TECHNICAL REPORT

Volume 2 - 2006

ITTO PD277/04/ Rev.3 (I)

Promoting Selected Non-Timber Forest Products Based on Community Participation Approach to Support Sustainable Forest Management in East Kalimantan

Biopharmaca Research Center
Bogor Agricultural University

Ministry of Forestry

PT Inhutani I

International Tropical Timber Organization

MARCH 2006

TECHNICAL REPORT II - 2006

ITTO PD277/04/ Rev.3 (I)

Promoting Selected Non-Timber Forest Products Based on Community Participation Approach to Support Sustainable Forest Management in East Kalimantan

Project Management Unit (PMU)

1. Project Leader : Prof. Dr. Suminar S Achmadi
2. Secretary : Irmanida Batubara, SSi., MSi
3. Treasurer : Dr. Yulin Lestari

National Consultants

1. Prof. Dr. Dudung Darusman
2. Prof. Dr. Latifah K Darusman
3. Dr. Munif Ghulammahdi
4. Dr. Hardjanto
5. Ir. Yulius Hero, MSc
6. Dr. Didik Suharjito
7. Ir. Ervizal A M Zuhud, MS
8. Ir. Tajudin Edy Komar, MSc
9. Tri Wahyudiyati, SH, LLM
10. dr. Irma H. Suparto, MS
11. Prof. Mustafa Agung S.
12. Ir. Hidayat Salim, MMA
13. Dr. Kade Yasa

Program Coordinator / Technical Advisory Committee

1. Ir. Siswoyo, MS BRC – IPB
2. Dr. Dyah Iswantini, M.Agr BRC – IPB
3. Drs. Edy Djauhari, MSi BRC – IPB

Biopharmaca Research Center – Bogor Agricultural University

Kompleks IPB Taman Kencana, Bogor, Indonesia 16151

Phone/Fax : +62 251 373 561

e-mail : bfarmaka@indo.net.id

ISBN No. 979-25-1203-9

Contents

Preface

The Project entitled "Promoting Selected Non-timber Forest Products Based on Community Participation Approach to Support Sustainable Forest Management in East Kalimantan", Serial Number PD 277/04 Rev. 3(I) is progressing in the second half of the first year. The project was commenced in April 2005 and the first volume of the technical report was published in September 2005.

Now, seedlings have been growing healthy in the nurseries and will be ready to be transferred to the field when the climate is suitable. Farmers, with sincere assistance from BIOMA personnel, are enthusiastically carried out the herbs cultivation, practicing the transformation from raw herb to prospective commodities, and gaining their knowledge in marketing. Serial workshops have been conducted amongst the stakeholders, and all participants seemed to take benefit of them.

Progress in on-farm nursery shows high growth rate of the seedlings. On the other hand, some other studies have been carried out in the laboratories, performed by researchers of Biopharmaca Research Centre. All information will be compiled to enrich the knowledge about traditional remedies, both from farmers land and from non-timber forest products.

Once again, appreciation is given to BIOMA for keeping the farmers' interest and all the stakeholders in East Kalimantan for making this successful progress.

Bogor, March 2006

Suminar Setiati Achmadi

Project Leader

GROWTH, FERTILIZER REQUIREMENT, AND CULTIVATION DESIGN OF MEDICINAL PLANTS ON BALIKPAPAN DRYLAND, EAST KALIMANTAN

Munif Ghulamahdi[1,2], Yadi Iswadi[2]

[1]*Department of Agronomy and Horticulture &* [2]*Biopharmaca Research Centre*
Bogor Agricultural University

ABSTRACT

Ninety to 95% of java tea plants (*Orthosifonis aristatus, kumis kucing)* grew in the nursery and 80% for ginger. Although the level of soil fertility on dry land in this study location were low in organic matter, low cation exchangeable capacity, low in nitrogen, phosphorus, and potassium nutrients, and inferior soil structure, applications of lime, manure, and urea, SP36, and KCl fertilizers on this dry land have increased the soil fertility level.

Java tea, ginger, and kaempferia (*Kaempheria galanga*) were cultivated in polyculture system, intercropped with food crop or horticulture or plantation crops. The cultivated area was 6.0 hectares in the first year and 14.1 hectares in the second year. Number of farmer involved in the first and the second year were 40 and 94, respectively. The farmer gets received production inputs from ITTO project, i.e. seed, lime, fertilizer, and pesticide. The production inputs are expected to be returned by the farmer when they harvested their crops.

INTRODUCTION

The dry land area in Indonesia was 192 million hectares, distributed on islands of Kalimantan 28.1%, Sumatra 25.7%, Irian Jaya 22.0%, Sulawesi 9.9%, Java and Madura 6.9%, Bali dan Nusa Tenggara 4.6%, and Maluku 3.9% (Rumawas 1983). Dryland in the tropical rain forest have low exchangeable capacity and base saturation, low phosphorous, calcium, and magnesium nutrients, but high in aluminium, manganese, and iron concentrations. Application of lime, organic matter, and fertilizer increased crop production (Rumawas 1983; Tisdale *et al*. 1985).

According to Hornok (1992), some external factors affected the medicinal plants growth from two different directions, i.e. (1) as limiting factors, by their extent compare to the optimum, (2) by their extreme values as stress effects. The level of active substance has been at the centre of attention since the start of medicinal plant ecological research. Qualitatively and quantitatively, active substances differ within the species, depending on the growing stages.

In 2002, the production of ginger (*jahe*) and kaempferia (*kencur,* a root crop resembling ginger, used as spice and medicine) in Indonesia were 118 496 tons and 12 848 tons, with productivity of 15.0 ton/ha and 12.0 ton/ha, respectively. Production centres of ginger and kaempferia in Indonesia were at Central Java and West Java provinces (Directorate of Horticulture & Biopharmaca 2004). The farmers in Boyolali (Central Java) usually cultivate kaempferia using polyculture system. Kaempferia was intercropped with maize or peanut (Nogosari Agriculture and Forestry Service 2001) In

Karanganyar, ginger was intercropped with coffee or clove (Directorate of Horticulture and Biopharmaca 2003).

The objective of this research were (1) to study the growth of kaempferia and ginger at nursery, (2) to study the growth of java tea at nursery and demonstration plot, and (3) to calculate fertilizer requirement and design of java tea, ginger, and kaempferia cultivations.

METHODS

Nursery of Java Tea, Ginger, and Kaempferia

Nursery of java tea was established at six villages in various sizes (Table 1). Java tea was planted in the plastic polybag 10 cm x 12 cm under 55% 'paranet' shading. Cuttings of java tea with size 10-12 cm were planted in the polybag planting media. Media for java tea cuttings was made of soil and manure with composition of 3:1.

Table 1. Planting Date and Size of Java Tea Nursery House

Village	Planting Date	Nursery House Size
Bukit Bangkirai	2 May 2005	12 m x 5 m
Karya Merdeka	2 May 2005	10 m x 6 m
Sungai Merdeka	12 August 2005	10 m x 8 m
Semoi I	12 October 2005	10 m x 8 m
Semoi IV	28 October 2005	10 m x 8 m
Mentawir	December 2005	10 m x 8 m

Maintenance of java tea in the nursery house consisted of irrigation, weeding, insect management, foliar application, and flower pruning. Irrigation was carried out 2 times per day every 2 days per week in mornings and in afternoons. Weeding and insect management were carried out by manual system. Weed grew on the polybag media and insect which attacked the leaves of java tea were picked by hand. Foliar fertilizer was applied every 2 weeks after 1.5 months planting, and flower was cut every 2 weeks after 2 months planting. The java tea growth was observed based on growth percentage and plant height.

Nursery of ginger was established at Semoi I and nursery of kaempferia at Sungai Merdeka. Seed of ginger and kaempferia were obtained from the local farmer land to get varieties that have been adaptive to the local soil and climate condition. Ginger and kaempferia were harvested at 9-10 months after planting, and used as seed source. Seed of ginger and kaempferia were planted in the nursery 1-2 month with planting media composition soil and compost 1:1.

Demonstration Plot of Java Tea

Demonstration plot of java tea was established at Bukit Bangkirai village during the Workshop I activities ("Medicinal Plant Cultivation System") on 12 July 2005. Java tea was planted by farmers on 100 m^2 area, with planting distance of 60 cm x 60 cm. Java tea was fertilized using 20 ton/ha manure, 6 g/plant N-P-K at planting date, and 20 ton/ha manure, 200 kg/ha urea, 200 kg/ha SP36, 200 kg/ha KCl, and 5 ton/ha dolomite at 3 months after panting.

Irrigation was carried out 2 times per day every 2 days per week in mornings and in afternoons. Weeding and insect management were practiced manual system. Flowers were cut every month after 2 months planting. Variables observed from java tea growth were growth percentage, plant height, plant branch, and number of leaf.

Fertilizer Requirement and Cultivation Design

Data of fertilizer requirement and cultivation design were collected from farmer in the field from May 2005 to December 2005. Variables observed regarding fertilizer requirement consisted of soil type, land condition (dry- or wetland), and medicinal plant commodities. Variables observed for cultivation design consisted of cropping pattern (monoculture, polyculture), food crop, horticulture and plantation crops, planting distance, and climate (wet and dry month).

RESULT AND DISCUSSION

Nursery of Java Tea, Ginger, and Kaempferia

Cuttings of java tea plant for Bukit Bangkirai and Karya Merdeka were obtained from Bogor, and developed at other villages as well. Cuttings of java tea for Sungai Merdeka, Semoi I, and Semoi IV were collected from Karya Merdeka, and for Mentawir were from Bukit Bangkirai. The growth percentage of java tea in the nursery falls in the highest category, i.e. 90-95 % (Table 2).

Table 2. Cuttings Material, Source of Cuttings and Growth Percentage of Java Tea

Village	Number of Cuttings	Source of Cuttings	Growth (%)
Bukit Bangkirai	7000	Bogor	90
Karya Merdeka	6000	Bogor	90
Sungai Merdeka	5000	Karya Merdeka	95
Semoi I	5000	Karya Merdeka	90
Semoi IV	5000	Karya Merdeka	95
Mentawir	5000	Bukit Bangkirai	-

Growth of java tea was normal at Bukit Bangkirai, Karya Merdeka, Sungai Merdeka, and Semoi IV, except Semoi I at 3 months after planting. Plant height of java tea at Semoi IV was higher than the other villages, and Semoi I was stunt only 40 cm at 3 months after planting. This problem was caused by low irrigation frequency (Table 3).

Growth percentage of ginger was 80% or 40-50 plants/kg seed weight. However, growth of kaempferia was not conducted yet, because the nursery will still be established at the end of January 2006.

Table 3. Height of Java Tea Plants in the Nursery (cm)

Village	Month after Planting							
	1	2	3	4	5	6	7	8
Bukit Bangkirai	20	40	60	80	120	125	130	135
Karya Merdeka	20	40	60	80	140	150	155	160
Sungai Merdeka	20	40	60	80	120	-	-	-
Semoi I	15	30	40	-	-	-	-	-
Semoi IV	20	60	100	-	-	-	-	-
Mentawir	-	-	-	-	-	-	-	-

Demonstration Plot of Java Tea Plantation

Growth of java tea in the demonstration plot was low at 1-2 months after planting, because the top soil was taken for planting media for other nursery. The growth of java tea at 3 months after planting was stunt with plant height, primary branch, secondary branch, number of leaf/secondary branch, and number of leaf/plant were only 40 cm, 3, 27, 90, and 2430, respectively. Growth of java tea was recovered after fertilized by 20 ton/ha manure, 200 kg/ha urea, 200 kg/ha SP36, 200 kg/ha KCl, and 5 ton/ha dolomite at 3 months after planting. The growth of java tea at 5 month after planting was normal with plant height, primary branch, secondary branch, number of leaf/secondary branch, and number of leaf/plant were 80 cm, 6, 54, 180, and 9720, respectively (Table 4).

Table 4. Growth of Java Tea Plants in the Demonstration Plot

Variables	Month after Planting				
	1	2	3	4	5
Plant Height (cm)	30	35	40	50	80
Primary Branch	-	-	3	4	6
Secondary Branch	-	-	27	34	54
Number of Leaf/Secondary Branch			90	113	180
Number of Leaf/Plant	-	-	2430	3842	9720

Fertilizer Requirement

The soil type in this study location was ultisol. The level of soil fertility on this dry land were low in organic matter, low cation exchangeable capacity, low nitrogen, phosphorous, and potassium nutrients, and inferior soil structure. Application of lime, manure, and urea, SP36, and KCl fertilizers in this dry land will increase the soil fertility level. The dosage of lime, manure, urea, SP36, and KCl for java tea, ginger and kaempferia is presented in Table 5.

Table 5. Seed, Pesticide, and Fertilizer Dosage per Hectare for Java Tea, Ginger, and Kaempferia Plants in the Polyculture System

	Production Input	Java Tea	Ginger	Kaempferia
1	Seedling	20 000	800 kg	800 kg
2	Urea	180 kg	400 kg	200 kg
3	SP36	75 kg	200 kg	100 kg
4	KCl	75 kg	300 kg	200 kg
5	Lime	1000 kg	2000 kg	2000 kg
6	Organic Fertilizer	2500 kg	20000 kg	20000 kg
7	Agrimisin	-	1 l	1 l
8	Insecticide	1	1 kg	1 kg
9	Fungicide	0.5	1 l	1 l

Design for Java Tea, Ginger and Kaempferia Cultivation System

Farmers in the study location usually cultivate food crop, horticulture and plantation crops. The commodities of food crops are rice, maize, cassava, and sweet potato; of horticulture crops are banana, pineapple, eggplant, cucumber, and of plantation crops are rubber trees, pepper, coffee, and coconut trees. Farmers usually cultivate ginger and kaempferia in the early year, in January or February when it is rainy season. The cultivation of java tea, ginger, and kaempferia were designed with polyculture system, intercropping with food crop or horticulture or plantation crop. In the first year, this polyculture were cultivated on 6.0 ha by 40 farmers (Table 6) and in the second year on 14.1 ha by 94 farmers (Table 7). The farmers received production inputs from ITTO, namely, seeds, lime, fertilizer, and pesticide. Each farmer at each village works on 1500 m^2, consisted of (1) ginger + food/horticulture/plantation crop = 750 m^2, (2) kaempferia + food/horticulture/plantation crop = 500 m^2, and (3) java tea + food/horticulture/plantation crop = 250 m^2.

It is expected that the farmers will returned the equivalent values of production inputs when have harvested their crops. These production inputs will be used by other farmers to enlarge the number of farmers involved in the project area.

Table 6. Cultivation of Java Tea, Ginger, and Kaempferia at Each Village in the First and the Second Year

Village	Area/Farmer (m^2)	Number of Farmer/Village	Area/Village (m^2)
Karya Merdeka	1 500	3	4 500
	1 500	8	12 000
Sungai Merdeka	1 500	7	10 500
	1 500	20	30 000
Semoi I	1 500	10	15 000
	1 500	22	33 000
Semoi IV	1 500	10	15 000
	1 500	22	33 000
Mentawir	1 500	10	15 000
	1 500	22	33 000
Total		40	60 000 = 6.0 ha
		94	141 000 = 14.1 ha

CONCLUSION

The growth percentage in the nursery was 90-95% for java tea, and 80% for ginger. The level of soil fertility on dry land in this study location were low in organic matter, low cation exchangeable capacity, low in nitrogen, phosphorus, and potassium nutrients, and inferior soil structure. Applications of lime, manure, and urea, SP36, and KCl fertilizers on this dry land have increased the soil fertility level.

Java tea, ginger, and kaempferia were cultivated in polyculture system, intercropping with food or horticulture or plantation crop. In the first year, this polyculture were cultivated on 6.0 ha by 40 farmers and in the second year on 14.1 ha involving 94 farmers. The farmer gets received production inputs from ITTO project, i.e. seed, lime, fertilizer, and pesticide. Each farmer in the each village works on an area of 1500 m^2.

Reference

Nogosari Agriculture and Forestry Service [Dinas Pertanian, Perkebunan, dan Kehutanan, Nogosari]. 2001. *Pola Tumpangsari Tanaman Kaempferia dengan Jagung dan Kacang Tanah di Lahan Kering*. Intercrop Pattern of Kaempferia, Maize and Peanuts. Boyolali: Cabang Dinas Pertanian, Perkebunan, dan Kehutanan

Directorate of Horticulture & Biopharmaca [Direktorat Tanaman Sayuran dan Biofarmaka]. 2003. *Pedoman Good Agricultural Practices pada Tanaman Obat* Guidelines on Good Practices on Medicinal Plants. Jakarta: Direktorat Jenderal Bina Produksi dan Hortikultura. Departemen Pertanian

Directorate of Horticulture & Biopharmaca [Direktorat Tanaman Sayuran dan Biofarmaka]. 2004. *Informasi Pengembangan Agribisnis Tanaman Biofarmaka*. Information on Agribusiness of Biopharmaca Plants. Jakarta: Direktorat Jenderal Bina Produksi dan Hortikultura. Departemen Pertanian

Hornok L. 1992. *Cultivation and Processing of Medicinal Plants*. Chichester: John Wiley and Sons

Rumawas F. 1983. *Ekologi Tanaman* (Plant Ecology). Bogor: Faculty of Agriculture IPB

Tisdale SL, Nelson WL, Beaton JD. 1985. *Soil Fertility and Fertilizers*. New York: Macmillan

PRODUCING SUPPLEMENT FROM JAVA TEA AND KAEMPFERIA AS ANTIDIURETIC

Dyah Iswantini[1,2], Afrina WR[2], Deden Saprudin[2], Latifah K Darusman[1,2], Salina Febriany[2], Irmanida Batubara[1,2]

[1] *Biopharmaca Research Center, Bogor Agricultural University*
[2] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University*

ABSTRACT

Java tea plants (*Orthosifonis ariststus, kumis kucing)* has been known as an effective remedy for kidney problems and infection of bladder. In addition, kaempferia (*Kaempferia galanga* Linn.) is also well known as a traditional remedy. The aim of this study was to prepare a diuretic formula from a mixture of java tea and kaempferia. The extract was obtained using water and ethanol. The result obtained indicates that java tea can be a remedy for kidney stone. Water extract of java tea is more effective in reducing kidney stone content compared with ethanol extract based on turbidity value of the two extracts. Combination of extract of java tea and kaempferia is not the proper remedy in treating kidney stone as the mixture of the extracts lower the turbidity value.

INTRODUCTION

Health is an important factor in our life for carrying our daily activities properly. Good supporting health facilities are needed to maintain and improve health, one of which is supply of curing, safe, and good quality medicines such as traditional medicines. Traditional medicine is defined as materials or ingredients in the forms of plants, minerals, *genelic* supply or combination of all those mentioned, which have, for generations, traditionally been used for curing remedies. So far traditional medicine has mainly been used in maintaining health itself or a preventive purpose though it has also been sometimes used as a remedy for a disease. Along with development of traditional medicine and the trend of "going back to nature", which is getting more popular, this type of medicine is also becoming better known. This becomes more apparent as there have been more herbal drink industries and pharmaceutical industries that produce traditional medicine to meet public's need. It is then necessary to find out a good way in producing traditional medicine that can maintain our health.

METHODS

Materials

The materials research was java tea plants (*Orthosifonis ariststus, kumis kucing)* and kaempferia (*Kaempferia galanga* Linn., *kencur*), urea, $CaCl_2 \cdot 2H_20$, ethanol, sodium oxalate, NaOH, and HCl.

Extraction

Extraction was done by macerating 100 grams of java tea powder by deep immersion in aqueous ethanol for 24 hours, and strained afterwards. The process was repeated three times. The extract was then concentrated using rotary evaporator under the temperature of 60 °C and 133 rpm speed. The result obtained was weighed and determined for its yield. Kaempferia was also treated in the same way.

Analysis Using Turbidimeter

Preparation of artificial urine

36.4 grams of urea was added into 1.5 L of distilled water and stirred until the crystals dissolved. Then, 15 grams of NaCl and 9 grams of KCl were stirred to get clear solution. Acidity was measured by using and indicator paper in order to keep the pH of urine in a normal range of 5-7. When it was beyond the range, HCl 1 N solution or and NaOH 1 N solution was added to adjust the pH.

Determination of turbidity

A turbidity cell was filled with 13.6 mL solution of calcium chloride and 1.4 mL of sodium oxalate was quickly added to it. The mixture was shaken for 10 seconds and then the cell was put into the compartment of turbidimeter and its turbidity value was measured.

The activity testing of herbal plants was done by mixing 0.1 % extract (1 mL) with calcium chloride solution and oxalate solution was added too. This was done to ethanol extract and water extract of java tea, the mixture of kaempferia extract and that of java tea in several concentrations, which was then observed for the turbidity value. The reading was done for ten minutes.

RESULTS AND DISCUSSION

Java tea has long been used as a remedy for kidney problems and infection of bladder. Traditionally, the leaves of the plant are used by boiling to cure infection of urinal tract and kidney stone (Anonymous 1980). The process of making traditional medicine has to be done in accordance with CPOTB (a principle of applying a proper way in producing traditional medicine) covering aspects related to the production of traditional medicine. CPOTB is aimed at giving a guarantee of good products meeting requirements assigned when later used to maintain health among general public.

Prior to being used, herbal plants have to be checked for their *symplicia* (dried plant materials) quality. A *symplicia* is considered having good quality when it meets all the requirements stated in SNI (Indonesian National Standard). A *symplicia* that is not dry enough and covered with microbes, slime, experiences colour change or smells bad cannot be used as raw material in making traditional remedies. When all the requirements are met, it will then undergo an extraction process. The extraction of java tea was done using maceration method in water and ethanol solution. The filtrate was then thickened using a rotary evaporator. This concentrate extract was dried into powder.

The method used in identifying whether a plant is potential a kidney stone breaker is time consuming and costly. Meanwhile, a parameter in observing the process of crystallization of calcium oxalate can be determined by the rate of crystal formation. A common approach often used in studying the crystallization process is by observing

crystallization of over saturated passing solution. A simple method in observing a precipitation process is by using a turbidimeter.

Calcium oxalate is one type of kidney stones in a kidney forming itself as a result of consuming foods containing calcium oxalate and purine. The compounds found in java tea can be a potential remedy in breaking kidney stone. Based on a pytochemical assay of water and ethanol extract, java tea contains compounds of alkaloids saponins, flavonoids, steroids, tannins, Na^+ and K^+.

Turbidity measurement was done at concentration levels of calcium chloride of 4 mM and 6 mM. The higher the concentrates used, the faster the formation of calcium oxalate. Oxalate stayed stable after calcium thoroughly reacted toward it. It can be identified in figures 1 and 2 showing the result of the measurement of turbidity value that java tea extract can decrease the calcium oxalate content, and decreasing calcium oxalate of water extract of java tea is more compared with that using java tea ethanol extract. This resulted from the reaction between calcium and organic substances that dissolved in water.

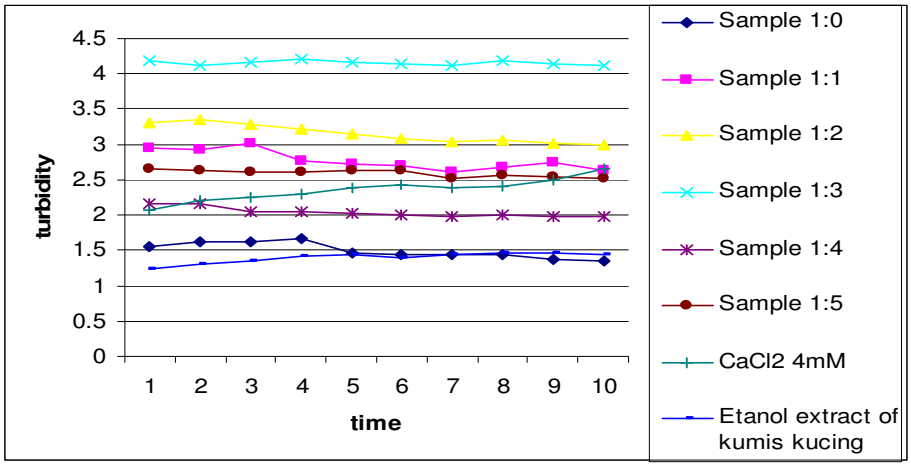

Figure 1. The turbidity of several ratios between extracts of java tea and kaempferia vs. Time (seconds) with concentration of $CaCl_2$ of 4 mM.

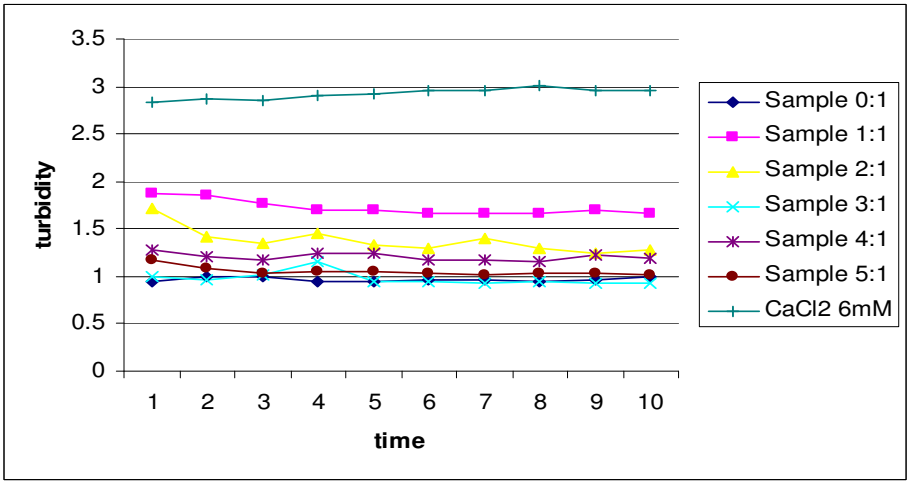

Figure 2: The turbidity of several ratios between extracts of java tea and kaempferia vs time (seconds) with $CaCl_2$ concentration of 6 mM.

The result of the measurement of turbidity value of java tea and kaempferia mixture in several ratios (v/v) of 1:1; 1:2; 1:3; 1:4; and 1:5 indicated that mixture of java tea and kaempferia was not a good remedy for kidney stone because the mixture decreased the activity of single extract of java tea. The proportions of java tea:kaempferia of 0:1; 1:1; 2:1; 3:1; 4:1 and 5:1 also lowered the activity of single extract of "java tea. Hence, the result indicates that java tea extract is better used individually as herbal medicine. kaempferia has so far been mainly used to create a specific aroma but not to cure kidney stones.

Water is more effective in promoting the breaking of kidney stone compared with ethanol. Soedibyo (1998) suggests that water extract has better dissolving capacity for kidney stone compound with ethanol extract. The Ca compound in calcium oxalate will break because of the presence of water and the calcium formed will react to organic substances in the leave extract.

The making of the supplement was further done by formulating an extract with addition of filler material. The filler material used can be amylum, but dry extract from the raw material can also be used, and the next step was drying the extract in an oven. The dry extract obtained was put into capsules, and is ready to consume.

CONCLUSION

The result obtained indicates that java tea can be a remedy for kidney stone. Water extract of java tea is more effective in reducing kidney stone content compared with ethanol extract. This can be identified by the turbidity value of the two extracts. Combination of extract of java tea and kaempferia is not the proper remedy in treating kidney stone as the mixture of the extracts lower the turbidity value.

REFERENCES

[Ministry of Health] *Departemen Kesehatan*. 1995. *Farmakope Indonesia* (Indonesian Pharmacopea. 4th Ed. Jakarta: Ministry of Health

[Ministry of Health] *Departemen Kesehatan*. 1986. *Sediaan Genelik* (Genelic Preparation). Jakarta: Ministry of Health

Harborne JB. 1973. *Phytochemical Methods.* New York: Halsted Press

Soedibyo M.1998. *Alam Sumber Kesehatan, Manfaat, dan Kegunaan* (Nature: Hiealth Source, Benefits, and Uses. Jakarta : Balai Pustaka.203-208

Supriadi *et al.* 2001. *Tumbuhan Obat Indonesia, Penggunaan dan Khasiatnya* (*Indonesian Medicinal Plants, Use, and Efficacy*). Jakarta. Pustaka Populer

FORMULATION OF SUPPLEMENT *JAMU* FROM *PASAK BUMI* (*EURYCOMA LONGIFOLIA JACK*) AND GINGER, AND *IN VITRO* PHARMACOLOGICAL ASSAY OF ANTIOXIDANT

Dyah I Pradono[1,3], Yulin Lestari[2,3], Deden Saprudin[1], Dani Firmansyah[1], Salina Febriany[3], Latifah K Darusman[1,3]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, IPB
[3]Biopharmaca Research Centre, LPPM-IPB

INTRODUCTION

Drugs and traditional medicines have been used since the ancient until now in modern era. Traditional medicines have multifunction in human life such as maintaining health and fitness, as an alternative medicine, cosmetics, food supplement, and aromatics therapy. Many nations have their own opinion on drugs and traditional medicines. For developing countries, the use of drugs and traditional medicines has become their habits, and about 80% of the population consume these for curing diseases and maintaining their health. However, for developed countries, which are economically more established, the use of traditional medicines is caused by increasing trend to go back to nature.

The application of drugs and traditional medicines is an alternative treatment and complementing the modern drugs (*complementary-alternative medicine*, CAM). The WHO survey analysis in various countries indicates that CAM is still interested because it is proven to prevent diseases and can be used for disease therapy. In developing countries, generally, expenses for drugs and traditional medicines are higher than for modern drugs. In Africa and developing Asian and American countries, most people are still using medicines and traditional medicines because of their difficulties in having formal medical treatment services. In developed countries such as in America and Europe, about 50% of the population also use medicines and traditional medicines.

In Indonesia, traditional medicines are known as *jamu*. The *jamu* has been consumed since long time ago and has been inherited from their ancestors with their belief to get benefits to their health. Although its scientific back up is still limited, *jamu* is still their choice to give therapeutics, physically, and suggestive effects. Suggestive effect is psychosomatics effect and this placebo effect has an important role, because in reality, there is chemical brain reaction toward their health recovery.

In year 2003, data indicated that global selling value for herbal/phytopharmaca reached USD$ 50 millions, meanwhile, the modern drugs closed to USD$ 310 millions. In Indonesia, the selling of traditional medicines reaches Rp 2 billions, increasing 12% annually. In percentage, market demand for herbal medicines is only 10.5% as compared with the modern drugs that reach 89.5%. Currently, the use of natural materials, in wider terminology is known as biopharmaca (natural materials from plants, animals and microbes, which have pharmacological effects, use as food/drink, food supplement, cosmetics and drugs) indicates an increasing figure. This increase is triggered by several factors such as:

a. The increasing trend to go back to nature.
b. Indonesia has the second largest biodiversity in the world after Brazil, having more than 28.000 species of plants. Among them, only about 1000 species registered in the Institute of Food and Drugs Administration (*Badan Pengawasan Obat dan Makanan,* BPOM*)* and have been used to produce medicines mainly for *jamu*.

The use of traditional medicines in many countries including Indonesia, its market potential, and the presence of many stimulant factors cause the traditional medicines become widely used. This situation gives chances for academicians and businessman to further develop this field. Consequently, many research activities have been conducted, and the results can be used as basic considerations for businessman to start their traditional medicines business. This research aim to understand the process required in using plants as raw materials for making commercial product, namely supplement *jamu* which has safety, quality, and efficacy assurances.

METHODS

Materials and Equipment. *Symplicia* of *pasak bumi* (*Eurycoma Longifolia Jack*) and ginger (*Zingiber officinale Rhizoma*) were used as main source of raw materials and as additional warm sensation of supplement product, respectively. Ethanol, distilled water, and dextrin/amylum were used as solvents. Maceration equipments and evaporator were used during this process.

The process employed several steps, e.g. pre-treatment of *symplicia*, extraction using maceration technique, concentrating extract, making of supplement powder, and pharmacological analysis of the product for antioxidant medical function.

Pre-treatment. Dried *pasak bumi symplicia* were grounded into powder. The powder was filtered through 30-40 mesh filtration equipment. The powder obtained was then used for extraction process.

Extraction. Maceration was used to extract the two *symplicia*, namelly *pasak bumi* and ginger using ethanol and water, respectively. The materials were macerated for 24 hours at room temperature, and repeated 3 times to obtain maximum extract. The extract was filtered, concentrated in a rotary evaporator, and dried. The yield was then calculated. *Pasak bumi* and ginger extracts were then combined at certain compositions. The mixture was dispersed in amylum or dextrin, dried to powder, then filled into capsules.

Water content. About 2-3 g of *pasak bumi* powder was weighed and put in a dry aluminium disk which is previously weighed. The powder was dried in an oven at 105^{o}C for 3 hours. The dry disk was placed in a desiccator to reach room temperature. The disk was weighed to get final weight and the following calculation was used:

$$\text{Water content} = \frac{\text{initial weight (g)} - \text{final weight (g)}}{\text{initial weight (g)}} \times 100\%$$

Yield. The yield was determined based on weight of extract obtained from the total extracted materials. The yield was presented in % (w/w).

Pharmacological: Assay on antioxidant activity

In vitro pharmacological assay for antioxidant was carried out to each composition of *pasak bumi* and ginger extracts.

Equipments used for the assay of antioxidant were measuring glass, aluminium foils, spectrophotometer, micropipettes, vortex, plastic tray, scissors, incubator, and Erlenmeyer.

Method of Assay

Antioxidant activity was assayed using DPPH method. Ethanol extract of *pasak bumi,* water extract of *pasak bumi*, ginger extract, and samples at various compositions of *pasak bumi*-ginger mixed were used for this assay. The concentrations were between 10 to 200 ppm, and BHT was used as a comparison. In detailed, the method is presented in Figure 2.

Antioxidant activity is represented by IC_{50} (*inhibition concentration*). From the absorbance value of various samples, the percentage of inhibition was obtained. Logarithmic equation obtained from the relationship between concentration of antioxidant sample and percentage of inhibition of the sample to its free radicals is calculated as the following:

$$\text{Logarithmic equation: } y^* = B \ln(x) + A$$

Notes:
* y value= 50 (50% inhibition), A value and B are known, thus x (IC_{50}) value can be calculated.

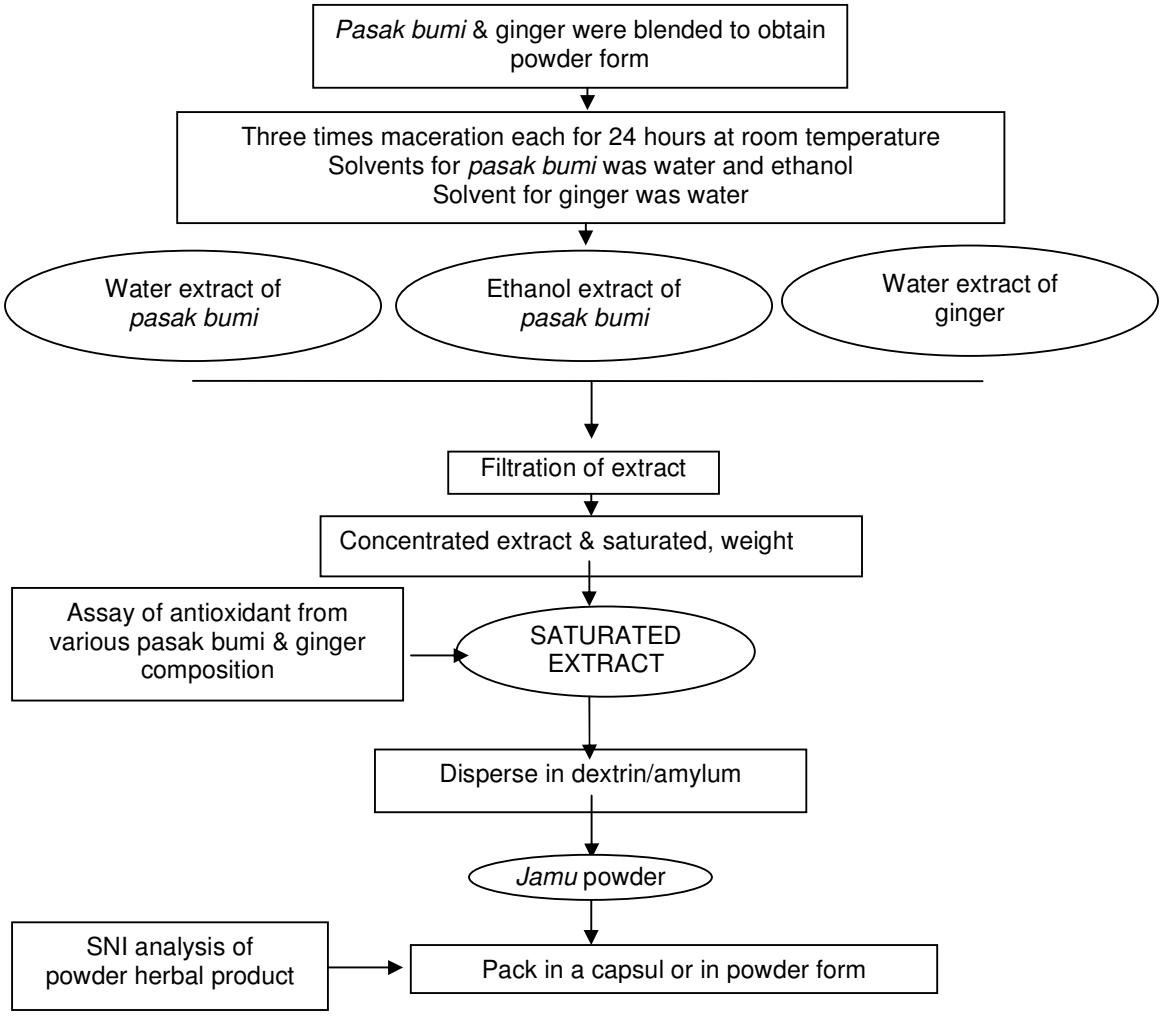

Figure 1 Steps in Research

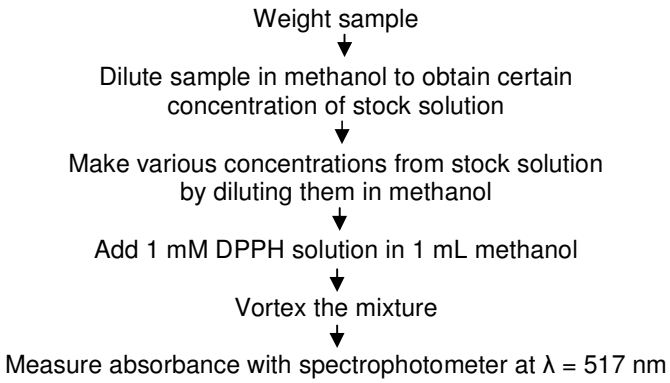

Figure 2 Measurement of antioxidant activity using DPPH method

RESULTS AND DISCUSSION

Making of *Jamu* and Assay

Generally, this research activity was divided into 4 steps: extraction, assay of antioxidant medical function, SNI based analysis of supplement product made of phytopharmaca raw materials, and cost analysis to predict its business potential. Extraction of raw materials was based on maceration technique. Two solvents which are commonly used for material extraction, namely water and ethanol, were used for macerating of *pasak bumi symplicia*.

The aim of using two different solvents was to measure the effectiveness of plant extract movement or to select the best solvent. The effectiveness of extraction was based on yield and antioxidant activity of the extract. Maceration has also been applied for ginger *symplicia* using water solvent.

The yield of ethanol extract of *pasak bumi* was 0.90%. The low yield may due to less concentrated active compounds in the wood. For that reason, other experiments were carried out to obtain higher yield from other parts of *pasak bumi* plant, namely roots and leaves.

Determination assay of antioxidant activity using DPPH. Measurement of antioxidant activity using this method was based on DPPH *free radical scavenging activity.* The DPPH (1,1-diphenyl-2-picrylhydrazil) at 1 mM concentration functions as synthetic free radical source and the concentration will decrease upon addition of sample solution as antioxidant. Antioxidant activity of *pasak bumi* extract (and or ginger extract addition) will inhibit free radicals in DPPH. Using UV-spectrophotometer, at 517 nm wavelength, the absorbance of free DPPH was very strong showing violet colour of solution and change into yellow when the free radicals become inactive due to pairing its single electron. The level of inhibition (%) of active concentration in the sample to the presence of free radical DPPH can be determined by measuring this non-inhibition DPPH radical absorbance. The measurement of antioxidant activity of BHT (butylated hydroxytoluene) solution was used as a comparison.

Antioxidant activity is presented as IC_{50} (*inhibition concentration*), which is concentration at 50% inhibition level. The percentage of inhibition was obtained from sample absorbance at various concentrations. The equation logarithmic obtained from the relationship between anti-oxidant sample concentration and percentage of inhibition to its free radicals is calculated as follows:

$$Y = B \ln(x) + A$$

By including 50% (y=50) inhibition value, the x as IC_{50} value or antioxidant activity was determined. The result of absorbance measurement and calculating antioxidant activity of ethanol and water extracts of *pasak bumi*; ginger extract and combination of both of them can be seen in Table 1.

Calculation of inhibition level:

$$\text{Level of inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

The inhibition level and also determination of IC_{50} indicate that *pasak bumi* has antioxidant activity, but at the lower level as compared with BHT solution, which is known as stable antioxidant. Meanwhile, it is also known that ethanol extract of *pasak bumi* has higher antioxidant activity as compared with water extract of *pasak bumi*. This indicates that antioxidant compounds are more soluble in ethanol than in water. Meanwhile, ginger that have warm sensation has weaker antioxidant activity as compared with ethanol extract of *pasak bumi*, but stronger than water extract of *pasak bumi*.

Table 1 Antioxidant activity of *pasak bumi*, ginger, and their combination

Sample	Concentration (ppm)	A	Inhibition (%)	Equation	$IC_{50} = x$
BHT	0	0.539	-	$y = 18.534 \ln(x) - 13.156$ $r^2 = 0.916$	30.2
	5	0.469	12.987		
	10	0.411	23.748		
	25	0.247	54.174		
	50	0.167	69.017		
	100	0.138	74.397		
	200	0.135	74.954		
EtOH Extract of *Pasak Bumi*	0	0.937	-	$y = 4.3532 \ln(x) – 7.1978$ $r^2 = 0.9301$	508.516
	10	0.915	2.348		
	50	0.830	11.419		
	100	0.827	11.740		
Water Extract of *Pasak Bumi*	0	0.937	-	$y = 0.923 \ln(x) + 4.1065$ $r^2 = 0.9873$	3.9×10^{21}
	10	0.879	6.190		
	100	0.857	8.538		
	200	0.854	8.858		
Water extract of Ginger	0	0.937	-	$y = 1.7166 \ln(x) + 0.5321$ $r^2 = 1$	3.3×10^{12}
	10	0.895	4.482		
	50	0.869	7.257		
	100	0.858	8.431		
Pasak Bumi:Ginger 1:1	0	0.495	-	$y = 2.9472 \ln(x) – 4.9565$ $r^2 = 0.893$	1.2×10^{8}
	10	0.483	2.424		
	50	0.465	6.061		
	100	0.460	7.071		
	200	0.435	12.121		
Pasak Bumi:Ginger = 1:3	0	0.495	-	$y = 2.4863 \ln(x) – 4.1095$ $r^2 = 0.7517$	2.8×10^{9}
	10	0.481	2.828		
	50	0.479	3.232		
	100	0.461	6.869		
	200	0.442	10.707		
Pasak Bumi:Ginger = 3:1	0	0.562	-	$y = 3.8311 \ln(x) – 1.7366$ $r^2 = 0.7078$	732.612
	10	0.511	9.075		
	50	0.506	9.964		
	100	0.484	13.879		
	200	0.439	21.886		

The influence of adding ginger extract into main *pasak bumi* material was determined using IC50 assay from the mixture at various comparable compositions. Table 1 shows that the more ginger concentrations in the mixture, the lower the antioxidant activity. Instead of that, at the composition where proportion of *pasak bumi* was higher than ginger (3:1), its activity was weaker than the activity of single *pasak bumi* ethanol extract. Therefore, addition of ginger will decrease the antioxidant activity of *pasak bumi* as the main *jamu* material.

Cost Analysis

Cost production was calculated on overall production process. The example of cost calculation for *pasak bumi* capsule production (single material without ginger addition) is presented in Table 2.

Tabel 2 Cost production analysis of *pasak bumi* capsule

Step	Cost (Rp)	Remark
Raw *pasak bumi*	15000	3 kg of raw materials
Washing & cutting	3000	Washing & cutting 3 kg raw materials
Oven drying	9000	1 kg *symplicia* from 3 kg raw materials
Crude grinding	3000	Grinding 1 kg *symplicia*
Maceration/extraction	25000	10 g (1% from 1 kg *symplicia*) yield
Extract dispersion with filler	3200	200 g (20% from *symplicia*) filler
Oven drying	3000	
Fine grinding	6000	210 g total extract in powder form
Capsule filling	3360	420 capsules obtained from 210 g powder
Total Cost Production	70560	420 capsules

Calculation was based on price raw material price and cost of raw material processing and filling into capsules, as described bellow:

Price of raw materials:

1 Raw *pasak bumi*	Rp 5,000/kg
2 Capsule gelatine	Rp 25/piece
3 Bottling	Rp 2,000/kg
4 Filler, e.g. corn amylum	Rp 16,000/kg

Cost calculation for processing:

1 Washing & cutting	Rp 1,000/kg
2 Oven drying	Rp 3,000/kg
3 Crude grinding	Rp 3,000/kg
4 Extraction	Rp 25,000/kg
5 Fine grinding	Rp 6,000/kg
6 Capsule filling	Rp 8/piece

Remark:
- Proportion of *symplicia* and filler = 5 : 1
- 500 mg extract powder in 1 capsule
- Yield of *pasak bumi* extract (in ethanol) from *symplicia* is 0.90% and for approximation, it is assumed as 1% yield.

At Biopharmaca, selling price of capsule supplement product with single material is from Rp 660 to Rp 960 per capsule exclude the price of bottle (price Rp 35,000-Rp 50,000 per bottle, with capacity of 50 capsules per bottle) depending on the price of raw materials and degree of difficulty during processing.

Based on cost production (excluding other cost components such as transport) from the above calculation, the price of *pasak bumi* supplement capsule:

$$\text{Basic price of capsule} = \frac{\text{Cost production}}{\text{Number of capsule produced}} = \text{Rp 7560/420 capsules}$$

$$= \text{Rp 168 per capsule}$$

If the lowest selling price per capsule is considered Rp 660 thus the percentage of profit (gross) of cost production:

$$\text{Profit (gross)} = \frac{\text{Rp 880} - \text{Rp 168}}{\text{Rp 660}} \times 100\% = 74.5\%$$

CONCLUSION AND RECOMMENDATION

Extraction of *pasak bumi* using ethanol solvent gave relatively low yield (0.9014%). The level of antioxidant activity of ethanol extract of *pasak bumi* was higher than that of water extract. Meanwhile, ginger has lower activity compared with ethanol extract of *pasak bumi*. Adding ginger extract will decrease antioxidant activity of *pasak bumi* as main *jamu* material.

The use of *pasak bumi* plant as raw material for making supplement capsule is potential to be developed either for national and international market. The minimum profit (gross) of supplement *pasak bumi* capsule reaches 74.5% or the cost production is only about 25.5%.

REFERENCES

Darusman LK *et al*. 2005. *Technology in Herbal Medicine Production in Relation to Biopharmaca Development Based on Invention and Innovation.* Text in Indonesian. Module for Retooling Program.

Ministry of Health, the Republic of Indonesia. 1994. *Kodifikasi Peraturan Perundang-undangan Obat Tradisional.* Codification on Traditional Remedies Regulations. Volume I. Directorate of Traditional Remedies Administration. Directorate General Food and Drugs Administration.

Ministry of Health, the Republic of Indonesia. 2000. *Pedoman Pelaksanaan Uji Klinik Obat Tradisional.* Guidelines on Clinical Tests for Traditional Remedies. Directorate of Traditional Remedies Administration. Directorate General Food and Drugs Administration.

Soedibyo M. 1998. *Alam Sumber Kesehatan: Manfaat dan Kegunaan.* Uses of Natural Resources for Health. Jakarta: Balai Pustaka.

Supriadi *et al*. 2001. *Tumbuhan Obat Indonesia: Penggunaan dan Khasiatnya*. Indonesian Medicinal Plants: Use and Efficacy. Jakarta: Pustaka Populer Obor.

SAPONINS OF ALBUTRA (*Arcangelisia flava* (L.) Merr) AS A HEPATOPROTECTOR

Suminar Setiati Achmadi, Irmanida Batubara, Sulistiyani

Biopharmaca Research Centre, Bogor Agricultural University

ABSTRACT

Albutra (*akar kuning* plant, *Arcangelisia flava* (L) Merr) contains two major chemical constituents, namely saponins and alkaloids. Saponins of albutra show hepatoprotector activity better than its alkaloids. The best extracting solvent was mixture of methanol:dichloromethane (2:1), followed by refluxing in a mixture of ethyl acetate:chloroform (1:1) medium, and precipitating in acetone. Yield of the glycoside was 8%. Through an *in vitro* test on Chang liver cells, saponin extract was able to regenerate damaged cell by paracetamol 8% better than that by a commercial medicine available in the market. Test on bioactivity showed that glycoside fraction was able to inhibit 50% of increasing aspartate transaminase and alanine transaminase enzyme activities in damaging liver cells of laboratory rats *Sprague dawley* by paracetamol. A compound that has been deduced is 6-hydroxyarcangelisin that is bound to glucomanoside moiety.

INTRODUCTION

Albutra is popular by its vernacular name as *akar kuning* (*Arcangelisia flava* (L) Merr.), belongs to family Menispermae. This species is distributed in Java, Sumatra, Kalimantan, Sulawesi, Papua, and Maluku. Albutra is a wild plant, which is usually found in stony coastal area or in primary or secondary forest, or bushy thicket, on 100-1000 m above sea level. It is an herbaceous perennial, climbing liana, up to 20 m long. Stalk round, twining, coarse, dark brown in colour, and the wood is bright yellow (Figure 1).

Figure 1. Plant of Albutra (*Arcangelisia flava* (L.) Merr.)

Parts of the plant mostly used are root, flower, stalk, fruit, leaf, and wood. The root is effective for curing bronchitis, anthelmintic, depurative, dysentery, syphilis, diabetes, ulcer, and hepatitis; the flower is good for dysentery; the stalk is good for chicken pox, fever, and sprue; the fruit is effective for sprue; the leaf is for anaemia and sprue; while the wood is good for aphrodisiac, antiseptic, ulcer, anthelmintic, skin irritation, malaria, enhance digestive enzyme, antiinfection, stomach-ache, emenagog, disinfectant, analgesic, demulcent, rheumatism, hepatitis, and sprue. Names of symplicia for the traditional remedy are *Arcangelisiae flavae cortex* (bark of albutra), *Arcangelisiae flavae radix* (root of albutra), *Arcangelisiae flavae lignum* (wood of albutra).

HEPATITIS AND HEPATOPROTECTOR

At least 58 plant species have been summarised by Dalimartha (2000) as having bioactivity to cure liver disorder (hepatitis). One of the plant species is albutra. Albutra is a native plant of South East Asia that is empirically proven to cure hepatitis in Indonesia. Many traditional remedies containing albutra for this illness are sold in many places, particularly in Kalimantan. Traditionally, about 15 cm of dried stem or root is decocted using two glasses of water until about one glass of thick liquid left. The hepatitis patient must take this liquid one to three times a day, depends on the degree of symptom, until the symptom disappear. Today, hepatitis medicines in the market are expensive, not to mention that the effect is questionable. Therefore, many patients are using traditional remedies for treating this liver disorder.

Hepatitis is inflammation of the liver, characterised by jaundice, and usually accompanied by fever and other systemic manifestations. Hepatitis suffers about 12 millions of Indonesian, the third place in Asia Pacific (Anonymous 2002). The main cause of hepatitis is virus, in addition to other agents such as bacteria, parasites, nematodes, drugs (such as paracetamol, statin), natural and synthetic chemicals, and malnutrition. Up to now, effective medicine for liver cure has not been known, mainly that is caused by virus except by administering interferon (Levinson 1997). So far, medicine for hepatitis is symptomatic treatment, just to lighten the symptoms. In addition, supportive and promotive treatments are also practiced to help liver functions. The medicines are usually had hepatoprotective, lipotropic, choleretic, and colagogum properties. Hepatoprotector is effective compounds that can protect liver cells from toxic agents to liver cells. The mechanism is by detoxication of the toxic compounds, regeneration of liver cells, antiinflamation, and immunomodulator (Dalimartha 2000).

One of quick methods to test a compound as hepatoproptector is using liver cells in an artificial environment outside the living organism (*in vitro* test). Rat can be used to test a hepatoprotector by measuring AST and ALT enzyme activities. Damage liver cells are indicated by increasing these enzyme activities.

Utilisation as hepatoprotector has been registered in the Directorate General for Intellectual Property Rights, under patent registration number of P00200100045 dated 22 January 2002, and publication number of 033.055.

CHEMICAL CONSTITUENTS

Alkaloid vs. Saponins. Root of albutra contains berberine, 8-hydroxy-berberine, jatrorrhisine, limacine, pinarrhine, and thalifendia, all belong to alkaloids (Nuryanti 1993). According to Kunii and Kagei (1985), the stem contains fibraurine, 6-hydroxyfibraurine, fibleucine, 6-hydroxyarcangelisine, 2-dehydroxyarcangelisinol, tinophylol, and 6-hydroxyfibleucin. Parts of plant may contain different chemical constituents as indicated by qualitative phytochemical test. The hanging root of albutra contains alkaloids but not saponins (Meistiani 2001), on the other hand, root or stem on the ground contain alkaloid and saponins as well (Suparto *et al.* 2000).

Moisture content of albutra symplicia sample was 4.5%, indicating that the dry material is relatively safe toward microorganism growth. Preliminary qualitative tests on wood mill of the stem part and root detected saponins, flavonoid, and alkaloids in high concentrations (Table 1).

Table 1. Qualitative phytochemical tests of albutra wood mill

Test	Result
Flavonoid	+
Alkaloid	+
Saponins	+++
Steroids	-
Terpenoids	-
Tannins	-

Remarks: +++: the highest concentration.

Alkaloids can be extracted with water at low pH. *In vitro* test of the alkaloid (Suparto *et al.* 2000) showed that alkaloid extract was able to regenerate liver cells damaged by paracetamol, but could not be considered as sufficient hepatoprotector. *In vivo* test of the alkaloid extract confirmed the conclusion about the ineffective alkaloids as the hepatoprotector (Meistiani 2001). These facts lead us to pursue the study on saponins.

Saponins. Saponins are glycoside that can have a function as natural detergent (active surface agent), have one to seven carbohydrate units and widely distributed in nature and marine organisms. Saponins are divided into three classes: steroid glycosides, triterpene glycosides, and alkaloid steroid glycosides, being the triterpene glycoside is the most abundant (Harborne 1991, Rao 1996). Essentially, saponins consist of sapogenins and sugar moieties.

Saponin extraction has been optimised. Some wood mill was refluxed using Beutler *et al*. (1997) methods (Figure 2) in ethanol, methanol, mixture of methanol to dichloromethane 1:1, or mixture of methanol:dichloromethane 1:2 for 30 minutes. Refluxing was carried out three times. The resulted crude extract was dried in a vacuum evaporator, followed by three times refluxing in *n*-hexane for 30 minutes, to remove fat and other nonpolar compounds. The crude extract was airdried for one hour to remove the residual solvent. The fat-free extract was refluxed in a mixture of ethyl acetate-chloroform 1:1, repeated for seven times. The filtrate was removed and the residue was dissolved in ethanol to precipitate the glycoside. The extract solution was evaporated to half of the original volume and poured into acetone under stirring until all glycoside precipitate. The precipitate was filtered and dried.

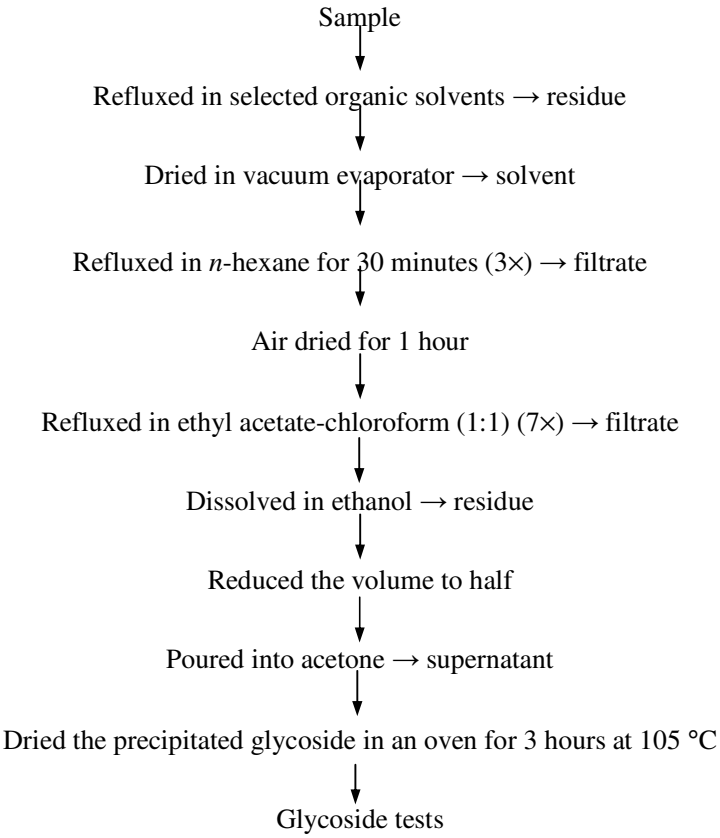

Figure 2. Saponin extraction

Saponins concentration in the glycoside fraction was evaluated by measuring surface tension of the aqueous solution (Rosen 1987), and compared with a saponin standard. Saponins from quilaja bark have been widely used as standard compound (Martin and Briones 2000). Surface tension was determined using capillary tube. Glycoside solution (50 mL) was placed in a tube and a capillary tube was partially immersed in the solution. Solution in the large tube was blewed to raise water surface in the capillary tube; kept for one hour to see whether the water surface in the capillary tube stayed still. Difference of the two surface levels was measured and the surface tension was calculated using the following equation:

$$\gamma = \rho \times g \times h \times \tfrac{1}{2} r.$$

where ρ = density of water

g = gravitational acceleration

h = difference of surface levels

r = diameter of capillary tube.

The highest yield of saponins was obtained from extraction using mixture of methanol:dichloromethane 2:1 (Table 2). Ethanol extract was not evaluated further due to assumption that the extract has been chemically modified (Dey and Harborne 1991).

Table 2. Effect of extracting solvent on yield and saponin concentration

Solvent	Yield (%)		Saponin (% of Glycoside Fraction)
	Crude Extract	Glycoside*	
Ethanol	80.0**	21.1	**
Methanol	40.6	10.8	14.60
Methanol-dichloromethane 1:1	41.0	7.3	15.80
Methanol-dichloromethane 2:1	40.7	7.4	18.65

* contains small amount of alkaloids
** considered as modified saponins

Purification of saponin fractions using column and thin layer chromatography methods followed by deduction using infrared and ultraviolet spectra as well as liquid chromatography-mass and gas chromatography spectra, a sapogenin having molecular weight of 390, namely 6-hydroarcangelicine (Figure 3), has been detected, bound to a glucomannose (Batubara *et al*. 2004).

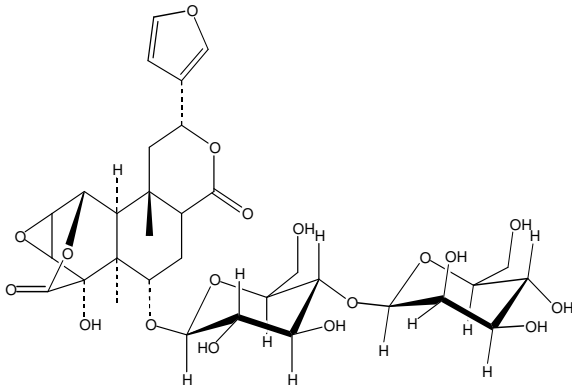

Figure 3. Glucomanoside of 6-hydroxyarcangelicine

Sapogenins from crude extract that were column chromatographed using mixture of chloroform:methanol:2-butanol (2:1:3) are also moieties belong to triterpenoids with molecular weight of 517, 428, and 340. An alkaloid was also detected, having molecular weight of 517. Sugar moieties attached to these sapogenins were glucose, fructose, mannose, xylose, ribose, and sucrose.

IN VITRO TEST

Methods of Study. Hepatoprotector activity of the saponins was studied outside living organism using Chang cells ATCC CCL-13 that were previously treated with paracetamol as a negative control and those using Cursil®-70 as a positive control (Figure 4).

Prior to the test, concentration level of the saponins extract was determined. The saponins extracts were incubated on the liver cells until 50% growth confluence and the liver cells were counted at day-3. The concentration titration was carried out to determine safe concentration of saponins and Cursil®-70 on liver cells.

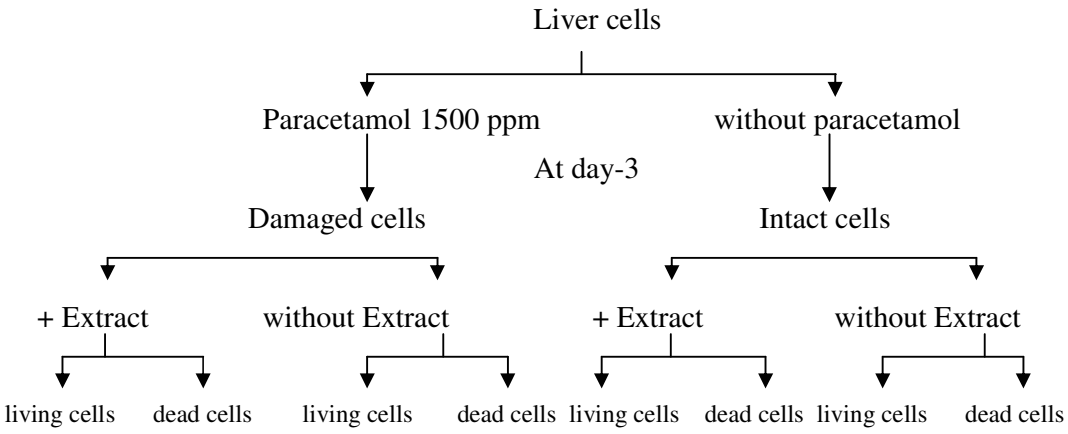

Figure 4 *In vitro* test (Suparto *et al.* 2000)

The safe dose. The results (Figure 5) indicated that saponin concentration above 500 ppm because it will destroy liver cells using control cells and solvent effects as references. The safe dose for further experiment was up to 200 ppm.

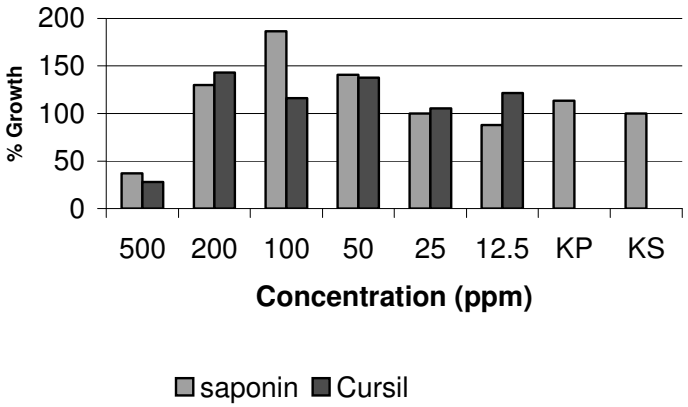

Figure 5. Determination of safe concentration for *in vitro* test of saponins.

To evaluate regeneration potency of liver cells as the effect of saponins or Cursil administrations, the cells were spoilt with 1500 ppm paracetamol (Suparto *et al*. 2000). Saponins and Cursil at 50 and 100 ppm that were administrated at day-4 have maintained the number of cells at day-7 (Figure 6). On the other hand, concentration of 250 ppm and higher did not give good effects. The effect of Cursil seems to be better as compared to saponins from albutra. The safe dose for Cursil was also at 200 ppm, which is close to the suggested dose by the Cursil manufactures (224 ppm). Therefore, the safe dose for saponins is between 100 and 200 ppm.

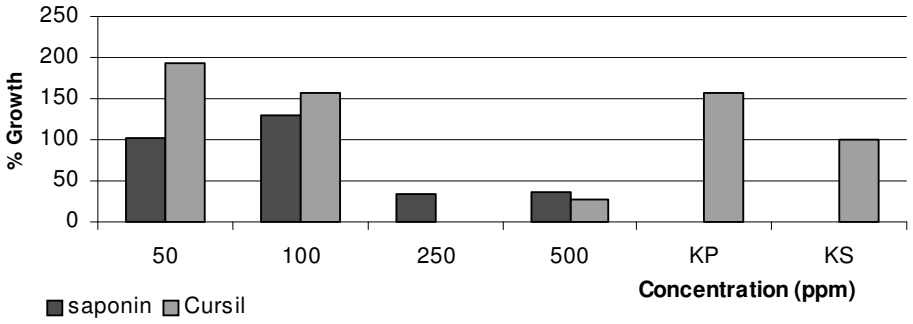

Figure 6. Effect of saponins administrated at day-4 on liver cells that have been 3-days spoilt by paracetamol

Ability to regenerate damaged liver cells. When the saponins extracts were given at day-7 after paracetamol treatment, the regeneration ability of the cells three days after saponins administration is illustrated in Figure 7. Without any treatment, the damaged cells due to paracetamol could only regenerate up to 2.3%. However, saponins and Cursil have regenerated the cells up to 40%. The ability of saponins in regenerating damaged cells is different from Cursil. Curcumin contained in Cursil is able to increase gall secretion and accelerate lipid degradation, therefore, smoothen digestion and ease the liver work (Murray 1995). Oleum xanthorhizae may act as liver protector from toxic agents and along with silimarine in Cursil®-70 stabilise the cell membranes and protect the liver cells from oxidative agents (Conti *et al*. 1994). The mechanism of albutra's saponins in regenerate damage liver cells has to be studied further.

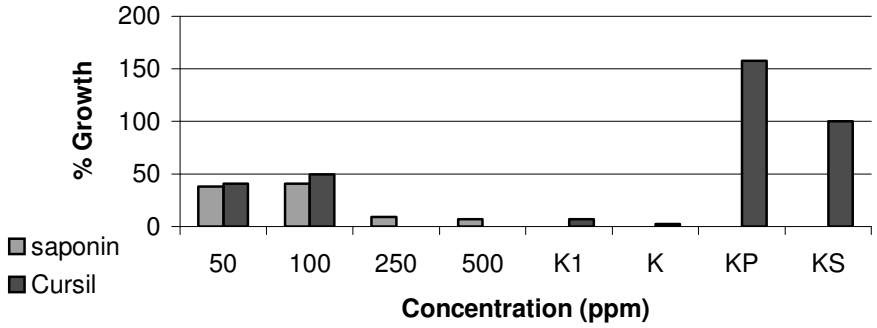

Figure 7. Effect of saponins administrated at day-4 on liver cells that have been 7-days spoilt by paracetamol

IN VIVO TEST

Methods of study. Animal model for studying liver damage was Sprague Dawley rat administered with paracetamol at a dose of 500 mg/kg body weight. Male rats were two months-old, having an average of 150-200 g body weight. After treatment, aspartate transaminase (AST) and alanine transaminase (ALT) of the serum were measured. When the liver cells are damaged or necrosis, these enzymes move out the cells and cause high concentrations of them in blood serum. According to Amin (1995), AST and ALT activities are good indicators for liver cell damage because high concentrations of

these enzymes happen in early stage and usually in enormous levels compared to other enzymes.
Before the treatment, the rats were conditioned for one month in a cage to reach suitable age and weight. It was also aimed to reduce the animal stress. The rats were divided into three groups: control, hepatotoxic, and treated group. Each group has five rats. The control group was given standard feed, and the hepatotoxic group was given paracetamol without saponins for 14 days, started at day-8 until day-21. The treated group was given saponins for seven days, started at day-0 to day-7 and followed by paracetamol administration for 14 days, started at day-8 until day-21.

Measurement of ALT and AST Activities. Saponins as hepatoprotector were evaluated based on AST and ALT enzymes activity in the rat blood serum. At day-0, -7, -14, and –21, AST and ALT activities in the blood were measured using Bergmeyer method. For AST measurement, the blood serum was taken (0.2 mL) and mixed with Bergmeyer reagent (1.0 mL) and incubated at 25 °C for one minute. Absorbance was read at the first, second, third, and fourth minutes at 340 nm wavelength in an ultraviolet spectrophotometer. For ALT activity, the serum sample (0.1 mL) and the reagent (1 mL) were mixed and incubated for 37 °C for one minute. The absorbance was read at 340 nm wavelength and after incubated, the measurement was performed at the first, second, and third minutes after the first readings. The difference of incubation temperature between AST and ALT measurement was due to AST activity in the rat that was greater than that of ALT, so that incubation temperature for AST measurement had to be decreased to 25°C, so as to read the activity. The reagent for AST analysis consisted of Tris buffer, L-aspartic, LDH, 2-oxoglutaric, NADH, and MDH, whereas for ALT reagents consisted of Tris buffer, L-alanine, LDH, 2-oxoglutaric, and NADH.

AST or ALT levels were calculated using the following equations:

$$\text{ALT concentration} = 1745 \times {}_{\Delta}A \text{ 340 nm/minute}$$

$$\text{AST concentration} = 952 \times {}_{\Delta}A \text{ 340 nm/minute}$$

Remark: ${}_{\Delta}A$ = average absorbance at the first, second, and third minutes.

Dose of Albutra Extract. Man with average weight of 60 kg, usually consume about 15 cm stem with an average of 45.2 g. The material was boiled with two glasses of water until one glass of liquid remained. With an assumption those 5 grams of wood mill contained saponins of 0.18 g, the weight of saponins in 15 cm albutra (45.2 g) was

$$\frac{0.18 \text{ g}}{5.0 \text{ g}} = \frac{X}{45.2 \text{ g}}$$

$$X = 1{,}63 \text{ g}$$

Therefore, 1.63 g was a dose for a man and for the rat with an average of 200 g body weight, the dose of saponins was 5.4240 mg. This amount of saponin glycoside extract was dissolved in 0.5 mL distilled water and was administered to the rat.

Effect of Saponins Extracted from Albutra. AST and ALT activities in the blood serum were measured before and after treatment with saponins and paracetamol. The average AST in the blood serum before treatment was 53.4 U/L, which was normal for the rat according to Girindra (1989), i.e. 45.7–80.8 U/L, indicated that the rats were in healthy condition. The average ALT level was also in normal range (17.0–30.2 U/L).

Saponins were given 7 days to the second group, followed by paracetamol administration. Saponins did not give significant difference on AST and ALT activities, before and after administration (Figure 8a and 8b), meaning that saponins do not damage the liver.

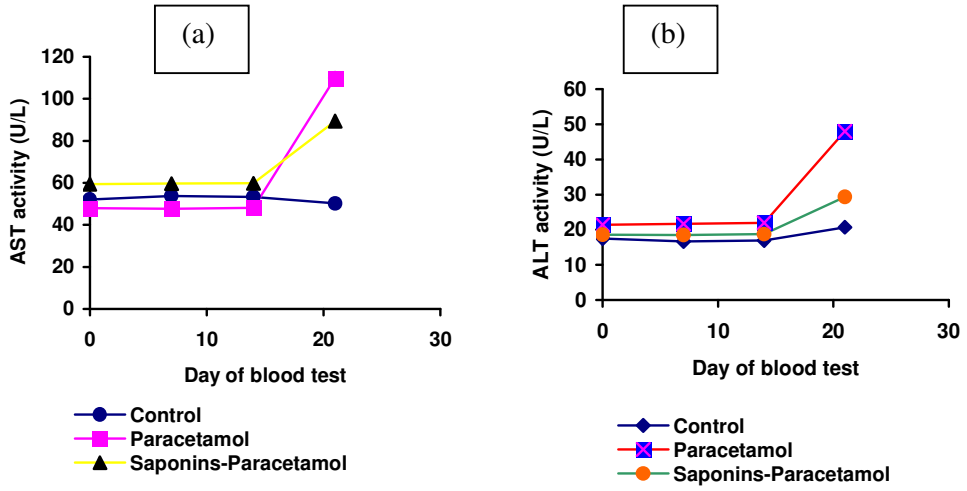

Figure 8. AST (a) and ALT (b) activities in rat's blood serum treated with saponins.

Paracetamol was given to the second and the third groups for 14 days, started at day-8 until day-21. Figures 3 and 4 indicate that paracetamol administration for 7 days did not give significant effect on increasing AST and ALT activities of the hepatotoxic group. However, AST and ALT activities in the hepatotoxic group were significantly different after 14 days administration. AST activity increased 130.4% and ALT activity increased 122.3% as compared to before treatment (Figure 9a and 9b). Difference of AST and ALT activities after paracetamol administration to the group receiving saponins was smaller than those without saponins administration.

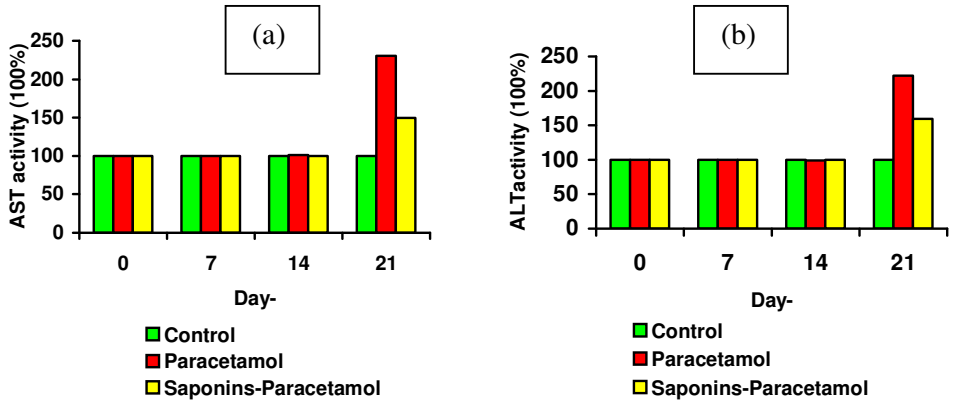

Figure 9. Percent activities of AST (a) and ALT (b) enzyme activities in the rat blood serum treated with saponins

Saponins administration prior to paracetamol treatment was able to give significant effect on the increasing AST and ALT activities in the blood serum. When we compare the activities between the treated and the untreated groups, saponins was able to inhibit activity increasing 58% for AST and 52% for ALT after 14 days of saponins administration. This is an indication that saponins have biological activity as hepatoprotector or liver protecting agent. The mechanism of how saponins protect the liver is may be of its antioxidant function that can inhibit cytochrome P-450 activity (Lacaille-Dubois 1996). In addition, saponins activity as an antiinflammation (Harborne 1991) can inhibit increasing AST and ALT activities in the blood serum.

ACKNOWLEDGEMENT

Appreciations are given to *Hibah Bersaing* X for the funding, Ir. Ervizal AM Zuhud, MS for the albutra sample, Saras Purbo Kayun, Pramudito Adji, Nuraini Ridwan, and Iwan Firmansyah for data collecting.

REFERENCES

[Anonymous]. 2002. *Indonesia Endemi Tinggi Hepatitis B*. High Endemic of Hepatitis B in Indonesia. *Kompas.* 10 October 2002.

Amin I. 1995. *Pengaruh pemberian seduhan rimpang kunyit (Curcuma domestika, VAL) terhadap activity SGPT dan SGOT ayam.* Effect of decoction of cucumin rhizomes on SGPT and SGOT in chicken. [research report]. Bogor: Bogor Agricultural University, Faculty of Mathematics and Natural Sciences.

Batubara I, Achmadi SS, Iskandriati D, Hanafi M. 2004. *Glikosida akar kuning (Arcangelisia flava (L) Merr) sebagai hepatoprotektor: Ekstraksi, Pemisahan, dan Bioactivitynya*. Albutra glycosieds as hepatoprotector: Extraction, Purification, and Bioactivity. *Jurnal Natur Indonesia* 7:14-20

Beutler JA, Kashman Y, Cardellina II JH, Alexander MRA, Balaschak MS, Prather TR, Shoemaker RH, Boyd MR. 1997. Isolation and Characterization of Novel Cytotoxic Saponins from *Archidendron ellipticum*. *Bioorganic & Medicinal Chemistry* **5:**1509-1517

Conti M, Marozzoni P, Bombardelli E. 1994. *Sylibum marianum* (L.) Gaertn. Roma: INDENA Spa, Scientific Department.

Dalimartha S. 2000. *Ramuan Tradisional untuk Pengobatan Hepatitis.* Traditional Formulae for Hepatitis. Jakarta: Penebar Swadaya.

Dey PM, Harborne JB**. 1991. *Method in Plant Biochemistry.* Volume-7. London: Academic Press.**

Girindra A. 1989. *Biokimia Patologi Hewan.* Biochemistry of Animal Pathology. Bogor: Institut Pertanian Bogor, Pusat Antar Universitas.

Harborne JB. 1987. *Metode Fitokimia. Penuntun Cara Modern Menganalisis Tumbuhan.* Translated by K. Padmawinata & L. Soediro. Bandung: Bandung Technology Institute.

Kunii T, Kagei K, Kawakami Y, Nagai Y, Nezu Y, Sato T. 1985. Indonesian medicinal plants. I. New furanoditerpenes from *Arcangelisia flava* Merr. *Chem. Pharm. Bull* 33:479-487

Lacaille-Dubois HW. 1996. A review of the Biological and Pharmacological Activities of Saponins. *Phytomedicine* **2:**363-386.

Levinson MJ. 1997. Hepatitis A-G Prevention, Diagnosis & Therapy. *Comprehensive Therapy*. **23**: 482-490

Martin RS, Briones R. 2000. Quality control commercial quilaja (quillaja saponaria) extracts by reverse phase HPLC. *J. Sci. Food. Agric.* 80: 2063-2068.

Meistiani Y. 2001. Isolasi dan Identifikasi Senyawa Alkaloid dari Akar Kuning (*Arcangelisia flava* (L) Merr). Isolation and Identification of alkaloids from albutra [Research Report]. Bogor: Faculty of Mathematics and Natural Sciences.

Murray RK. 1995. *Biokimia Harper*. Harper's Biochemistry. Jakarta: IKAPI.

Nuryanti S. 1993. Identifikasi Senyawa Alkaloid dari Batang Kayu Kuning. Identification of alkaloid from albutra. [Thesis]. Yogyakarta: Universitas Gajah Mada.

Rao AV. 1996. Anticarcinogenic Properties of Plant Saponin. *Second International Symposium on Role of Soy in Preventing and Treating Chronic Disease*. Brussels, September

Rosen MJ. 1987. *Surfactants and interfacial phenomena*. New York: Wiley-Interscience.

Suparto IH, Achmadi SS, Iskandriati D, Zuhud EAM, Batubara I. 2000. Pengembangan Akar Kuning sebagai Obat Asli Indonesia Tahap II. Development of albutra as Indonesian Remedies. Report on Join Research between Bipharmaca Research Centre and PT Indofarma Phase II.

EXTRACT OF JAVA TEA LEAVES AS ACTIVATOR IN LITTER DECOMPOSITION AND EFFECT OF JAVA TEA INTERCROPPING ON *DAMAR* PLANTATION

Wijanarko, Dudung Darusman, Latifah K Darusman, Yahya Fakuara

Biopharmaca Research Centre

Bogor Agricultural University

ABSTRACT

Leaves of java tea, a medicinal plant, could be extracted and used as activator in composting mahogany leaves. Extract of mixture of java tea leaves and leaves of *meniran,* also a medicinal plant, (in a ratio of 75:25) gave the highest nutrient content N, P, K, Ca, and Mg in the compost made of mahogany litter. The calcium content is significantly higher than in other mixtures. Two kilograms compost of mahogany litter activated by extract of java tea leaves and *meniran* leaves (75:25) administered per hole for *damar* plant resulted to 4.7 cm growth during the first four months in the field.

Intercropping java tea and maize crops with 2-year old tree plantation of *damar* (*Agathis loranthifolia*) resulted better nutrient balance as compared to cultivation without intercropping. Intercropping gave higher benefit per hectare per year as compared to without intercropping (Rp 12,375,105 vs. Rp 10,956,822).

INTRODUCTION

One of ways to improve quality of seed in field is quality improvement of plant media. The quality of plant media can be improved by administering compost activated by extract of java tea leaves (*Orthosipon aristatus* Bl. Miq.). Java tea is famous for its efficacy in eliminating gallbladder and for land conservation due to its capability in binding soil particles. Parameters used in analysing the media improvement were changes of nutrient (N, P, K, Ca, Mg, C/N ratio) and pH. Good quality of compost is very essential for fulfilling nutrient requirement of *damar* (*Agathis loranthifolia*) crop in field. Quality improvement of *damar* crop was evaluated through growth of the crop and nutrient level in its leaves. Cultivation of java tea alternately with the *damar* trees was studied from the nutrient balance. This study includes estimation on benefit of intercropping system using sweet maize and java tea plants.

Aims of this research are (1) to obtain the best concentration of java tea leaves extract as decomposition activator that gives the highest nutrients, (2) to obtain optimum compost dose for the benefit of *damar* seed in field, (3) to obtain nutrient balance in soil with java tea as intercrop, and (4) to estimate the benefit of java tea intercropping.

METHODS

There were are two activities in this study, namely evaluation of extract of java tea leaves as activator in litter decomposition of mahogany leaves for growth of *damar* crop and evaluation of java tea plants as intercrop toward soil nutrient balance and analyse its economic benefit.

Study on Extract of Java Tea Leaves as Activator of Litter Decomposition

The effect of level of extract of java tea and *meniran* (*Phyllanthus urinaria* Linn., leaves on nutrient content (N, P, K, Ca, and Mg) of the compost were evaluated using Complete Randomised Design (RAL) with three replicates. Levels of leaf extract were as followed: $K_{25}M_0$ = 25 g java tea leaves; $K_{50}M_0$ = 50 g java tea leaves; $K_{75}M_0$ = 75 g java tea leaves; $K_{100}M_0$ = 100 g java tea leaves; K_0M_{25} = 25 g *meniran* leaves; $K_{25}M_{25}$ = mix of 25 g java tea leaves + 25 g *meniran* leaves; $K_{50}M_{25}$ = 50 g java tea leaves + 25 g *meniran* leaves; $K_{75}M_{25}$ = 75 g java tea leaves + 25 g *meniran* leaves; and $K_{100}M_{25}$ = 100 g java tea leaves + 25 g *meniran* leaves. The least significant difference (LSD) at 5% level was employed to determine the response.

Amount of compost per-hole for *Damar* crop was optimised using RAL with single treatment amount of compost per hole with five replicates. The amount of comÿÿst for each hole to be planted varied from 0 kg (control), 1, 2,ÿÿ, ÿÿÿÿnd 5 kg. LSD method ÿÿ 5% level was again employed to analyse the response.

Condition of soil nutrient balance in intercropping system using java tea plants in rows alternating with maize was assessed by measuring level of nutrients. Level of nutrients could be increased due to various sources and could be decreased for various reasons as well. Increasing nutrient value could be due P1 = litter came from *damar* crop; P2 = litter from java tea crop; P3 = litter from sweet maize crop; P4 = from direct rainfall, and P5 = from fertilizer. Decreasing nutrient value could be due to M1 = absorption by crops; M2 = carried away by erosion; and M3 = leaching.

Economic Analysis of Intercropping System

Net benefit was calculated by subtracting total cost from total benefit. Benefit from intercropping with java tea was calculated from harvest of maize and java tea crops. Whereas, benefit from treatment without java tea intercrop was from harvest of maize only. Cultivation cost consisted of fixed cost (media and seeds) and variable costs (media preparation, labour, and tending cost). The net benefit was calculated using the formula:

$$\pi = R - C = \Sigma \{(PY \times Y) - (FC + VC)\}$$

where π = net benefit
R = total benefit
C = total cost
PY = output price
Y = volume of output
FC = fixed cost
VC = variable cost.

RESULTS AND DISCUSSIONS

Extract of Java Tea Leaves as Activator for Litter Decomposition of Mahogany Trees

This experiment was aimed to determine the best extract to produce mature compost (C/N ratio < 20) with the highest nutrients of N, P, K, Ca, and Mg. The first step was to measure macronutrient (C, N, P, K, Ca, and Mg) levels in leaves of mahogany, java tea, and *meniran.* Table 1 shows that litter of mahogany trees has C/N ratio more than 20, meaning that this material is not good as organic fertilizer in the field. Additional treatment should be employed to obtain reasonable C/N ratio of mature compost.

Table 1. Levels of C, N, P, K, Ca, and Mg in Leaves of Mahogany, Java Tea, and *Meniran*

Species	Level of Nutrients (%)						
	C	N	P	K	Ca	Mg	C/N
Mahogany	44.17	1.06	0.10	0.10	2.35	0.25	41.67
Java tea	53.16	3.00	0.34	0.93	1.48	0.44	17.75
Meniran	56.24	2.29	0.27	0.63	1.28	0.26	24.60

Macronutrient Level in Extract

Result of chemical analysis to each mixture of java tea and *meniran* as compost raw materials is listed in Table 2. It is obvious that the higher proportion of java tea leaves in the mixture, the higher the extracted macronutrients. Addition of *meniran* leaves in the mixture resulted more macronutrients dissolve into water. Therefore, mixture of 100 g java tea leaves and 25 g *meniran* leaves boiled in 1000 mL water (to leave 500 mL solution) gave 1.36% C, 0.11% N, 0.03% P, 0.30% K, 0.09% Ca, and 0.04% Mg in the extract.

Flavonoids (Quercetin) in Java Tea Leaves and *Meniran* Leaves

Chemical analysis of java tea and *meniran* leaves showed that quercetin content were 0.703% and 0.317%, respectively. Quercetin belongs to flavonoid group, known as activator in litter decomposition. Qualitative test on the extracts revealed that other secondary metabolites, namely saponins, were present (Table 3). Flavonoids are usually present as glycosides that are easily dissolved in water. Quercetin itself dissolves better in hot water.

Table 2. Levels of C, N, P, K, Ca, and Mg Elements in Leaf Extracts

Extract Type	Nutrient Content (%)						
	C	N	P	K	Ca	Mg	Total
$K_{25}M_0$	0.28	0.02	0.01	0.10	0.02	0.01	0.44
$K_{50}M_0$	0.68	0.05	0.02	0.17	0.04	0.02	0.99
$K_{75}M_0$	0.79	0.06	0.02	0.18	0.05	0.02	1.12
$K_{100}M_0$	1.15	0.10	0.03	0.27	0.07	0.04	1.66
K_0M_{25}	0.30	0.02	0.01	0.07	0.02	0.01	0.42
$K_{25}M_{25}$	0.55	0.04	0.01	0.14	0.03	0.02	0.79
$K_{50}M_{25}$	0.85	0.04	0.02	0.21	0.06	0.03	1.21
$K_{75}M_{25}$	1.27	0.08	0.03	0.27	0.09	0.05	1.79
$K_{100}M_{25}$	1.36	0.11	0.03	0.30	0.09	0.04	1.93

Table 3. Qualitative Tests on Secondary Metabolites in Leaf Extracts

Extract Type	Secondary Metabolite						
	Triterpenoids	Steroids	Hydroquinones	Flavonoids	Saponins	Tannins	Alkaloids
$K_{25}M_0$	-	-	-	+	+	-	-
$K_{50}M_0$	-	-	-	+	+	-	-
$K_{75}M_0$	-	-	-	+	+	-	-
$K_{100}M_0$	-	-	-	+	+	-	-
K_0M_{25}	-	-	-	+	+	-	-
$K_{25}M_{25}$	-	-	-	+	+	-	-
$K_{50}M_{25}$	-	-	-	+	+	-	-
$K_{75}M_{25}$	-	-	-	+	+	-	-
$K_{100}M_{25}$	-	-	-	+	+	-	-

Note: + = detected; - = not detected

Nutrients, C/N Ratio, and pH of Compost

Nutrients, C/N ratio, and pH of litter of mahogany leaves composted in one month under anaerobic condition are presented in Table 4. All treatments resulted mature composts, indicated by C/N ratio of less than 20 and pH about alkaline or neutral (6.86-8.12) matured compost distinguished by C/N ratio < 20, pH about alkali or neutral. On the other side, some nitrogen element would be in the form of NO_3^- and a few in form of NH_4^+ (Indranada 1986). Mature compost is also characterise by its crumb structure, rather free-flowing and do not lump, dark brown in colour, and its aroma like humus or soil.

Table 4. Levels of Macronutrients, C/N Ratio, and pH of Compost at 1-Month Age

Treatment	Macronutrients in Compost (%)*						
	N	P	K	Ca	Mg	C/N Ratio	pH
K_0M_0	1.07	0.08	0.07	2.11	0.22	22.02	6.87
$K_{25}M_0$	1.09	0.08	0.20	2.29	0.24	18.88	6.86
$K_{50}M_0$	1.09	0.07	0.21	2.54	0.25	17.09	7.17
$K_{75}M_0$	1.28	0.15	0.41	2.81	0.30	15.98	7.13
$K_{100}M_0$	1.18	0.11	0.50	2.11	0.25	17.70	7.31
K_0M_{25}	1.05	0.04	0.08	2.54	0.22	19.21	7.20
$K_{25}M_{25}$	1.10	0.07	0.21	2.54	0.25	17.61	7.35
$K_{50}M_{25}$	1.19	0.07	0.34	2.86	0.22	16.34	7.32
$K_{75}M_{25}$	1.41	0.12	0.46	3.69	0.32	15.77	8.04
$K_{100}M_{25}$	1.25	0.09	0.34	2.60	0.26	16.84	8.12

Note: * average of 3 replicates

Alkaline condition of the compost was anticipated as the effect of organic substance decomposition of the litter to become inorganic components. The pH of the compost tends with the increasing amount of extract given. This phenomenon can be explained because more substance are readily converted to soil nutrients such as salts containing C, N, P, K, Ca and Mg. C, N, and P are elements in enzymes necessary for inducing (activating) microorganisms growth in compost. Other minerals such as K, Ca, and Mg are important for microorganisms as well. In short, type and amount of elements will influence the pH of compost.

Further statistical analysis (Table 5) showed that level of extract administration gave significant effect on level of N, P, K, Ca, and Mg (except P) in the compost. This condition may be due to different type microorganisms that can be active in different nutrient content in the organic substrate. Extracts of $K_{75}M_{25}$ and K75M0 gives similar level of nutrient, which is the best amongst the treatments. The mixture K75M24 was then chosen for the subsequent experiment in preparing compost for *damar* plants in the field because of higher level of Ca as compared to $K_{75}M_0$. Calcium ions will reduce the porosity of cell membrane of the crop and this will prevent some toxic elements, such as manganese, copper, zinc, and molybdenum enter the cell (Nyakpa 1988).

Table 5. Statistical Difference of Macronutrient Levels in Compost

Treatment	Macronutrient in Compost (%)				
	N	P	K	Ca	Mg
K_0M_0	1.07 cd	0.08 abc	0.07 e	2.11 f	0.22 e
$K_{25}M_0$	1.09 c	0.08 bc	0.20 d	2.29 ef	0.24 cde
$K_{50}M_0$	1.09 bcd	0.07 abc	0.21 d	2.54 de	0.25 bcd
$K_{75}M_0$	1.28 ab	0.15 a	0.41 b	2.81 d	0.30 a
$K_{100}M_0$	1.18 bcd	0.11 abc	0.50 a	2.11 f	0.25 bc
K_0M_{25}	1.05 d	0.04 c	0.08 e	2.54 cd	0.22 cde
$K_{25}M_{25}$	1.10 d	0.07 bc	0.21 d	2.54 cd	0.25 bcd
$K_{50}M_{25}$	1.19 bcd	0.07 bc	0.34 c	2.86 b	0.22 de
$K_{75}M_{25}$	1.41 a	0.12 bc	0.46 ab	3.69 a	0.32 a
$K_{100}M_{25}$	1.25 abc	0.09 abc	0.34 c	2.60 c	0.26 b
LSD 5%	0.196	0.076	0.063	0.184	0.033

Note: The number in same column which is followed by the same letter is not significant different in 5% level

Chemical Properties of Soil Planted with *Damar*

Soil condition of the field to be cultivated with *damar* and intercropped with java tea plants was considered acidic (Table 6). In general, nutrients are more readily absorbed by the plants because most nutrients are easily dissolved when the soil pH is about neutral. In acidic soil, phosphorous will be bound (fixation) by aluminium. Other reason is that at very low pH, very toxic microelements that are required by the plants at low concentration might be dissolved more than needed (Hardjowigeno 1995).

Nutrient levels in the soil were considered low to medium, indicating that the fertility is medium. Value of cation exchange capacity that was 20.55-20.65 implied that the planting site would not need heavy fertilizing. Excessive fertilizing would give negative impact for *damar* plants.

Soil texture is also an important aspect to be considered in supporting tree growth. The soil was dominated by clay (64-67 %). This type of soil has good ability in holding water and nutrients. Absorbed water will be difficult to be released; therefore, water will be less available for plants. Clayey soil is also referred to as heavy soil due to its difficulty to be tilted (Islami and Utomo 1995).

Table 6. Chemical Condition of Initial Soil Condition in Field

Depth	Sample	pH	C Total (%)	N Total (%)	P-Bray (ppm)	Cation Content (me/100 g)				Texture (%)		
						Ca	Mg	K	CEC	Sand	Dirt	Clay
0-20 cm	1	4.8	2.78	0.33	12.3	4.48	2.09	0.48	24.59	7	30	63
	2	4.8	2.77	0.37	11.7	4.62	1.60	0.35	16.88	7	27	66
	3	5.1	2.64	0.26	12.6	4.71	1.68	0.33	20.18	5	33	62
Average		4.9	2.73 (M)	0.32 (M)	12.2 (L)	4.60 (L)	1.79 (M)	0.39 (M)	20.55 (M)	6	30	64
20-40 cm	1	4.8	2.75	0.34	12.6	4.41	2.04	0.40	23.68	7	30	61
	2	4.6	2.74	0.37	12.0	4.55	1.46	0.37	17.98	7	27	71
	3	5.0	2.61	0.25	12.9	4.64	1.72	0.28	20.29	5	33	60
Average		4.8	2.70 (M)	0.32 (M)	12.5 (L)	4.53 (L)	1.74 (L)	0.35 (M)	20.65 (M)	7	26	67

Note: L = Low; M = Medium, H = High

Growth of Crop and Nutrient Content in *Damar* Leaves

The effects of $K_{75}M_{25}$ compost administration on *damar* tree height as well as nutrient content in the leaves are presented in Table 7. Statistical analysis showed that amount of $K_{75}M_{25}$ compost per plant hole from 0 kg to 5 kg were significantly different from one to another in terms of plant height and nutrient level in the leaves (dry basis). Plant height resulted from administration of 5 kg compost per plant hole was not significantly different with those of control and treatment with 1 kg compost. Compost prepared by Perhutani gave similar results to those using compost prepared in this experiment at the same amount (2 kg).

Nutrient Content of *Damar* Leaves

Level of compost resulted significant different in nutrient content in *damar* leaves when it was given from 0 to 5 kg per plant hole. The tendency of increasing level of nutrient as the result of increasing amount of compost on one year old damar seedlings is obviously seen in Table 7. High nitrogen absorption by the crop can be due to high fertilization (Nyakpa 1988). The crop can also absorb high concentration of nitrogen when level of phosphorous in the soil is low. This might the case in the soil on the research location (12.2-12.5 ppm).

Nitrogen is important for crop growth, because nitrogen is the main element in all proteins and nucleic acids, which are constituents of protoplasm that will affect cell dimensions. The cells become bigger and the cell walls become thinner that make the plant will grow faster [Russel (1963) in Siswoyo (1999)]. Nitrogen has a role in inducing the crop growth as a whole especially the stem, branch, and the leaves, also in producing chlorophyll for photosynthesis (Lingga 1991). In almost all crops N plays a role in regulating the use of K, P, and other nutrients (Soepardi 1983).

Table 7. Effect of $K_{75}M_{25}$ Compost per Plant Hole toward Plant Height and Nutrient Content in *Damar* Leaves

Compost per Plant Hole (kg)	Increasing Plant Height (cm)	Nutrient Content in Dry Leaves (%)*				
		N	P	K	Ca	Mg
0	2.0 c	1.51 c	0.15 b	1.12 b	0.56 c	0.23 b
1	2.1 c	1.53 c	0.16 b	1.17 b	0.59 c	0.27 b
2	4.7 a	1.76 b	0.22 a	1.41 a	0.82 a	0.39 a
3	4.6 ab	1.79 b	0.21 a	1.42 a	0.74 b	0.38 a
4	3.0 bc	1.98 a	0.23 a	1.48 a	0.74 b	0.37 a
5	2.8 c	2.05 a	0.24 a	1.52 a	0.75 b	0.39 a
LSD 5%	1.650	0.164	0.040	0.125	0.060	0.053
2**	**4.2**	**1.77**	**0.21**	**1.39**	**0.81**	**0.37**

Note: *average of 5 replicates; ** As compared to administration of 2 kg compost prepared by Perhutani per plant hole. The number in same column which is followed by the same letter is not significantly different in 5% test level

Table 7 also shows that there is tendency of increasing height with increasing absorption of nutrient when the plants were given $K_{75}M_{25}$ compost at the right dose. Excess nutrient will be stored in vacuole to a certain limit. However, excessive storage of nutrients will give poisoning effect due to imbalance in photosynthesis process and respiration of crop. Referring to the soil condition that is considered medium fertile, medium level of cation exchange capacity (20.55-20.65), the planting site does not need heavy fertilization. Based on these facts, 2 kg $K_{75}M_{25}$ compost will be sufficient dose for each *damar* seedling.

Effect of Java Tea Intercropping to Nutrient Balance

The following experiments were employed in the field having chemical properties at various depths as seen in Table 8. The soil was considered acidic, having a pH of pH 4.6. All properties of the soil is similar to that of earlier mentioned substrate, namely CEC of 22.73-22.76 (relatively responsive to fertilization), clayey soil (68-70% clay), high ability to absorb water and nutrition, difficult to release water (less available for crop), and considered as heavy soil (difficult to be tilted).

Table 8. Chemical Properties of Soil in the Planting Field

Depth	Sample	pH	C Total (%)	N Total (%)	P-Bray (ppm)	Cation Content (me/100 g)				Texture (%)		
						Ca	Mg	K	CEC	Sand	Dirt	Clay
0-20 cm	1	4.6	1.78	0.20	4.3	2.48	1.09	0.98	21.59	9	30	61
	2	4.4	1.77	0.18	3.9	2.49	1.24	0.13	22.13	7	22	71
	3	4.9	2.58	0.26	6.6	6.71	1.88	0.24	22.18	9	27	64
	4	4.2	1.83	0.20	2.8	1.84	0.64	0.88	21.66	3	24	73
	5	4.6	2.17	0.25	3.9	3.12	1.10	0.81	24.43	3	23	74
	6	4.7	2.37	0.20	3.2	3.64	1.14	1.22	24.56	8	25	67
Average		4.6	2.10 (M)	0.22 (M)	4.1 (VL)	3.38 (L)	1.18 (M)	0.71 (H)	20.55 (M)	7	25	68
20-40 cm	1	4.6	2.54	0.28	4.2	3.07	1.04	0.91	22.93	7	25	68
	2	4.6	2.16	0.26	5.2	3.43	1.42	0.12	21.66	6	26	68
	3	4.8	1.93	0.18	5.3	4.71	1.52	0.17	20.17	8	22	70
	4	4.3	2.21	0.25	3.7	2.05	0.67	0.94	24.12	4	23	73
	5	4.7	2.80	0.31	2.9	4.17	1.32	0.86	24.27	3	25	72
	6	4.7	1.60	0.17	4.0	2.94	1.17	1.12	23.23	10	22	68
Average		4.6	2.10 (M)	0.24 (M)	4.2 (VL)	3.40 (L)	1.19 (M)	0.69 (H)	22.73 (M)	6	24	70

Note: VL = Very Low; L = Low; M = Medium, H = High

Based on data in Table 8, reserved nutrients in soil can be calculated (Table 9). Nitrogen element was the most abundant, but phosphorous was the least. High nitrogen content might be linked to high precipitation in the location under study. Low level of phosphorous might be correspondence to acidic condition of the soil (pH 4.6). At acidic soil, most phosphorous is bound (fixed) by aluminium and mostly reside in lower part of the soil.

Table 9. Reserved Nutrients in the Soil (L = Low; M = Medium; H = High)

Depth	Nutrient Content									
	me/100 g					kg/ha				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
0-20 cm	0.22 (M)	4.10 (L)	0.71 (H)	3.38 (L)	1.18 (M)	3608	6.72	454.12	1108.64	232.22
20-40 cm	0.24 (M)	4.20 (L)	0.69 (H)	3.40 (L)	1.19 (M)	3984	6.97	446.71	1128.80	237.05
Total	0.46	8.30	1.40	6.78	2.37	7592	13.69	900.83	2237.44	469.27

Economic Analysis of Intercropping System

Java tea is a commercial crop. Net benefit per hectare field intercropped and without intercropped with java tea is estimated and the results in Indonesian rupiah currency are presented in Table 10. Net benefit from the field intercropped with java tea is only Rp 3,165,000.while without java tea intercrop is higher (Rp 3,779,167).

Table 10. Net Benefit per Hectare Field in 4 Months

System	Plot	Benefit (× Rp 1000)			Cost (× Rp 1000			Net Benefit	B/C Ratio
		Maize	Java Tea	Total	Maize	Java Tea	Total	(x Rp 1000)	
Java Tea Intercropped	**1**	5,625.0	1,675.0	7,300	2,847.5	955.0	3,802.5	3,497.5	1.92
	2	5,625.0	1,600.0	7,225.0	2,902.5	972.5	3,875.0	3,350.0	1.86
	3	4,875.0	1,550.0	6,425.0	2,757.5	1,027.5	3,785.0	2,640.0	1.70
	Avg.	5,375.0	1,608.0	6,983.3	2,835.8	985.0	3,820.8	3,162.5	1.83
Without Java Tea Intercropped	**1**	6,937.5	-	6,937.5	2,942.5	-	2,942.5	3,995.0	2.36
	2	6,900.0	-	6,900.0	3,042.5	-	3.042.5	3,857.5	2.27
	3	6,412.5	-	6,412.5	2,927.5	-	2,927.5	3,485.0	2.19
	Avg.	6,750.0	-	6,750.0	2,970.8	-	2,970.8	3,779.2	2.27

However, it is important to note that the calculation is based on the first four-month of cultivation (first quarter) in which the first two months there is no java tea leaf-harvest. If the estimate is extended to one year with an assumption that there is no price escalation in the next two quarters and there is no depreciation on equipment, the net benefit of java tea intercropping is presented in Table 11. In one year cultivation using java tea and maize intercrops the net benefit is Rp 12,930,834, which is four times higher than the net benefit in the first four months. If it is only maize intercrop, the net benefit during one year cultivation is Rp 11,337,501, or three times higher than that of the first four months. Comparing the net benefit per hectare for one year cultivation, java tea intercropping is Rp 1,593,333 higher than maize intercropping only.

Analysis Based on Nutrient Balance and Net Benefit

It is previously described that estimated net benefit per hectare per year of java tea intercropping is higher as compared that without. However, the net benefit value must be corrected to the input fertilizers in order to maintain the level of K, Ca, and Mg nutrients. Considering an estimated fertilizer to be added in one year cultivation, and with an assumption that KCl is Rp 3,000/kg and dolomite is Rp 1,000/kg, the estimated net benefits from the field intercropped with and without java tea are presented in Table 12 and Table 13, respectively. The net benefit is Rp 12,375,105 for java tea intercropping and only Rp 10,956,822 without java tea intercropping. The difference of Rp 1,418,283 is due to java tea as intercrop.

Table 11. Estimated Net Benefit per Hectare in 1 year

Items	Net Benefit in Quarter (Rp/ha) I	II	III	Total
With Java Tea Intercropping				
Benefit				
Maize	5,375,000	5,375,000	5,375,000	16,125.000
Java Tea	1,608,333	3,216,667	3,216,667	8,041,667
Total	6,983,333	8,591,666	8,591,666	24,166,667
Cost				
Maize	2,835,833	2,835,833	2,835,833	8,507,499
Java Tea	985,000	871,667	871,667	2,728,334
Total	3,820,833	3,707,500	3,707,500	11,235,833
Net Benefit				
Maize	2,539,167	2,539,167	2,539,167	7,617,501
Java Tea	623,333	2,345,000	2,345,000	5,313,333
Total	3,162,500	4,884,167	4,884,167	12,930,834
Without Java Tea Intercropping				
Benefit	6,750,000	6,750,000	6,750,000	20,250,000
Cost	2,970,833	2,970,833	2,970,833	8,912,499
Net Benefit	3,779,167	3,779,167	3,779,167	11,337,501

Table 12. Estimated Net Benefit per Hectare by Quarter from Java Tea Intercropping System Corrected by Fertilizer Cost

Items	Net Benefit in Quarter (Rp/ha) I	II	III	Total
Benefit				
- Maize	5,375,000	5,375,000	5,375,000	16,125.000
- Java Tea	1,608,333	3,216,667	3,216,667	8,041,667
Total	6,983,333	8,591,666	8,591,666	24,166,667
Cost				
- Maize	2,995,394	3,033,917	3,033,917	9,063,228
- Java Tea	985,000	871,667	871,667	2,728,334
Total	3,820,833	3,707,500	3,707,500	11,235,833
Net Benefit				
- Maize	2,379,606	2,341,083	2,341,083	7,061,772
- Java Tea	623,333	2,345,000	2,345,000	5,313,333
Total	3,162,500	4,884,167	4,884,167	12,375,105

Table 13. Estimated Net Benefit per Hectare by Quarter without Java Tea Intercropping System Corrected by Fertilizer Cost

Items	Net Benefit in Quarterly year (Rp/ha) I	II	III	Total
Benefit	6,750,000	6,750,000	6,750,000	20,250,000
Cost	3,097,726	3,097,726	3,097,726	9,293,179
Net Benefit	3,652,274	3,652,274	3,652,274	10,956,822

RECOMMENDATION

If *meniran* leaves are not available, java tea extract alone can be used as an activator in composting mahogany leaves. However, further observation must be made on the growth of *damar* as the main crop. It is recommended to develop intercropping system between java tea plant and *damar* plantation to get better growth of the plantation, and better economic income, as well.

REFERENCES

Hardjowigeno S. 1995. *Ilmu Tanah (Soil Science)*. Jakarta: Akademika Presindo

Indranada HK. 1986. *Pengelolaan Kesuburan Tanah (Soil Fertility Management*). Jakarta: PT Bina Aksara

Islami T & Utomo WH. 1995. *Hubungan Air Tanah dan Tanaman (Correlation between Soil Water and Plants)*. Semarang: IKIP Semarang Press

Lingga P. 1991. *Petunjuk Penggunaan Pupuk (Fertilizing Guidelines)*. Jakarta: Penebar Swadaya

Nyakpa MY. 1988. *Kesuburan Tanah (Soil Fertility*). Bandarlampung: Penerbit Universitas Lampung

Soepardi G. 1983. *Sifat dan Ciri Tanah (Properties and Characteristics of Soils*). Bogor: Faculty of Agriculture, Bogor Agriculture

THE CHALLENGES TO THE UTILIZATION, TRADE, AND CONSERVATION OF FOREST MEDICINAL PLANT

Tajudin Edy Komar

Forest and Nature Conservation Research and Development[1]

ABSTRACTS

Non-timber forest products including medicinal plants have been becoming important products. These products are harvested from both natural and artificial forest. However, market is still a key barrier for its further development, especially for medicinal plants and some other products. Exceptions for these are rattan, agarwood, natural honey, and some others. Some authors have summarized characteristics and strategy to deal with the barrier as well as conservation needs for their sustainable utilization.

INTRODUCTION

Floristic diversity in natural and artificial forests now receives growing attention. This is because many Non-Timber Forest Products (NTFP) including medicinal plants which originally from natural and planted forests have been widely promoted and demanded even though their specific uses is still under observation for some species. Some of those have been readily available in the market are growing popular for both traditional and modern uses.

Until the advent of modern medicine, human life is dependent mostly on the plants for treating various ailments in human and livestock. Human communities around the globe have accumulated a huge stock of indigenous knowledge on medicinal uses of plants and its related uses. This includes as poison for fish catching and animal hunting, purifying water for drinking purposes and for controlling pests and diseases of crops and livestock (Rao *et al*. 2004).

Various traditional uses for medicinal plants by some local community or ethnic group have been explored in East Kalimantan (Shell *et al* 2002) and some other areas. Some local communities in Sumatra, such as Kubu in Jambi have utilized extracts of plants to cure various ailments and or other diseases related syndrome, such as dragon blood, a substance extracted from certain rattan species. Local community in South Sumatra has used a plant species as poison to catch wild fish living in the river.

Technology has given a vast contribution to the change in the use of the medicinal plants from traditional uses to modern day related life not only to cure various ailments but also to improve healthiness, stamina, and other corresponding healthy life. Many countries have officially encouraged the use of extracts from medicinal plants to substitute the modern-day medicines.

From a number of species, it is predicted that there are still huge number of species remain unexplored for NTFP and source of medicinal extracts. Several efforts to provide medicinal plant explored from forest diversity have been carried out in various way. Forestry and other related institutions have managed to explore those from a

[1] Jalan Gunung Batu No. 5, Bogor, Indonesia; Email: raminppd87@yahoo.com

wide range of natural forest types: mangrove, peat swamp until mountain forest, even in agroforestry systems (Rao *et al.* 2004). The modern herbal industry has stimulated this endeavour. These industries, such as jamu in Java, have long been interested in the investment of developing medicinal plant industry for many purposes from traditional uses to modern usage to substitute relatively higher price medicine. In Indonesia, there are some examples for these types of medicine extracted from plants: *Morinda citrina* (Java nonny), buah merah (extract of fruits from a species of Pandanaceae naturally growing in Papua), and some others. The extracts of these species are believed to be useful to cure high blood pressure, heart related diseases, diabetes, etc.

THE UTILIZATION AND TRADE OF MEDICINAL PLANTS: PROBLEMS RELATED TO ITS DEVELOPMENT

Primary problem related to the development of medicinal plants is the discontinuous market needs and market networks. As other most NTFP products, with some exception for rattan, bamboo, and agarwoods, the market share is relatively small and the value is also relatively low. This low value causes lack of cultivation efforts. Supply which is solely dependent on wild sources is in general unable to compete with other similar products.

Marshall *et al.* (2006) identified that lack of market information is the key barrier in the trade of NTFPs. In most cases, little could be done to deal with this trade barrier. Below is the summary of their key findings in the trade of NTFP, which are believed to be applicable for the trade of medicinal plants:

1 The fact that in most cases there are lacks of market contacts and knowledge. In Indonesia the role of the third party, called *tengkulak*, is dominant in determining market for most agricultural products produced in rural areas. The traditional producers are also mostly having little financial capability and poor infrastructures, which will, in turn, limit the weak producers, processors and traders to reach advanced market.

2 The real value of market information is to ensure that the commercialization process is equitable, efficient, and sustainable.

3 The important of good organization of NTFP producers and processors could contribute to the improvement of product quality and quantity, more cost effective in transportation and the increase in negotiating ability.

4 The available access to financial support, such as credit, could enable poor people to improve their NTFP based income generation through increased volume of trading, and improved quality.

5 The general improvement of market, transports, and communications infrastructures could facilitate commercialization of the NTFP, including medicinal plants.

6 There is no significant difference in formal education between household engaged in NTFP commercialization and those that are not, although NTFP traders often have significantly higher levels of education than producers.

7 Existing traditional knowledge can be very important in determining a community's interests and capacity to successfully commercialize an NTFP.

THE VALUE OF NTFP AS ALSO IN MEDICINAL PLANT

The products from medicinal plants vary and the consumption is highly dynamic and probably similar to the rest of Non-timber forest products with some exception. Bamboo, rattan, agarwood, etc. are relatively constant in demand and high in economic value per unit quantity. Marshall *et al*. (2006) observed highly dynamic of value chain in NTFP with the following characteristics:

1 Innovation, both in term of resources management and product processing and marketing, is often critical to maintaining market share. This phenomenon occurred in many places. Pineapple production in East Kalimantan and ginger production (a species of Zingiberaceae) in some places in West Java and South Sumatra are some examples.

2 A specialized market niche and product quality could contribute against substitutions. Some products may capture consumer interest last longer than some other products. When the scarcity and prices go up there will be a tendency to see other product substitutions.

3 Most NTFP value chains (this includes other products) are demand-driven. Therefore, the production of a new one solely on the basis of existing supply will be unlikely to be successful.

4 The viability of a particular NTFP value chain may also depend on demand for other products.

5 Entrepreneurs could play a key role in facilitating access to markets by providing information, skill, and financial supports.

6 Concentration of power in the hands of a few is most likely in the value chain of highly processed or perishable products for an International market.

CONSERVATION OF NTFP PRODUCING PLANTS

The unwise and unlimited exploitation of products for both NTFP and medicinal plants will cause the degradation of the population and the habitat. In the long term, these will reduce their natural production capability. Some species may have gone to extinction. A good example for this case is the hunting of agarwood, *gaharu*, in Kalimantan and other places in Sumatra. This market-driven activity has made local community to search the naturally growing plants of *Aquilaria malaccensis* and other agarwood producing species to be cut down without any prior knowledge on the presence or absence of the agarwood in the stem. Similar situation occurred to the dragon blood hunting in Sumatera. Its high economic value has attracted local community to leave their previous activity to be NTFP product hunters. This phenomenon is found in many places, especially in developing community.

Donor investments, not only those for producing NTFP and medicinal plants but also other forestry related activity, mostly failed to assist poverty alleviation, especially for local community. They also least contribute to the improvement in the conservation of natural resources (Marshall *et al.* 2006). Therefore, intervention in the managing NTFP including medicinal plants to ensure the contribution to community prosperity and

conservation is critical important. Government rule and regulation on the exploitation of previously abundant in source need to be provided before becoming scarcity. Species listing in CITES appendix has been valuable in reducing uncontrolled International trade of wild fauna and flora. However, 'trade mafia' has destroyed those valuable efforts as occurred to *Aquilaria* spp.

Domestication (followed by cultivation) efforts could contribute to the conservation of the NTFP producing species and medicinal plants. Community participation in the conservation and cultivation is a great important to achieve the overall objective in the sustainable uses of natural resources. Biopharmaca (in cooperation with ITTO) has initiated this effort and choose some wild species of flora to begin with (*Eurycoma latifolia* (pasak bumi), *Ficus deltoidea* (tabat barito) and some others). Conservation of species in their natural habitat, *in situ*, has been incorporated into the management of conservation area (national park, nature reserves, and botanical gardens). Conservation in outside the natural habitat, *ex-situ*, has been growing popular in the form of home gardens by both traditional and modern community. However, strong human intervention in their life cycles, need to be considered for long term conservation.

REFERENCES

Marshall E, Scheckenberg K, Newton AC. 2006. *Commercialization of Non-timber Forest products*. The Overstory # 168. www.agroforestry.net/events

Rao MR, Palada MC, Becker BN. 2004. *Medicinal and aromatic plants in Agroforestry*. The Overstory #135. www.agroforestry.net/events

Shell D *et al*. 2002. *Exploring biological diversity, environment and local people's perspectives in forest landscape*. CIFOR

Nurrochmat D, 2005. Peluang Usaha Pengambangan Tanaman Obat. Presented in Training Workshop. Biopharmaca-ITTO.