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The role of *Annona* spp philogeni tree DNA in the development of natural material drugs

Hamidah[1]*, Thin Soedarti[1] and Mulyadi Tanjung[2]

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia
[2]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia

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ABSTRACT

Annona is a plant that is widely found in the tropical country, like Indonesia, with many advantages that can be extracted such as its chemical potential. This study aims to determine (1) the relationship between *Annona muricata, Annona squamosa*, and *Annona reticulata* through a molecular engineering approach. (2) the role of *Annon*a spp DNA philogeni tree in the Development of Natural Material Medicine. This research is an obsevational study by isolating the DNA of the leaves of *Annona muricata, Annona squamosa* and *Annona reticulata* and then made a philogeni tree and the results of the dendrogram are translated. Based on the development of biosystematics and bioinformatics science can determine to find natural medicinal ingredients through the kinship of DNA of a plant in a way that has a close kinship. Plants that have close kinship in phytochemicals contain the same bioactive and functional content.

Key words : *Annona sp, DNA, Philogeni DNA tree, Phytochemicals*

Introduction

Annonaceae, the custard apple, or annona, family, the largest family of the magnolia order (Magnoliales) with 129 genera and about 2,120 species. The family consists of trees, shrubs, and wood climbers found mainly in the tropics, although few species extend into temperate regions. *Annona* is systematically included in the divisions of Magnoliophyta, Angiosperma subdivisions, Magnoliopsida class, Magnoliidae sub-class, Order Magnoliales.

The *Annona* clan consists of 120 well-known species including *Annona muricata* (sour soup), *A. squamosa* (sour sweet), *A. reticulata*, and *A. cherimola*. Among these *Annona, A. muricata* and *A. squamosa* are currently more common when compared with *Annona reticulata*. because a lot is cultivated to be used by the fruit. If it does not get special attention, it is not impossible to become extinct (Simpson, 2006). The three plants are one clan and have close kinship. Furthermore, the indigenous people of Indonesia have relied on medicinal plants for their health needed through generations (Wahyuni *et al.,* 2017).

Determination of kinship with DNA (phenetic) is qualitatively determined by comparing the similarities and differences in characteristics possessed by each taxon: using a number of character equations (morphology, anatomy, DNA, embryology, palinology, cytology, chemistry, reproductive biology, ecology and physiology). Generally, closely related plants have similar anatomy, morphology and physiological processes. Morphological charac-

terization is the most accurate way of determining to assess agronomic traits and plant taxonomic classification (Sudarsono *et al.*, 2012). Kinship relationships between types of plants can be analyzed to determine the extent of dissimilarity by calculating the correlation coefficient, similarity index, taxonomic distance, and can also use group analysis. The Research problem is How the relationship between *Annona muricata, Annona squamosa*, and *Annona reticulata* through a molecular engineering approach? and whatthe role of *Annon*a spp DNA philogeni tree in the Development of Natural Material Medicine?

Methodology

Leave samples of *A. muricata, A. squamosa* and *A. reticulata*, this research were taken from Surabaya. The leaves used for DNA isolation are derived from the leaves which are located on the ends of the stem branches. The number of samples used for each species is one.

The chemicals used in this study consisted of hexadecyl tri methyl ammonium bromide (CTAB), CIA (24: 1) cation buffer, chloroform: isoamil alcohol (PCI) (25: 24: 1), polyvynilpyrollidon (PVP), ammonium acetate, absolute ethanol, 95% ethanol, 70% ethanol, tris buffer - ethylene diamine tetra acetic acid (TE) pH 8, β-mercaptoethanol, 0.8% agarose gel (for genomic DNA) and 1.5% (for amplicons), tris - boric acid ethylene diamine tetra acetic acid (TBE) 1X, ethidium bromide, loading dye, 10 pmol / uL primer (OPP 12, OPP 4, OPO 15, OPO 18, OPT 11,Q 12, and C 11) first base brand, double distilled water (ddH2O), PCR Mix of Promega brand Go Green, and 10 mg/mL of bovine serum albumin (BSA).

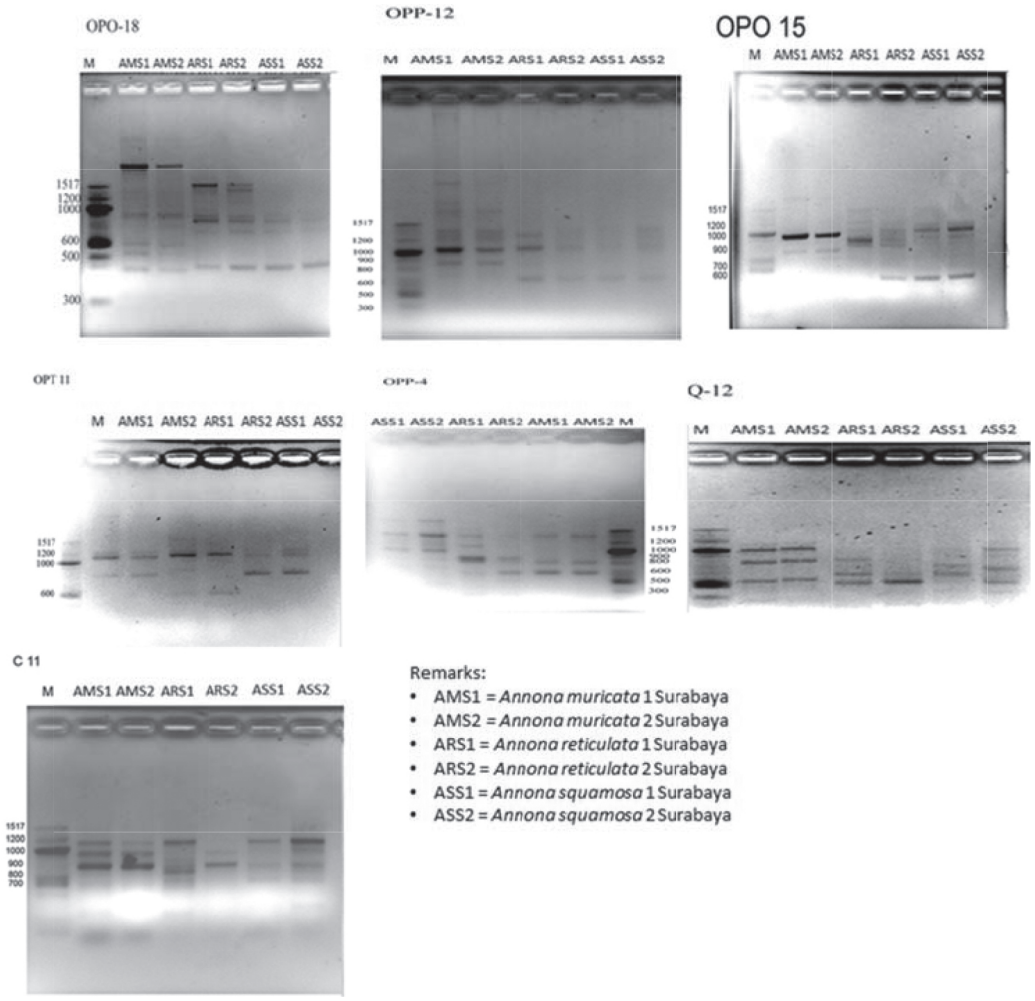

Fig. 1. Results of sample DNA amplification

Procedure

DNA Isolation

First, DNA was isolated. The ratio of purity and concentration of obtained DNA isolate was measured. The purity and concentration of DNA was determined qualitatively and quantitatively. Quantitative measurements were determined by reading spectrophotometers at wavelengths λ 230, λ 260, and λ 280. Qualitative testing was carried out by electrophoresis using agarose gel concentration of 0.8%. Electrophoresis was done by 100 volts charging for 20 minutes.

DNA Amplification

DNA amplification was carried out by random amplified polymorphic DNA (RAPD) PCR method. The PCR results then analyzed by electrophoresis (MUPID), using 1.5% agarose gel. Electrophoresis results are then documented using gel documentation (BioRad).

Amplification Analysis

Visualized DNA bands are translated into numerical (binary) data based on the appearance of the ribbon. The band that appears is translated into one (1) and the band that does not appear is translated to zero (0). Numerical data is then entered into the Multi-Variate Statistical Principle (MVSP) version 3.22 for cluster analysis using clustering methods, namely Unweighted Pair Group Method with Arithmetic Mean (UPGMA), simple matching.

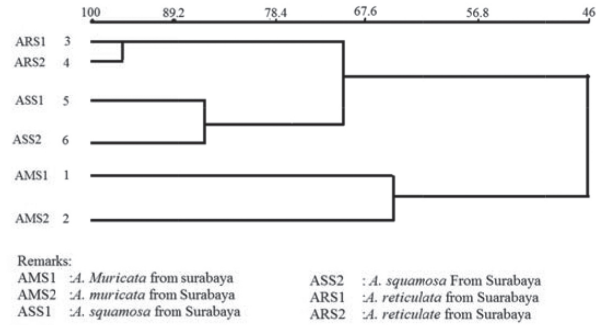

Fig. 2. Dendrogram between *A. muricata, A. squamosa, A. reticulata* and outgroup

Results and Discussion

Resulted DNA banding pattern was used as parameter for the *Annona spp.* grouping into the dendrogram. The DNA bands were then scaled by observing DNA bands appearance, as listed in Table 1.

The high similarity of *Annona reticulata* and *Annona squamosa* in molecular level was also obeserved in morphological level, such as the similarity of their fruit and flowers, but not their habitus.

Fig. 3. Habitus *Annona squamosa* (A), Habitus *Annona reticulata* (B), Habitus *Annona muricata* (C)

The criteria to choose DNA fragment to do scoring are reproducibility, thickness and size. The characters showed that *M. umbellate* was more primitive than *M. peltata* and *M. mammosa* (Kundu *et al.*, 2011). It is supported by Hamidah (2009), that species of *A. muricata* is more primitive because it has many characters than *A. squamosa* and *A. reticulata*.

Table 1. Number of DNA array patterns from each RAPD primer used from samples of *Annona muricata*, Annona *squamosa*, and *Annona reticulata* species

Primer	Order 5′ 3′	Total Patten Running	Poli Morfik	%	Mono Morfik	%
OPO18	AGG TGA CCGT	10	8	80	2	20
Q 12	AGT AGG GCA C	6	6	100	0	0
OPP 12	AAG GGC GAG T	8	6	75	2	25
OPO15	TGG GGT CCT T	8	8	100	0	0
OPP 4	GTG TCT CAG G	7	6	85.7	1	14.3
OPT 11	TTC CCC GCG A	5	2	40	3	60
C 11	AAT GCT GCG G	5	1	20	4	80
TOTAL		49	37	76	12	24

Conclusion

Annona squamosa and *Annona reticulata* have a closer phenetic relationship so that they can grouping into one large group while *Annona muricata* separates far from the other *Annona* groups. All three species can be used as a source of medicinal ingredients because they are closely related

Acknowledgements

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References

Hamidah, 2009. Biosystematics *Annona muricata* L., *Annona squamosa* L., and *Annona reticulata* L. with numerical approach, *Dissertation*, Gadjah Mada University, Yogyakarta, Indonesia.

Kundu, A., Sarkar, D., Bhattacharjee, A., Topdar, N., Sinha, M.K. and Mahapatra, B.S.A. 2011. Simple ethanol wash of the tissue homogenates recovers high-quality genomic DNA from *Corchorus* species characterized by highly acidic and proteinaceous mucilages. *Electron J Biotechnol*. 14 1-7.

Simpson, Michael, G. 2006. *Plant Systematics*. Elsevier, London.

Sudarsono, Soenarsih, Djoefri and Wahyu, 2012. Keragaman Spesies Pala (*Myristica* spp.) Maluku Utara Berdasarkan Penanda Morfologi dan Agronomi. *Jurnal Litri*. 18 (1): 1-9.

Wahyuni, D.K., Ansori, A. N.M. and Vidiyanti, F. 2017. GC-MS analysis of phytocomponents in methanolic extracts of leaf-derived callus of *Justicia gendarussa* Burm.f., *Bioscience Research*. 14 : 668-677.