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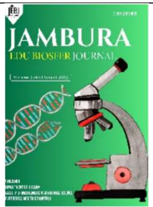

PHYLOGENETIC OF TAPAK DARA (*Catharanthus roseus*) RELATIVE IN FAMILY APOCYNACEAE BASED ON matK AND rbcL SEQUENCES

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ABSTRAK

Famili Apocynaceae, salah satunya Tapak Dara (*Catharanthus roseus*), merupakan salah satu tanaman obat populer karena metabolit sekunder yang dimilikinya. Metabolit sekunder secara alami di produksi dalam jumlah sedikit, namun bernilai ekonomi sangat tinggi. Sehingga, eksplorasi terhadap jenis-jenis tanaman dalam satu famili menjadi hal yang menarik untuk dilakukan. Tujuan dari penelitian ini adalah untuk menemukan anggota famili Apocynaceae yang memiliki kekerabatan dekat dengan *Catharanthus roseus* sehingga diperkirakan memiliki kandungan senyawa aktif yang sama. Adapun metode yang dilakukan yaitu dengan analisis filogenetik dengan menggunakan sekuen DNA daerah maturase K (matK) dan riboluse 1,5-bifosfat karboksilase/oksigenase subunit besar (rbcL). Sejumlah 22 tanaman famili Apocynaceae digunakan, dan sekuen kedua gen dari tanaman diambil dalam *database* NCBI. Konstruksi pohon filogenetik menggunakan aplikasi MEGA 11 dengan dua spesies Rubiaceae sebagai *outgroup*. Hasil studi didapatkan 10 kelompok tanaman terbentuk berdasarkan super marga pada kedua pohon yang dihasilkan. Secara konsisten diperoleh bahwa Rauvolfia serpentina berada dalam satu kelompok dengan *C. roseus* dalam super marga Vinceae, sub famili Rauvolfioideae. Secara morfologi, kelompok tanaman ini memiliki kesamaan karakter bunga dan daun. Analisis filogenetik dengan sekuen DNA mendukung pengelompokan data morfologi, serta mengindikasikan *Rauvolfia serpentine* memiliki profil biokimia yang mirip dengan Tapak Dara.

Kata kunci: Apocynaceae, Filogenetik, matK, rbcL

ABSTRACT

The Apocynaceae family, including Tapak Dara (*Catharanthus roseus*), is one of the most popular medicinal plants due to its secondary metabolites. Secondary metabolites are naturally produced in small amounts but have very high economic value. Therefore, exploration of plant species within the same family is an interesting issue to be conducted. The purpose of this study is to discover members of the Apocynaceae family that have close relatives with Catharanthus roseus so that expected to have the similar active compounds. The method used was phylogenetic analysis using DNA sequences of maturase K (matK) and riboluse 1,5-biphosphate carboxylase/oxygenase large subunit (rbcL) regions. A total of 22 plants of the Apocynaceae family were used, and the sequences of both genes from these plants were retrieved in the NCBI database. Phylogenetic trees were constructed using MEGA 11 and two Rubiaceae species as outgroups. The study results showed that 10 plant groups were formed based on the suprageneric in both trees generated. It is consistently obtained that Rauvolfia serpentina is in one group with *C. roseus* in the Vinceae tribes of Rauvolfioideae subfamily. Morphologically, this group of plants has similar flower and leaf characters. It can be concluded that phylogenetic analysis with DNA sequences supports the grouping of morphological data and indicates that *Rauvolfia. serpentine* has a biochemical profile similar to Tapak Dara.

Keywords: Apocynaceae, matK, Phylogenetic, rbcL

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1. Introduction

The use of plants as a source of medicine becomes a sustainable part of cultural heritage. Saifuddin (2011); Handayani *et al.,* (2018); Nurmila *et al.,* (2019), reported that Indonesia has more than 30,000 species of high-level plants, but less than 300 plants are used as raw materials for the pharmaceutical industry (Yassir *et al.,* 2018; Siregar *et al.,* 2020). In contrast, the use of plants as medicine is believed to be more effective because it is considered to contain no toxins and have fewer side effects compared to chemical drugs. The Apocynaceae used to as a medicine because its known to contain alkaloid and flavonoid compounds (Azalia *et al*., 2023). In addition, this family also known to have a high level of diversity because it can grow in various tropical and subtropical climates. Tapak dara (*Catharanthus roseus*) which belongs to the family *Apocynaceae* is one of the important medical plants. Two magnificent secondary metabolites from Tapak dara leaves, vinblastine and vincristine, have anticancer activity, especially leukimia and lymphoma (Yusransyah *et al.,* 2016). Fitri *et al.,* (2023) proved that the leaf extract of Tapak dara could inhibit the growth of a variety of human cancer cells, and are safe for normal human cell. However, compounds in this plant have high economic value and are produced with very small levels. Therefore, exploration is essential to find Tapak dara relatives.

Cancer remains a major cause of morbidity and mortality in Southeast Asia, particularly in Indonesia (Kustanti et al., 2019). To date, existing drugs have not provided satisfactory results. In this study, in accordance with the invention of medicinal compounds in Tapak Dara*,* phylogenetic based on DNA sequences of *maturase-K* (*matK*) and *ribulose-1,5-bisphosphate carboxylase large* subunit oxygenase (*rbcL*) genes was conducted to screen the Indonesian plants. The aim of this study is to find a strong phylogenetics relationship with Tapak dara. Phylogenetics explains the diversity of organisms by reconstructing evolutionary relationship using molecular data such as DNA, RNA, and protein. It considers organisms sharing similar characteristics to be closely related, and probably evolved from the same ancestor (Hidayat et al., 2016). Thus, phylogenetics provides a basic information to which a new variant of secondary metabolites extracted plants might be established. The recommended sequence for DNA barcoding in plants are maturase-K (*matK*) and ribulose-1,5-bisphosphate carboxylase large subunit oxygenase (*rbcL*). The *matK* gene is found in introns with a length of 1500 bp (Dong *et al*., 2013). While the *rbcL* gene has a length of 1400 bp. In addition, this gene can be amplified with a high success rate (Kumar et al., 2016). r*bcL gene* has a lower mutation rate than other chloroplast DNA markers. Meanwhile, matK is one of the most rapidly evolving chloroplast coding regions and consistently shows a high level of discrimination (CBOL, 2009; Mursyidin *et al*, 2020).

2. Methodology

This study was conducted using the in-silico method by utilizing *matK* and *rbcL* gene sequence data from several families of Apocynaceae contained in the NCBI GenBank (Table 1).

Nucleotide sequence search and homology analysis

DNA sequences are traced from the GenBank (https://www.ncbi.nlm.nih.gov/) page by filling the species name along with the targeted gene (Example: *Catharanthus roseus* matK/rbcL) on query box. Data including accessions, nucleotide displays are stored in Ms.Word. Meanwhile, the nucleotide arrangement is stored in FASTA format (Notepad) (Hariri et al., 2021).

Alignment using CLUSTAL W

All DNA sequences that have been collected from NCBI are aligned. DNA sequence exploration was carried out using MEGA 11 for tree construction. It aims to determine the level of homology and identification of sequences that have the potential to be barcodes. Sequences that have the potential to be barcodes are different and distinctive sequences compared to others (Anzani et al., 2021).

Analysis of genetic relatedness

Genetic relatedness analysis of the family Apocynaceae was conducted using MEGA 11 software (Kumar et al., 2018). The analysis was carried out by entering all sequence data in FASTA format (for each gene used) into Mega format (.meg). Phylogenetic tree reconstruction was carried out using the Construct/Test Neighbour-Joining Tree method with bootstrap 1000 using the Maximum Composite Likelihood model on MEGA-11 (Anafarida et al., 2020).

Phylogenetics aims to determine the level of kinship of each variation of *C. roseus* species with plants of the family Apocynaceae and see the rate of evolution based on the evaluation of bootstrap values. Bootstrap values can be divided into several categories including high (>85%), moderate (70-85%), weak (50-69%) or very weak (<50%) (Lestari et al., 2018).

Table 1. Plant materials examined in this study

No	Species name	Gene	*Accession Number*
1	*Allamanda cathartica*	*matK*	Z70190.1
		rbcL	X91759.1
2	*Alstonia scholaris*	*matK*	Z70189.1
		rbcL	X91760.1
3	*Aspidosperma triternatum*	*matK*	AM295077.1
		rbcL	AJ419735.1
4	*Catharanthus roseus*	*matK*	DQ660507.1
		rbcL	X91757.1
5	*Kopsia fruticosa*	*matK*	Z70178.1
		rbcL	X91763.1
6	*Rauvolfia serpetina*	*matK*	KT955394.1
		rbcL	MG946861.1
7	*Vinca minor*	*matK*	DQ660553.1
		rbcL	AJ419768.1
8	*Schizozygia coffaeoides*	*matK*	DQ660540.1
		rbcL	AJ419760.1
9	*Tabernaemontana divaricata*	*matK*	Z70187.1
		rbcL	X91772.1
10	*Tabernanthe iboga*	*matK*	GU973973.1
		rbcL	AJ419763.1
11	*Diplorhynchus condylocarpon*	*matK*	DQ660514.1
		rbcL	AJ419743.1
12	*Molangum laxum*	*matK*	Z70185.1
		rbcL	X91765.1
13	*Carbera venenifera*	*matK*	DQ660509.1
		rbcL	AJ419740.1
14	*Plumeria rubra*	*matK*	Z70191.1
		rbcL	EU916731.1
15	*Thevetia peruviana*	*matK*	Z70188.1
		rbcL	X91773.1

No	**Species name**	**Gene**	***Accession Number***
16	*Carissa macrocarpa*	*matK*	DQ660505.1
		rbcL	DQ660635.1
17	*Adenium obesum*	*matK*	DQ660494.1
		rbcL	AJ002880.1
18	*Nerium oleander*	*matK*	EF456295.1
		rbcL	AJ002886.1
19	*Wrightia arborea*	*matK*	DQ660555.1
		rbcL	AJ002891.1
20	*Apocynum cannabinum*	*matK*	DQ660500.1
		rbcL	X91761.1
21	*Prestonia quinguangularis*	*matK*	Z70180.1
		rbcL	X91768.1
22	*Hollarrhena pubescens*	*matK*	EF456363.1
		rbcL	AJ002884.1
23	*Cinchona pubescens*	*matK*	Z70197.1
		rbcL	X83630.1
24	*Gardenia thunbergia*	*matK*	Z70198.1
		rbcL	X83637.1

3. Results and Discussion

3.1 Result

The phylogenetic tree was successfully constructed using the NCBI database of 22 *matK* and rbcL genes Apocenaceae family and 2 *outgroup sequences* from the family Rubiaceae. Based on the results of the phylogenetic tree reconstruction, both *matK* and *rcbcL* genes divide the Apocynaceae into 10 clusters based on suprageneric (tribes) (Figure 1). Cluster A consists of the Vinceae tirbes (*Rauvolfia serpentina, Catharanthus roseus, Vinca minor* and *Kopsia fruticosa*). Cluster B consists of the Alstonieae tribes (*Alstonia scholaris* and *Aspidosperma triternatum*). Cluster C consists of the Wrightieae tribes (*Wrightia arborea, Nerium oleander* and *Adenium obesum*). Cluster D consists of the Tabernaemontana tribe (*Tabernaemontana divaricata, Molangum laxum, Tabernanthe iboga* and *Schizozygia coffaeoides*). Cluster E and Cluster F consist of Carisseae tribe (*Carissa macrocarpa)* and the Melodineae tribe (*Diplorhynchus condylocarpon*), respectively. Plumerieae consist of *Plumeria rubra, Carbera venenifera, Allamanda cathartica* and *Thevetia peruviana* are clustered in G. Furthermore, Cluster H, I, and J, orderly consists of Malouetieae (*Hollarhena pubescens*); Apocyneae (*Apocynum cannabinum*), and Echiteae (*Prestonia quingualaris*). The consistency of the clusters formed is supported by a moderate - high bootsrap score (>70%) which indicates that the confidence level is good of grouping taxa. Both phylogenetic trees inform that the relative of *C. roseus* is *R. serpentina* with bootstrap score >90%.

3.2 Discussion

Phylogenetic analysis depicted the arrangement of phylogenetic relationships in a branching line like a tree (Subari *et al.,* 2021). The contructed tree also revelaed a closely relation among organism and told the biological evolution. Members whose share many characters in common is considered to be very closely related and is thought to be descended from a common ancestor. Both phylogenetic trees constructed indicate that *C. roseus* and *R. serpentine* are closely related. Both species have a monophyletic structural pattern, which means they share characters from a recent ancestor. The relationships consistent with known taxonomic groupings which combines both species in one tribe, Vinceae.

This grouping is based on the morphological characteristics of its members, which are characterized by trumpet-shaped flowers and downy surfaces (Endress, Schumann, & Meve., 2014; Simões et al., 2007). Furthermore, both plant species have lanceolate or ovate leaves, and funnel-shaped or infundibuliform petals (Simões et al., 2007). In addition, Nazar et al (2019) analyzed phylogenetic relationships in Apocynaceae based on PHYA gene sequences and *trnL-F* plastids, finding that Catharanthus and Rauvolfia are closely related based on the APSA clade (Apocynoidae, Peri-plocoideae, Secamonoideae and Asclepiadoideae).

In addition to showing the similarity of genetic patterns, the similarity of ancestors (monophyletic), also shows the similarity of biochemical properties (Hidayat et al., 2008). *C. roseus* is a medicinal plant that has activities as anti-inflammatory, antiangiogenesis, anti-malaria, anti-microbial, anti-oxidant, anti-cancer, anti-diabetic and cytotoxic (Pham et al., 2020; Ali et al., 2021). This plant is known to contain active compounds, namely Vindolline, Ajmalicine, Perivine, Chlorambucil, Acid, atharanthine, Serpentine, Lochnericine, Vinblastine and Vincristine (Vernanda et al., 2016; Cureno et al., 2020; Rani et al., 2021; Fernanda et al, 2023). Similarly, the species *R. serpentina* (local name: pule pandak) contains ajmaline (in leaf, root and stem), serpentine (in leaf and stem), 18-hydroxyyohimbine (in leaf, root and stem) (Kumar et al., 2023). *R. serpentine* traditionally utilized by local to cure various diseases as an anti-cancer, anti-diuretics, anti-hipertention, anti-oxydant as well as anti-inflammation (Malviya et al., 2016; Astuti, 2020). Identifying plants that are closely related to *C. roseus* is very important, considering that important compounds in this plant have very high economic value but are produced at very small levels. Meanwhile, *R. serpentine* are abundance and easy to cultivate that potentially developed. However, clustering data using DNA supported the existing data build up using morphology characters. For Apocynaceae family, the two DNA sequences used consistently grouped the members of the species within it. The difference in clustering sequences in the two trees correlated with the use of different genetic sequences.

4. Conclusion

The results of phylogenetic tree reconstruction based on *matK* and *rbcL* sequences, show that *Rauvolfia serpentina* is closely related to *C. roseus*. In addition, the data sinergically support the morphological dan biochemical data. Both plants also provide localities with advantageous metabolites for medicine. These findings can be used as an initial foundation to make *Rauvolfia serpertina* as an alternative new medicinal plant for cancer treatment.

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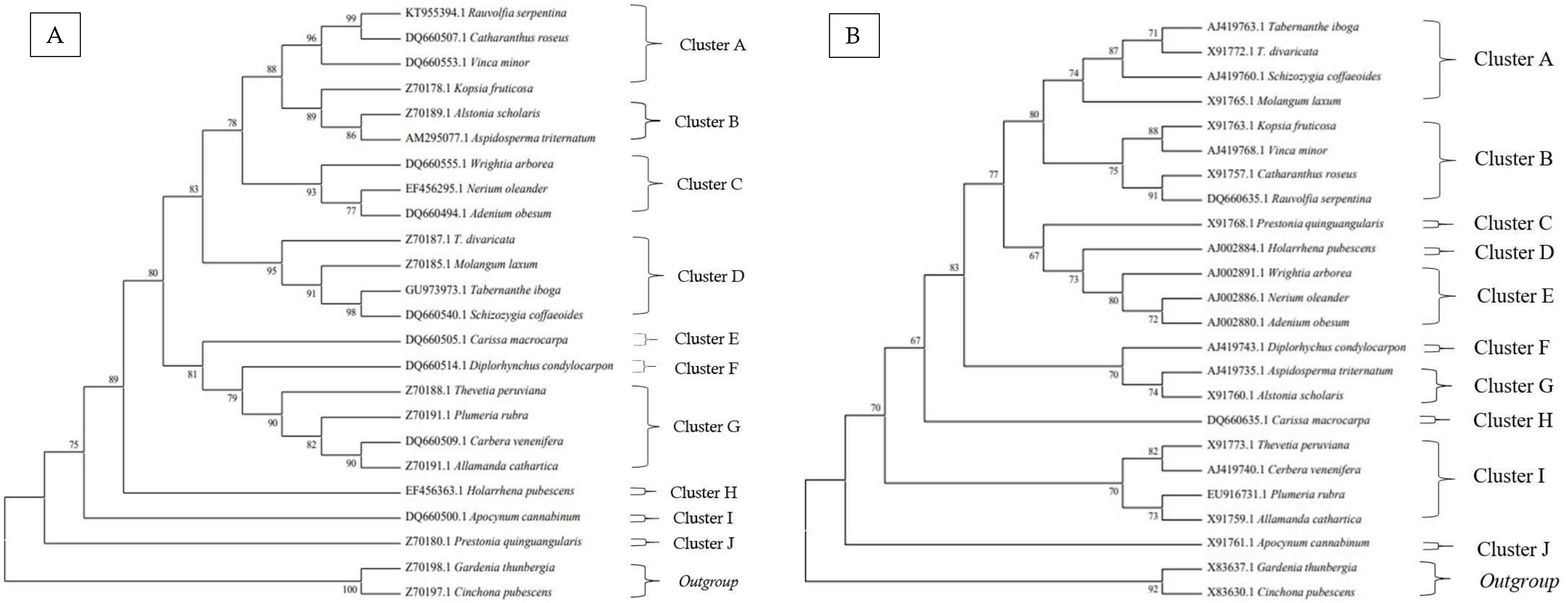

Figure 1. Phlogenetic tree of family Apocynaceae based on A) *matK* and B) *rbcL* sequences.
Bootstrap values are shown each branch; Apocynaceae clusters into 10 groups regarding suprageneric