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DNA identification of kayu kuning (yellow-fruited moonseed) from East Kalimantan, Indonesia

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ABSTRACT

Kalimantan is an island with an abundance of *kayu Kuning* (yellow-fruited moonseed). *Kayu kuning* is used for three different plant species, namely *Arcangelisia flava* (L.) Merr., *Fibraurea tinctoria* Lour., and *Coscinium fenestratum* (Gaertn.) Colebr. Therefore, it creates confusion and may cause improper use. It has properties such as anti-diabetic, antiplasmodial, antidiarrheal, hepatitis, and antidote. The study uses the DNA barcode technique to identify *kayu kuning* (yellow-fruited moonseed) from East Kalimantan, Indonesia. The genomic DNA of *kayu kuning* (yellow-fruited moonseed) was extracted, and *ITS* primers were used for identification using polymerase chain reaction (PCR). It was compared with *Arcangelisia flava*, *Fibraurea tinctoria*, and *Coscinium fenestratum* as a phylogenetic tree. DNA sequence alignment of ITS and phylogenetic reconstruction revealed that *kayu kuning* (yellow-fruited moonseed) from East Kalimantan was closely related to *A. flava*. The *kayu kuning* (yellow-fruited moonseed) had 94.16% of sequence similarity with *A. flava* according to the *ITS1* barcode.

Keywords: *Arcangelisia flava*, ITS, *yellow-fruited moonseed*, phylogenetic

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INTRODUCTION

Medicinal herbs have been widely used in Indonesia for treating various diseases and boosting the immune system since the ancestral period. Therefore, medicinal herbs are required as novel drug candidates. Moreover, medicinal herbs have an important role in further studies and application (Katiyar et al., 2012); for example, quinine, quinidine, cinchonine, and cinchonidine in cinchona as antimalarial and anticancer drug and vinblastine and vincristine in *Vinca rosea* Linn. as an anticancer drug (Huspa, 2009).

Kalimantan is an island with an abundance of yellow-fruited moonseed**.** It is the local name of three different plant species, such as *Arcangelisia flava* (L.) Merr., *Fibraurea tinctoria* Lour., and *Coscinium fenestratum* (Gaertn.) Colebr (Noorcahyati, 2017; Suzuki et al., 2011). In Indonesia, all three species are known as *kayu kuning* (Noorcahyati, 2017). It has antimalarial effects by inhibiting *Plasmodium falciparum* growth (Kresno, 2010). Furthermore, the adverse effect is also detected in the liver through the interference of cytochrome P3A4 by chloroform-soluble fraction extract, especially from *F. tinctoria* (Su et al., 2007). It has an anti-inflammatory effect (Su et al., 2007). In addition, the methanol extract of *C. fenestratum* exhibits a hepatoprotective effect against induced carbon tetrachloride (Venukumar & Latha, 2004).

However, these three species are known to contain berberine, which has a fairly dangerous toxicity value, considering that 50 mg/kg berberine sulfate causes diarrhea in 40% of rats and directly impacts the digestive tract. In cats, berberine 100 mg/kg (oral) causes vomiting within 6-8 hours and, at the same time, administration for 8-10 days, causes the death of all test animals with hemorrhagic inflammation of the small and large intestines (Sulistiarini et al., 2020). The berberine values of these three species are known to be different. Namely, in *Arcangelisia flava*, it is known that the berberine content is 0.0004% (Sulistiarini et al., 2020), in *Fibraurea tinctoria* it is 25.8% (Utami et al., 2017). In *Coscinium fenestratum* it is 1, 7 – 2.8% (Rojsanga and Gritsanapan, 2005). Therefore, it is very important to know the type of yellow wood referred to by the people of Kalimantan, which has medicinal properties so that it is known the effective dose that can cure without toxic effects by carrying out the identification process by using the basic components of plant DNA compared with *Arcangelisia flava* (L.) Merr., *Fibraurea tinctoria* Lour., and *Coscinium fenestratum* (Gaertn.) DNA.

Arcangelisia flava, *Fibraurea tinctoria*, and *Coscinium fenestratum* have similar morphologies (Noorcahyati, 2017), such as ovoid-leaf similar to *Piper betel* (Soemardji 2019), stem diameter of 5–10 cm, liana in trees and climb up until 40 m in height (Ali et al., 2010; Setyowati et al., 2014; Soemardji, 2009). Sharing the same familiar names and morphologies leads to confusion because different species may exhibit different pharmacological effects. Therefore, plant identification with a molecular marker is essential to avoid misidentification and misapplication.

DNA barcode is a highly standardized method that uses one or more short DNA sequences for molecular identification. The ideal barcode locus should have more interspecific variance than intraspecific variation. The internal transcribed spacer (ITS) is a highly polymorphic non-coding region with an adequate taxonomic unit that can discriminate an organism into species level. The ITS loci properties that are said to be helpful for phylogenetic reconstruction purposes are biparental inheritance, universality, intergenomic heterogeneity, simplicity, uniformity of intragenomic, and low functional constraints (Álvarez & Wendel, 2003). ITS-based barcode has been widely used to identify some plant taxa, such as *Durio* spp. and *Lansium* spp. (Sinurat & Siregar, 2016), and Piperaceae (Nagaraj et al., 2019).

The identification of yellow-fruited moonseed from East Kalimantan, Indonesia, was conducted using ITS. In biodiversity and conservation biology research, DNA sequence-based species identification is a method that is considered easy, reliable, and consistent, so it is essential. DNA sequence recognition was performed using molecular markers. The DNA bar code (DNA barcoding), a series of short DNA sequences that can display genetic variation within a population, is one of the molecular markers currently used in expressing taxonomy. Certain genes can be used as markers in the

genetic division of species and phylogenetic reconstruction during the DNA Barcoding process (Irawan et al., 2016).

The study aimed to identify *kayu kuning* (yellow-fruited moonseed) from East Kalimantan, Indonesia, by using a DNA barcode. In this present study, we identified *kayu kuning* (yellow-fruited moonseed) by comparing it with the sequences of *A. flava*, *F. tinctoria*, and *C. fenestratum*.

MATERIALS AND METHOD

Samples for DNA extraction

DNA was extracted from the stem of *kayu kuning* (yellow-fruited moonseed) in East Kalimantan. The stem of *kayu kuning* was obtained from *kayu kuning*'s collectors at the Citra Niaga Market in Samarinda City, East Kalimantan, whereas *A. flava*, *F. tinctoria*, and *C. fenestratum* were obtained from Samboja Conservation and Natural Resources, East Kalimantan (Figure 1A-B).

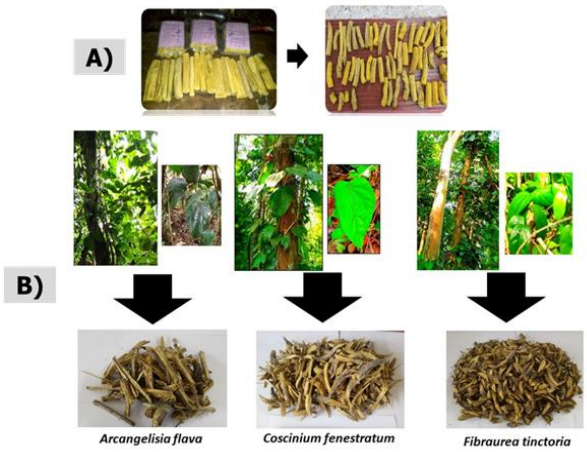

Figure 1. Plant materials used in the study. (A) The stem of *kayu kuning* was obtained from *kayu kuning's* collectors at the Citra Niaga Market in Samarinda City, East Kalimantan. (B) *A. flava, F. tinctoria,* and *C. fenestratum* (Sulistiarini et al., 2020)

Procedure

The genomic DNA extraction was performed according to (Aritonang et al., 2007) using Cetyltrimethylammonium Bromide (CTAB) buffer extraction. The 20–200 mg of fine wood powder was extracted using 200 mL CTAB, 40 µL of 26% PCP, and 10 µL of mercaptoethanol. The genomic DNA was used as a PCR template. The PCR reaction was conducted with 10 µL of DNA templates, 25 µL of Tax Master Mix (Vivantis), 2 µL of 1 mM reverse primer, 9 µL of 1 mM forward primer, 1.5 µL of nuclease-free water, and 2.5 µL of $MgCl_2$.

A pair of universal primers (ITS) was used in this study to obtain the target sequence. The primer pairs of ITS were 5'-ATGCGATACTTGGTGTGAAT-3' (forward) and 5'-GACGCTTCTCCAGACTACAAT-3' (reverse). The PCR setup was pre-denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a cycle of post-extension at 72 °C for 10 min (Abdullah et al., 2016; Lorenz, 2012). The PCR product was visualized in 2% agarose gel, observed uFsing UV-Transilluminator TFX-20.LM, and documented using a digital camera. The PCR products were sequenced using ABI® PRISM BigDye Terminator Cycle Sequencing Kit v3.1 with Sanger's method to the 1st BASE, Malaysia.

Data Analysis

The obtained sequences were aligned using Clustal Omega and analyzed using the BLAST program (https://blast.ncbi.nlm.nih.gov). Clustal Omega was used to obtain phylogenetic trees and the relationship of analyzed plant species (Dharmayanti, 2011; Thompson et al., 1994).

RESULT AND DISCUSSION

The electrophoresis results of ITS showed good and thick bands in all four tested samples (Figure 2). Based on the electropherogram, ITS bands in the range 200-340 bp showed clearly. The band size for *Fibraurea tinctoria*, *kayu kuning*, and *Arcangelisia flava* showed similar in ~300 bp. While the band size for *Coscinium fenestrate* showed longer than the three others, around 300-400 bp.

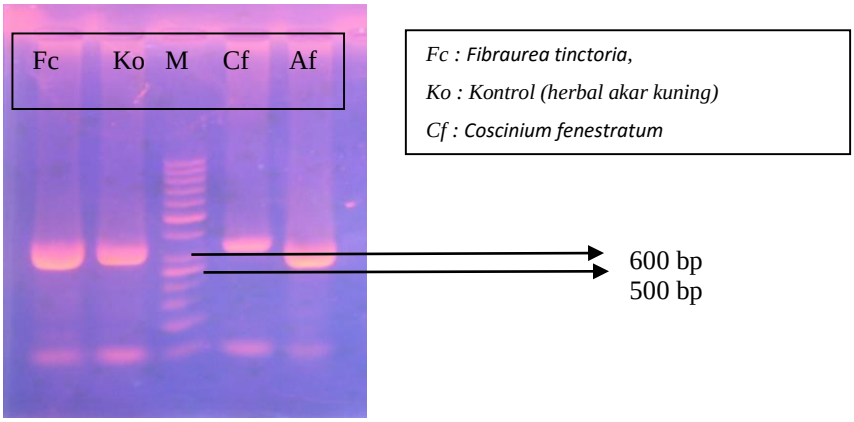

Figure 2. PCR products of *kayu kuning* (yellow-fruited moonseed) samples amplified using ITS primer (Fc, *Fibraurea tinctoria*; Ko, *kayu kuning* (yellow-fruited moonseed) herb from Samarinda, East Kalimantan; Cf, *Coscinium fenestratum*; Af, *Arcangelisia flava*; M, Marker

These four sequencing results have been submitted to the GenBank NCBI database with ID #2391101, #2391103, #2391104, and #2391105. The sequencing results were analyzed to evaluate the similarity of the base position at a certain size. Up to the 120th base, the sequence showed the same order among four sequences. The results also showed that up to the 275th base sequence was similar between yellow-fruited moonseed and *A. flava*. Then, the base of the 289th order showed a similar composition between *F. tinctoria* and *C. fenestratum* (Figure 3).

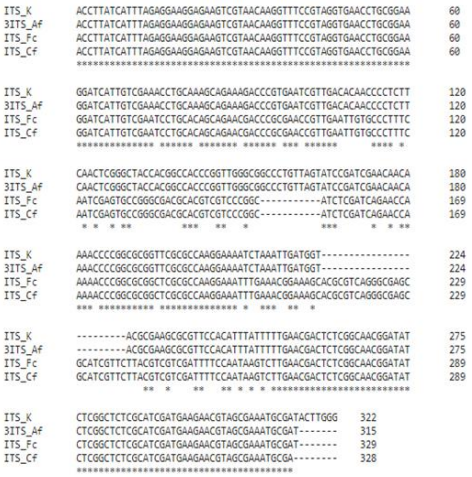

Figure 3. Sequence alignment (3ITS_Af, Arcangelisia flava; ITS_K, yellow-fruited moonseed herb; ITS_Fc, *Fibraurea tinctoria*; ITS_Cf, *Coscinium fenestratum*)

Based on the alignment shown until base 120 bp showed similar of four sequences of tested samples. However, after this base order, yellow-fruited moonseed herb (ITS_K), *Arcangelisia flava* (ITS_Af), then *Fibraurea tinctoria*, and *Coscinium fenestrate* showed similarly. This alignment also showed varied sequences length in yellow-fruited moonseed herb, *Arcangelisia flava*, *Fibraurea tinctoria*, and *Coscinium fesenstratum* of 322 bp, 315 bp, 329 bp, and 328 bp, respectively.

The matching sequences were conducted according to the database after identifying the ITS sequence composition from *kayu kuning* (yellow-fruited moonseed). The analysis result was compared between the sample sequence and the sequence in the NCBI database using MEGA-Blast. The National Center for Biotechnology Information (NCBI) provides an extensive suite of online resources for biological information and data, including the GenBank® nucleic acid sequence database and the PubMed database of citations and abstracts for published life science journals.

The matching results were selected based on the primer type and the largest percentage identity. The description result showed that the *kayu kuning* (yellow-fruited moonseed) from East Kalimantan was similar to *A. flava* with a percentage identity of up to 94.16% (Table 1).

Table 1. Description Sequences alignments of *kayu kuning* with BLASTN GenBank NCBI

Sample code	Description	Species	Percentage identity (%)*	Accession
ITS1_K	*Arcangelisia flava* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	*Arcangelisia flava*	94.16	FJ603109.1
3ITS_Af	*Arcangelisia flava* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	*Arcangelisia flava*	94.16	FJ603109.1
ITS_Fc	*Fibraurea tinctoria* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	*Fibraurea tinctoria*	95.53	FJ603110.1
ITS_Cf	*Coscinium fenestratum* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	*Coscinium fenestratum*	95.52	J5PMPHF5013

Phylogenetic reconstruction was supported by Clustal Omega analysis. A phylogeny tree is a tool used on earth to assess the kinship of living beings. A phylogeny tree is an arrangement in which species are organized based on evolutionary kinship in the form of branches linking them (Lubis, 2014). Yellow-fruited moonseed was closely related to *A. flava* with 0 genetic distance (Figure 4). The smaller genetic distance value indicates a close relationship between two species or more (Tallei & Kolondam, 2015). Based on Figure 4, *Coscinium fenestratum* (DF) and *Fibraurea tinctoria* assumed have closely related, even not identically similar.

Figure 4. Phylogenetic tree of yellow-fruited moonseed herbs (ITS_Af, *Arcangelisia flava*; ITS_K, yellow-fruited moonseed herb; ITS_Fc, *Fibraurea tinctoria*; ITS_Cf, *Coscinium fenestratum*)

In the results of this determination, in addition to the yellow wood herbs, which were identified to be similar to *Arcangelisia flava* from the alignment results, the description of NCBI and phylogenetic trees, two other types of yellow wood were identified to be similar to one another, namely *Fibraurea tinctoria* which was identified to be similar to *Coscinium fenestratum*. This is probably because there is still no complete base sequence data for these two types of plants due to the lack of tropical plant data in the NCBI GenBank. Because when viewed from the phylogenetic tree, these two plants are different.

CONCLUSION

Based on the results of molecular genetic analysis and the phylogenetic tree of the yellow wood, the yellow wood species used by the Kalimantan community has the most significant similarity to the Arcengelisia flava species.

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