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# Proceedings of

## *Humboldt Kolleg :*
## SYNERGY, NETWORKING AND THE ROLE OF FUNDAMENTAL RESEARCH DEVELOPMENT IN SOUTH EAST ASIA

## *in conjunction with :*
## THE INTERNATIONAL CONFERENCE ON NATURAL SCIENCES (ICONS) 2011

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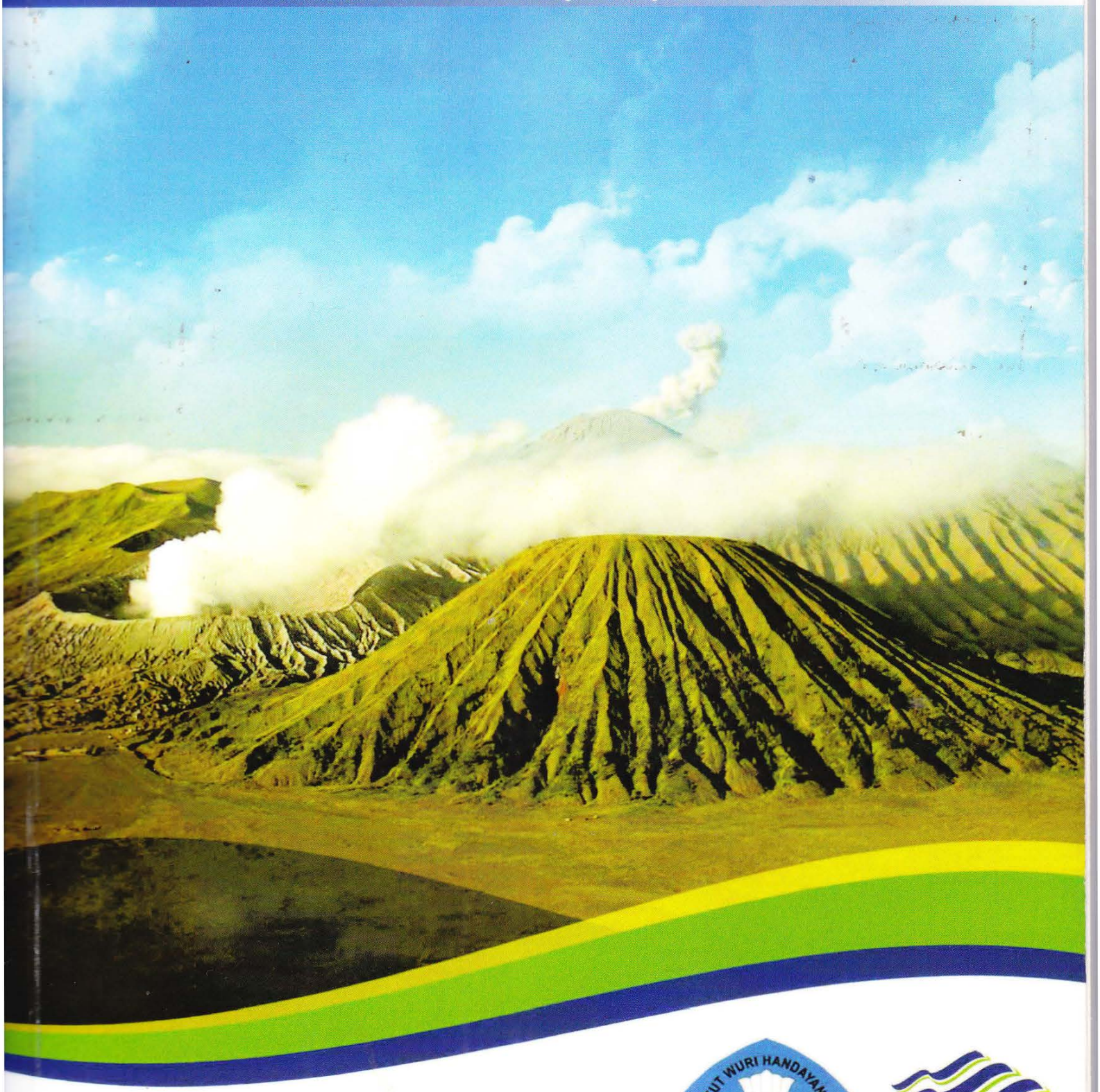

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# Proceedings of the International Conference on Natural Sciences (ICONS) 2011

09.–11. July 2011, Batu, East Java, Indonesia

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# FOREWORD

The First International Conference on Natural Sciences, July 9-11, 2011 in Batu, East Java, Indonesia, brought together scientists from nine countries from South-East Asia, Germany and Japan. South-East Asia is extremely rich in natural resources, many of them still untapped, but has also extremely densely populated areas that have to cope with the ensuing problems including infrastructure measures, intensive agri- and aquaculture, waste management, and nature preservation. Study, use and development of existing resources and coping with the aforementioned problems, requires interdisciplinary cooperation. Based on a network of Alexander von Humboldt alumni, the conference aimed at linking the wide professional expertise, at making the best use of existing equipment and pinpointing gaps, and at integrating basic and applied research.

This book is a mosaic of the impressive oral and poster presentations of the conference. It reflects the scientific diversity, existing contacts, and areas of promising new joint ventures. Editing such a wide scope of subjects was fascinating and challenging, allowing at the same time to reflect the many discussions during the meeting that encompassed a world of science. We trust that the book may serve a similar function among the participants, as well as for a wider scope of readers.

Thanks to all who contributed: Irfan Tri Raharjo as coordinator, our co-editors helping to review the submissions, the Alexander von Humboldt Foundation who gave financial and logistic support, and, last but not least, the Rector, Leenawaty Limantara, and the staff of Ma Chung University who had already organized the meeting so well and now relieved us of many formal and administrative tasks involved in making the book.

May this seed grow and bear rich fruit!

Malang, 15 March 2012

**Hugo Scheer**
*Editor-in-Chief*
*Ludgwig Maximilians University, Germany*

# VORWORT

Die "First International Conference on Natural Sciences" brachte vom 9.-11. Juli 2011 Wissenschaftler aus neun Ländern Südostasiens, Deutschland und Japan nach Batu in Ost-Java, Indonesien. Südostasien ist außergewöhnlich reich an natürlichen Ressourcen, von denen noch viele unangetastet sind. Es hat gleichzeitig außerordentlich dicht bevölkerte Gebiete, in denen die daraus erwachsenden Probleme der Infrastruktur, der intensiven Landwirtschaft und Aquakultur, und des Naturschutzes gelöst werden müssen. Exploration, Nutzung und Entwicklung der vielfältigen Ressourcen und die Lösung der häufig konfliktträchtigen Probleme ist nur durch interdisziplinäre Kooperationen möglich. Es war das Ziel dieser Konferenz, auf der Basis eines Netzwerks "Südostasien" der Alexander von Humboldt Alumni einschlägige Erfahrungen zu bündeln, vorhandene technische Ausrüstung Labor-übergreifend zu nutzen, Lücken zu definieren, und Wissenschaftler aus der Grundlagenforschung und aus technologischen Anwendungen zusammenzuführen.

Das vorliegende Buch gibt, als ein Mosaik, die thematisch breit gefächerten und beeindruckenden Vorträge und Poster wieder, die auf der Konferenz vorgestellt wurden. Es zeigt die Vielfalt der Forschung, bereits bestehende Kontakte, und Möglichkeiten zu neuen Kooperationen. Die Herausgabe eines solch weiten Spektrums von Arbeiten war zugleich beeindruckend und fordernd, es gab uns gleichzeitig die Gelegenheit, noch einmal die vielen Diskussionen zu erinnern, die eine Welt der Wissenschaft umfassten. Wir hoffen, dass dieses Buch bei allen Teilnehmern diese Funktion erfüllen wird, und die Themen und Teilnehmer einem weiteren Kreis von Lesern nahebringt.

Wir danken allen Beteiligten: Irfan Tri Raharjo für die Koordination des Buches, den Mitherausgebern für die kritische Durchsicht und Kommentierung der Manuskripte, der Alexander von Humboldt Stiftung für finanzielle und logistische Unterstützung. Nicht zuletzt danken wir der Rektorin der Ma Chung Universität, Leenawaty Limantara, und ihren Mitarbeitern; nach der ausgezeichneten Organisation der Konferenz haben sie uns durch Entlastung von vielen formalen und administrativen Aufgaben auch die Herausgabe dieses Buches leicht gemacht.

Möge dieses Samenkorn gut anwachsen und reiche Ernte bringen!

Malang, 15. März 2012

**Hugo Scheer**
*der Chefredakteur*
*Ludgwig-Maximilians-Universität, Deutschland*

# *Messages*

# MESSAGE FROM THE SECRETARY GENERAL OF THE ALEXANDER VON HUMBOLDT FOUNDATION

***Contribution of the Humboldt Foundation to the "Proceedings" on the occasion of the Humboldt Kolleg in Indonesia in 2011***

Knowledge creates development – this is not only the case in developing and emerging countries, but in industrialized countries, too. The major issues in areas like resource conservation, global warming, sustainable energy supplies and healthcare, as well as access to water can only be tackled jointly, which means across both borders and disciplines. The key to this are highly-qualified academics in the natural sciences and engineering as well as in the humanities and social sciences. By selecting and promoting the best researchers and creating and developing self-supporting networks the Alexander von Humboldt Foundation has set itself the task of making a significant contribution to development and thus to the improvement of living conditions. It does not base its selection on country, subject, religious belief or gender, but purely on academic eligibility. The Foundation sponsors individuals involved in both applied and basic research. The best chance of successfully addressing the problem areas described above lies in precisely this complementarity.

Good research results alone are not enough. What is crucial is whether they have been achieved in different cultural contexts, are introduced into different social contexts and have a long-term impact when they are implemented. Mutual trust is required if this is to be achieved. For this reason, the Alexander von Humboldt Foundation places great emphasis on engendering trust: the academics it sponsors and their families are embedded in a culture of mentoring and counseling not only during their stay in Germany but also after their return to their own countries. The foundation provides platforms for cross-disciplinary, cross-border networking; it offers its alumni a whole range of sponsoring opportunities that allow them to continue the research projects they have started in Germany in their own countries.

It is important to keep upgrading the portfolio of programmes. To this end, the Alexander von Humboldt Foundation regularly organizes round-table discussions with fellows, alumni and their hosts in order to tailor their programmes to genuine needs. The International Climate Protection Fellowships, which were introduced recently, are one example of this process. They seek to address the global challenge of climate change in the context of cross-border, international cooperation. Up to 20 of these fellowships are available every year for potential leaders from non-European emerging and developing countries in the field of climate protection and resource conservation.

The first Humboldt Kolleg in Indonesia under the heading "Synergy, Networking and the Role of Fundamental Research Development in ASEA", together with the International Conference on Natural Sciences (ICONS 2011) and the research results that were presented and discussed there, constitute an important response to surmounting cross-border challenges.

**Dr. Klaus Manderla**
*Head of Division for Asia*
*Alexander von Humboldt Foundation*

# MESSAGE FROM THE CHAIRWOMAN OF HUMBOLDT KOLLEG IN CONJUCTION WITH ICONS 2011

***Welcome to Humboldt Kolleg Synergy, Networking and the Role of Fundamental Research Development in South-East Asia in conjunction with the International Conference on Natural Sciences 2011***

The year 2011 is a monumental year for the Humboldt Fellow Indonesia for the success of the first Humboldt Kolleg in Indonesia which was held in conjunction with the International Conference on Natural Sciences 2011. Owing to the excellent cooperation between Humboldt Club Indonesia under the leadership of Dr. L. T. Handoko and Ma Chung University, the program was successfully and officially opened on 8$^{th}$ July, 2011 (for Humboldt fellows) and on 9$^{th}$ July, 2011 (for public). The program intended for Humboldt fellows, academics, and scientists from South-East Asia, was created in the form of a plenary lecture, invited lectures, oral presentations, a poster session, and an excursion to Mount Bromo in East-Java. The program consisted of four themes: (1) the role of natural sciences in conserving natural resources, (2) the role of natural sciences in overcoming global warming, (3) the role of natural sciences in developing science and technology, and (4) the role of natural sciences in improving human welfare. Through the three-day activities, three outcomes could be secured: (1) two conference proceedings to be published by Shaker-Verlag, Germany, and an Indonesian publisher; (2) a book entitled *Humboldtians in South-East Asia: Research Interests and Future Prospects,* and (3) the declaration of Malang Humboldt Resolution. Humboldt Kolleg I in Indonesia was attended by 23 Humboldt fellows from the 55 Humboldt Fellows invited, and 129 researchers and academics representing Germany, Japan, Indonesia, Singapore, the Philippines, Malaysia, Korea, and Vietnam.

The proceedings book was created to compile all the International Conference on Natural Sciences (ICONS) activities held as a single unit of activities of the first Humboldt Kolleg in Indonesia. In practice, several writers withdrew their articles because their articles were successfully published in national and international journals so that they are not mentioned in the proceedings.

We sincerely hope that this book can be used as a scientific reference for many scholars.

Malang, 15 March 2012

**Leenawaty Limantara**

*Chairperson*
*Humboldt Kolleg in conjunction with ICONS 2011*

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# DNA FINGERPRINTING ON ITS REGION OF *Sauropus androgynus*' DNA FROM EAST JAVA, BY RANDOM AMPLIFIED POLYMORPHIC DNA METHOD

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**ABSTRACT**

*The important effect of Sauropus androgynus / SA (katuk), as a lactagogum for increasing human breast-milk production in Indonesia, must face the reality that there are also many investigations revealing its side effect, associated with Bronchiolitis obliterans, in Taiwan and Japan.*

*This research had performed a genetic assessment, by the use of DNA fingerprinting with a RAPD method, for mapping the genetic pattern among SA accessions from East Java, Indonesia. The genetic map of SA accessions will become the supporting database on further research to ensure the safety of SA in Indonesia, as a lactagogum.*

*The DNA fingerprinting had been done by a Random Amplified Polymorphic DNA (RAPD) method which amplified the Internal Transcribed Spacer (ITS) region on DNA of SA, from different geographic locations at East Java, Indonesia. The amplification mixture, contained ITS region of DNA and RAPD primers (OPF-07, OPF-12, OPF-15), was cycled in a thermocycler. Amplification products were separated by agarose electrophoresis, visualized and imaged after staining with Ethidium bromide.*

*Statistical analysis using Cluster Analysis had shown high similarity (0.786 – 0.895) between SA samples. The result assumed that genetic material of SA accessions had not been influenced directly by different environmental conditions. Despite this result, genetic assessment by DNA fingerprinting, could distinguish SA accessions more clearly than morphological assessment.*

Key Words: *Sauropus androgynus, DNA fingerprinting, ITS region, RAPD*

## 1. INTRODUCTION

*Sauropus androgynus* (SA) (Indonesian name: katuk), also known as *Sauropus albicans* (Euphorbiaceae), is a small perennial shrub of 0.7-1.3 m in height and often found growing wild in many parts of Southeast Asia. The dark green leaves, 2-6 cm long and 1.5-3 cm wide, have various nutritive value and are commonly used for human consumption after cooking in Malaysia and Indonesia. They traditionally ingest boiled SA soup or stir fried dishes [1,2].

Indonesian people traditionally use this plant for increasing human breast milk production. There are many publications that show the lactagogum (agent for increasing breast milk production) effect of this plant. There are many products at the market, containing extract of the SA produced by pharmaceutical industry which are claimed to have function as a lactagogum [3,4].

Despite its important effect on the breastfeeding program, there are also many investigations that reveal the side effect of this plant in Taiwan and Japan. In these countries, people use this plant for reducing body weight. After a wide-spread, prolonged and unregulated use of this plant, a few patients have died and many have developed protracted chronic respiratory failure [5,6,7].

Herbal medicine materials cultivated in different locations might differ not only in therapeutic effectiveness, but also in side effects. Samples from the same localities are probably of the same strains, therefore, origin identification helps select the best strains of herbal medicine materials [8,9].

One of the most reliable methods for identification of herbal medicine materials is by analyzing the DNA. DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions or environmental factors. DNA analysis methods can be classified into three types, namely polymerase chain reaction (PCR)-based, hybridization-based and sequencing-based [9,10].

Techniques based on the PCR concept include random amplified polymorphic DNA (RAPD), which usually uses a 10 bp arbitrary primer at constant low annealing temperature (generally 34 -

37ºC). RAPD is a quick and easy method to screen a large number of loci for DNA polymorphisms in a single PCR. Polymorphic markers can be generated rapidly without sequence information [11,12].

This method had been used to identify herbal medicines' geographical origins, such as *Hordeum spontaneum* [13], *Glycine max* [14], *Pinellia ternata* [15], *Piper sp*. [16], *Strychnos ligustrina* [17] and *Costus speciosus* Koen ex. Retz. [18].

Yunita [19] had also performed RAPD method for identifying DNA polymorphisms among many cultivars of betelvine (*Piper betle* L.) from several locations at East Java, with different geographical conditions. The source of DNA template was not from the plant genome, but from the amplification result of nuclear DNA (DNA) by Internal Transcribed Spacer (ITS) primer. Phylogenetics of another *Piper sp.* (Piperaceae) had also been studied based on ITS regions of DNA [20].

ITS region of DNA is biparentally inheredited and it has proven to be a useful source of characters for phylogenetic studies in many Angiosperm families because its enormous number of DNA repeat unit arranged in tandem repeats in plant genome. The presence of highly conserved sequences flanking each of two spacers, can make this region easy to be amplified [21].

This research reports DNA fingerprinting of *Sauropus androgynus* (SA) with a RAPD method on ITS region of DNA, for mapping the genetic pattern among SA accessions from several locations at East Java, Indonesia. The genetic map of SA accessions will become the supporting database on further research to ensure the safety of SA in Indonesia, as a lactagogum.

## 2. MATERIAL AND METHODS

### *2.1 Sampling Method*

*Sauropus androgynus* were collected during June – July, 2010, from twelve locations at six areas with different geographic conditions (Surabaya, Trenggalek, Bojonegoro, Purwodadi-Purwosari, Batu). Almost all samples were obtained from individual gardens except sample from Batu was obtained from Balai Materia Medika. All the samples were authenticated by the Center of Information and Development of Traditional Medicine (PIPOT), Faculty of Pharmacy, University of Surabaya, East Java, Indonesia.

Leaves of *Sauropus androgynus* were collected, washed free of dirt, mopped dry and quickly stored at -80 ºC until used.

### *2.2 DNA Isolation*

Genomic DNA isolation, modified from [19]. Fresh leaves 1.0 g were ground and 500 µl of β-mercaptoethanol and 3500 µl of 2X CTAB solution (2% CTAB, 0.1 M Tris-HCl, 1.4 M NaCl) were added. The mixture was incubated at 55 ºC for 40 min with occasional inversion and then cooled at room temperature and further incubated at 55 ºC for 15 min. A 3500 µl of chloroform : isoamyl acetate (24:1 v/v) was added, incubated at room temperature for 30 min and then shaken for 10 min. After centrifugation at 4000 rpm for 30 min, 500 µl of the upper phase was subsequently mixed with 3500 µl phenol : chloroform : isoamyl acetate (25:24:1 v/v). 500 µl of the upper phase was mixed with 3500 µl of chloroform : isoamyl acetate (24:1 v/v) and then was mixed and centrifuged 4000 rpm for 30 min. Upper phase was mixed with 50 µl CTAB 10 % (65 ˚C) and 500 µl 65 ˚C ppt buffer (1% CTAB, 0.05 M Tris buffer pH 8, 0.01 M EDTA pH 8), then the DNA-CTAB complex was formed at room temperature for 15 min. The mixture was centrifuged at 13,000 g at 4 ºC for 15min and DNA pellet was mixed with 500 µl of 1 M NaCl-TE and incubated at 55 ºC until DNA dissolved. Isopropanol 500 µl was added in this mixture. After centrifugation 13000 g at 4 ºC for 15min, the DNA pellet was washed twice with 600 µl of 70 % ethanol and DNA was dissolved in 75 µl water free nuclease and kept at – 20 ºC until used.

### *2.3 Amplification of DNA fragment by PCR*

The isolated DNA, was then amplified with primer ITS Y-5 (5' TAGAGGAAG GAGAAGTCGTAACAA 3') and ITS Y-4 (5' CCCGCCTGACCTGGGGTCGC 3'). The DNA was pre-denatured at 95 ºC for 2 min, cycled 35 times at 95 ºC for 30 sec, 57 ºC for 1 min and 71 ºC for 2 min in a thermocycler. The final extension cycle allowed an additional incubation for 5 min at 71 ºC, as in [19]. The amplification reaction of the ITS region by RAPD primer, was modified from [22], while the mixture contained 12.5 µl GoTaq® Green Master Mix (Promega) which contain GoTaq® DNA polymerase supplied in 2X Green GoTaq® Reaction buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM $MgCl_2$.; 3.5 µl RAPD primer and 9 µl PCR result from PCR with ITS primers method in 25 µl reaction volume. The mixture was cycled 44 times at 94 ºC for

1 min, 35 ºC for 1.5 min and 72 ºC for 1.5 min in a thermocycler. The final extension cycle allowed an additional incubation for 5 min at 72 ºC.

### *2.4 Visualization of DNA pattern by Electrophoresis*

Amplification products were separated by electrophoresis through 1.5 % agarose gels in 0.5 X TBE buffer, visualized and imaged after staining with ethidium bromide.

### *2.5 Statistical Analysis*

Each amplification product (band) will be considered to be a RAPD marker. Number one will be attributed to band presence and zero to absence. The binary data set will be used to calculate the similarity index and to assemble the corresponding similarity matrix. The matrix obtained will be used to generate a dendogram. All the analysis will be performed with the aid of the *SPSS 11.5 for Windows* computer program.

## 3. RESULT AND DISCUSSION

### *3.1 Sampling*

*Sauropus androgynus* were collected from six areas with different geographic conditions at East Java: Surabaya, Trenggalek, Bojonegoro, Purwodadi, Purwosari and Batu, as summarized in Table I.

**Table I. Sampling Condition of *Sauropus androgynus* from East Java**

| LOCATION | COORDINATE | SAMPLE CODE | ALTITUDE*) (meter amsl) | TEMPERATURE (˚C) | RELATIVE HUMIDITY (%) |
|---|---|---|---|---|---|
| Surabaya | 7° 14' 57" South, 112° 45' 3" East | ST | 0 | 29.3-30.0 | 73-74 |
| | | SP | 0 | 25.7-33.7 | 47-74 |
| | | SB | 0 | 27.8-30.5 | 50-74 |
| Bojonegoro | 7° 9' 0" South, 111° 52' 0" East | BJ I | 50 | 24.8-30.0 | 52-66 |
| | | BJ II | 50 | 32.8-33.6 | 54-55 |
| | | BJ III | 80 | 32.1-35.4 | 48-57 |
| Trenggalek | 8° 2' 52" South, 111° 42' 31" East | T I | 120 | 24.1-33.3 | 31-82 |
| | | T II | 120 | 24.1-33.3 | 31-82 |
| Purwodadi | 7° 48' 7" South, 112° 44' 10" East | PWD | 320 | 29.1-33.5 | 64-73 |
| Purwosari | 7° 46' 13" South, 112° 44' 28" East | PWS I | 220 | 29.7-32.3 | 66-69 |
| | | PWS II | 240 | 29.2-33.5 | 64-73 |
| Batu | 7° 52' 12" South, 112° 31' 42" East | B I | 840 | 25.7-33.7 | 39- 74 |

*) relative altitude, compared to Surabaya

All plant samples were collected and authenticated as *Sauropus androgynus*, based on their morphological structures, as seen on figure 1.

This research only used young leaves of this plant, which were characterized by light green color, small size (length, ca. <3.5 cm; width, ca. <1.9 cm) and soft textures. Young leaves are preferentially chosen for DNA isolation, because young leaves contain fewer secondary metabolites than old leaves, that could interfere the DNA isolation.

All leaves were harvested in the morning, before 12.00 p.m, when the photosynthetic process was still happening. After harvesting, all leaves were transported into the laboratory, in the cold condition (-20 ˚C) for preserving their freshness.

Figure 1. *Sauropus androgynus* (Indonesian names: katuk)
[photographed by PIPOT research teams, at Batu, 17 June 2011]

### *3.2 DNA Isolation*

Genomic DNA from accessions of *Sauropus androgynus* was isolated using the method as in [19], for isolation the DNA of *Piper betle*. The modified method was performed in this research including the usage of phenol for lowering secondary metabolic contamination at DNA isolation process.

The DNA purity and concentration of isolated DNA from fresh leaves from many locations show many variations but proved amenable to PCR amplifications, with OD 260/ OD 280 ratio > 1.8 and range DNA concentration between 406 – 2731 ng/µl.

The isolated DNA of fresh leaves from Surabaya Timur (ST), was used for optimization the amplification process with ITS primers and screening for RAPD primers.

For amplification purpose, the amount of fresh leaves for DNA isolation is about 1.0 g because this amount of plant materials would give large yields without decrease their DNA purity. Large yield is very important in RAPD method because this method will need many sample DNAs.

### *3.3 PCR-RAPD on ITS region*

Our preliminary work had revealed that amplification the DNA of *Sauropus androgynus* with the RAPD primers directly often resulted the smear band (data not shown), but after amplification of the ITS region of *Sauropus androgynus*, we could get the distinct and clear bands on agarose gel after staining with ethidium bromide. Reference [19] assumed that the DNA genome is too long and complex so that the RAPD primers could anneal to many sites at the DNA so that the yield of amplification is too low; this was shown with the smear band at agarose gel after electrophoresis method.

The isolated DNA of fresh leaves of *Sauropus androgynus*, then was amplified with ITS primers by Polymerase Chain Reaction (PCR) Method. These primers amplify the entire ITS region of *Sauropus androgynus'* DNA.

ITS regions of DNA were subsequently amplified with twenty decamer primers by PCR-RAPD method for preliminary research to obtain the best primers which gave result clear and sharp profiles of DNA banding pattern after gel electrophoresis process.

Three primers (OPF-07, OPF-12,OPF-15) resulted clear and sharp profiles of DNA banding pattern after PCR-RAPD method on ITS region of *Sauropus androgynus'* DNA, as shown on figure 2.

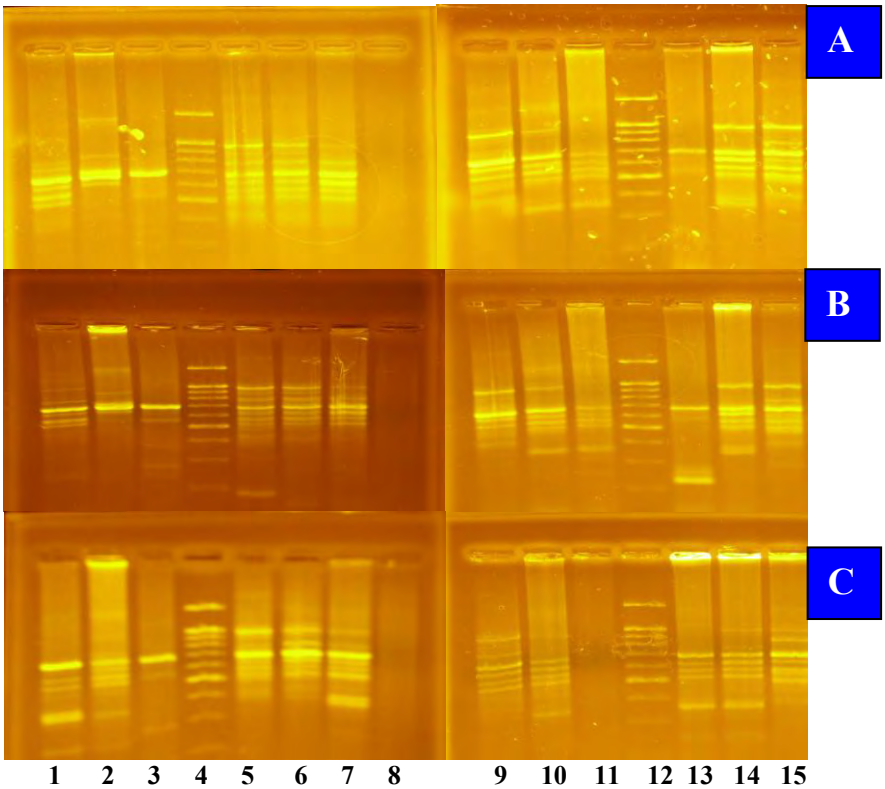


**Figure 2. RAPD profiles from ITS regions on DNA of *Sauropus androgynus* accessions, with primers OPF-7 (A), OPF-12 (B), OPF-15 (C)**
**1:SB; 2:SP, 3:ST, 4:marker 100 bp ladder, 5:T-I; 6:T-II, 7: B-I, 8:blank, 9:BJ-I, 10:BJ-II, 11: BJ-III, 12: marker 100 bp ladder, 13: PWS-I, 14:PWS-II, 15:PWD**

After visualizing by UV light transluminator, BioDocAnalyze Biometra helped to measure the weight of DNA bands comparing with the marker 100 bp ladder. The measurement result showed the monomorphic band, 600 – 700 kb in samples of *Sauropus androgynus*. In the next research, this band could be investigated further for obtaining specific biomarkers of *Sauropus androgynus*.

Examination of RAPD polymorphisms of the leaves samples by the use of arbitrary primers, was accomplished by primer OPF-07, OPF-12 and OPF-15. The results of statistical analysis indicate that RAPD patterns among the samples had high similarity (0.786-0.895), as indicated in figure 3.

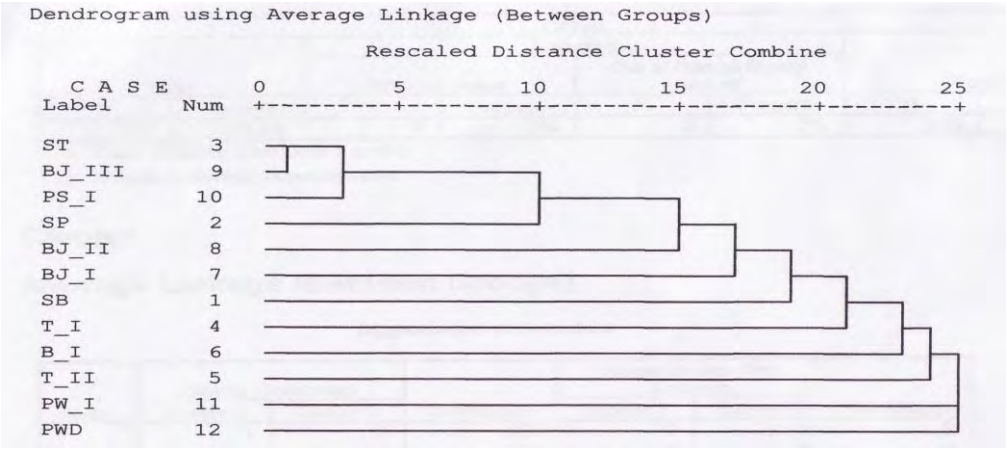


**Figure 3. Dendogram showing diversity of *Sauropus androgynus*' samples based on RAPD of ITS regions**

A dendogram of DNA banding pattern from *Sauropus androgynus'* samples revealed that genetic assessment by DNA fingerprinting, could distinguish SA cultivars more precisely than morphological assessment. There is no literature review could distinguish SA cultivars based on their morphological characteristics. All SA samples were authenticated by Center of Information and Development of Traditional Medicine at species level and they could not be differentiated at variety and locality level. Reference [18] stated that molecular identification could support the identification based on morphology for correctly designating different accessions of the same species. This method could support the assessment of raw materials in traditional medicine industry for assuring the quality of raw material and product.

DNA banding pattern of *Sauropus androgynus* from Surabaya Timur (ST) and Bojonegoro (BJ-III) show the highest similarity, although the samples were collected from two locations with different geographical conditions, such as relative altitude, temperature and relative humidity.

Despite the highest similarity, sample from Purwodadi (PWD) show the lowest similarity of DNA banding pattern comparing with other samples.

This research showed that environmental conditions might not be the major factor that influence the differences between accessions. The slight differences on DNA banding patterns of samples might be more influenced by different genetically accessions of *Sauropus androgynus*'s samples collected from several locations. Reference [20] also stated that human interference in plant domestication results in botanical evolution diversity.

For the next research, DNA fingerprinting of this plant must be done on the same genetically plant accession, which are cultivated on several locations with different geographical conditions, to analyze the influence of environmental conditions on genetic diversity of *Sauropus androgynus*.

## 3. CONCLUSION

Quality control is one of the key issues in the modernization of Indonesian traditional medicine. The early investigation at this research is to find the molecular system for controlling the quality of raw material on traditional medicine industry by using the RAPD method on ITS region of DNA of *Sauropus androgynus* accessions from several locations, at East Java, Indonesia.

Although the DNA banding pattern of several acessions show high similarity (0.786-0.895) with simple matching measure, this method could differentiate between accessions more precisely than morphological assessment.

DNA banding pattern of *Sauropus androgynus* from Surabaya Timur (ST) and Bojonegoro (BJ-III) show the highest similarity, although the samples were taken from two locations with different

geographical conditions. Meanwhile, sample from Purwodadi (PWD) show the lowest similarity of DNA banding pattern comparing with other samples. This result showed that environmental condition might not be the major factor that influence the differences between accessions.

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