeca-methy1	$C_{14}H_{42}O_7Si$	17.89	0.97	There is no report yet
9	2,4-di-tert- butyl-phenol	$C_8H_{16}N_2$ O	18.50	1.14	Antioxidant
10	Cetene	$C_{16}H_{32}$	19.82	2.41	Antimicrobial
11	Hexadecane	$C_{16}H_{34}$	19.94	0.19	There is no report yet
12	1-Octadecene	$C_{18}H_{36}$	22.98	1.65	There is no report yet
13	Cyclononasi-loxane, octadeca methyl	$C_{18}H_{54}O_9Si_9$	23.68	0.27	There is no report yet
14	Nonadecane	$C_{19}H_{40}$	24.52	0.71	There is no report yet
15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	24.84	0.82	Antifungal
16	n-hexa-decanoic acid	$C_{16}H_{32}O_2$	25.30	0.64	Antifungal
17	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	25.86	3.24	Antioxidant
18	Methyl 9- cis,11-trans-octadecadinoate	$C_{19}H_{34}O_2$	27.22	0.78	There is no report yet
19	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	7.29	1.57	Antioxidant
20	Phytol	$C_{20}H_{40}O$	27.47	0.68	Antioxidant
21	n-Propyl 9,12-octadecadinoate	$C_{21}H_{38}O_2$	28.10	0.46	Antioxidant
22	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	28.47	0.86	Food flavor enhancer
23	Gamma-sitosterol	$C_{29}H_{50}O$	28.99	3.81	Antimicrobial
24	Beta-sitosterol (Sterol tumbuhan)	$C_{29}H_{50}O$	9.13	6.00	Antifungal
25	Triacontyl acetate	$C_{16}H_{32}O_2$	30.87	0.13	There is no report yet

Description: RT (retention time): the time it takes for the compound to go from being injected to being detected by the detector; AUC: Area Under Curve, an abundance of compound. The function of the compound is traced from PubChem (2021).

The third dominant compound is Gamma-sitosterol (γ-sitosterol) with an AUC value of 3.81%, $C_{29}H_{50}O$, and molecular weight of 414.71 gmol-1 (Figure 5). Gamma-sitosterol is a phytosterol compound which is a compound in plants similar to cholesterol compounds. Gamma-sitosterol is a gamma-epimer of beta-sitosterol, so both have the same molecular formula. Gamma-sitosterol has antidiabetic, anti-inflammatory, anticancer, and cholesterol-lowering properties, and is also used as a cosmetic, namely skin and hair moisturizing ingredients (Manurung *et al.*, 2022).

Table 5. Functions of compounds contained in kayu tangi extract based on AU values

Number	Function of compound	AUC (Area Under Curve) (%)	Amount of supporting compound
1	Antioxidant	6.98	4
2	Antifungal	10.87	4
3	Antimicrobial	13.50	6
4	Insecticide	8.54	1
5	Herbicide	8.54	1

AUC (Area Under Curve): Abundance of compounds in the *Lagerstroemia tomentosa* extract

According to Hernandez *et al.* (2021), gamma-sitosterol can accelerate the healing of diabetes because it can reduce hyperglycemia, increase insulin production, and inhibit glucogenesis. Furthermore, the administration of gamma-sitosterol to diabetes-induced rats resulted in a marked decrease in blood glucose and glycosylated hemoglobin coupled with an increase in plasma insulin levels and body weight. Hexadecanoic ethyl ester is the fourth dominant compound in *Lagerstroemia tomentosa* extract with molecular formula $C_{18}H_{36}O_2$, molecular weight 284.48 gmol-1 (Figure 6). Hexadecanoic ethyl ester is a fatty acid ester also called ethyl palmitate in the form of a colorless liquid, insoluble in water, but soluble in organic solvents such as ethanol and ether.

Testing the Antioxidant Power of Kayu Tangi Extract

To determine the extract's antioxidant power, a DPPH test was conducted, and the results are shown in Table 6.

The regression equation formed based on Table 6. obtained the equation y = 24.7619x-2.500, which is the relationship between the strength of antioxidant power (y) and the concentration of the extract (x). Based on the equation, the IC_{50} value of 2.1 µgml-1 was obtained. This shows that 2.1 µgml-1 ethanol extract of tangi bark is able to inhibit DPPH (free radicals) by 50%. According to the category of Bakrim *et al.* (2022), the IC_{50} value is categorized as very strong. The category indicates that if IC_{50} >250 µgml-1 is classified as an inactive antioxidant compound, IC_{50} 100-250 µgml-1 is weak, IC_{50} 50-100 µgml-1 is moderate, IC_{50} 10-50 µgml-1 is a strong antioxidant compound, and IC_{50} <10 µgml-1 as a very strong antioxidant compound.

Currently, no one has studied the molecular identification of Lagerstromia tomentosa, so information on the genetic diversity of these plants is very minimal. Information on genetic diversity is very important in the process of developing plant breeding, especially in hybridizing in order to produce cultivars with superior traits. This can increase the selling value of the plant because it can be produced in large quantities. This plant is very useful as an alternative medicine because it is made from natural ingredients that are free from chemicals, so it is safe if consumed in the long term.

Table 6. The strength of *Lagerstroemia tomentosa* extract as an antioxidant based on binding to DPPH free radicals

Absorbance	Extract Concentration (x) (µg/ml)	Antioxidant Strenght Against DPPH (%)	Regression equation (y = ax+b)	IC_{50} (μgml^{-1})
0.560	0	0	y=24.7619x-2.500	2.1
0.481	0.2	14.10714286		
0.435	0.4	22.32142857		
0.355	0.6	36.60714286		
0.207	0.8	63.03571429		

Description: DPPH= *1,1-diphenyl-2-picrylhydrazyl*, free radical; IC_{50}=concentration of the extract that can oxidize 50% DPPH

Existing studies on Lagerstroemia tomentosa only discuss phytochemical analysis. The research conducted by Verma et al. (2023) discussed the Phytochemical and Antimicrobial Potential of Methanol Leaf Extracts from Acacia catechu and Lagerstroemia speciosa against *Escherichia coli* O157: H7. Currently, there is no research on the molecular identification of Lagerstroemia tomentosa, so this research is very useful for farmers who want to hybridize to get new cultivars that have superior properties because this research can add information to the cultivation of medicinal plants so that the plant breeding process is easier because there is a kinship relationship between species described in the phylogenetic tree. In addition, the results of phytochemical analysis and antioxidant activity power can be useful for researchers to obtain scientific evidence that Lagerstroemia tomentosa contains compounds that are useful for treating various diseases, so as to encourage the development of traditional medicines based on scientific standards that will contribute to health independence, especially researchers engaged in the pharmaceutical field.

CONCLUSION

Based on this study, it can be concluded that the kayu tangi located in Renon Park, Denpasar, Bali Province is a species of *Lagerstroemia tomentosa* with accession number MW044208.1. The phytochemical analysis showed that the compounds contained in kayu tangi bark with the largest AUC values were furfural (8.54%), beta-sitosterol (6.00%), gamma-sitosterol (3.81%), and hexadecanoic ethyl ester (3.24%), respectively. *Kayu tangi* extract has strong antioxidant and antimicrobial properties. The antioxidant power was measured by IC_{50} of 2.1 µgml-1 and included in the very strong category. Further study is needed on the development of kayu tangi breeding by using the sequences from this study as a reference in determining which species to cross, so as to produce superior cultivars. In addition, further research is needed on phytochemical analysis using different solvents to find out if there are differences in the compounds produced and molecular separation and function analysis of each compound.

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