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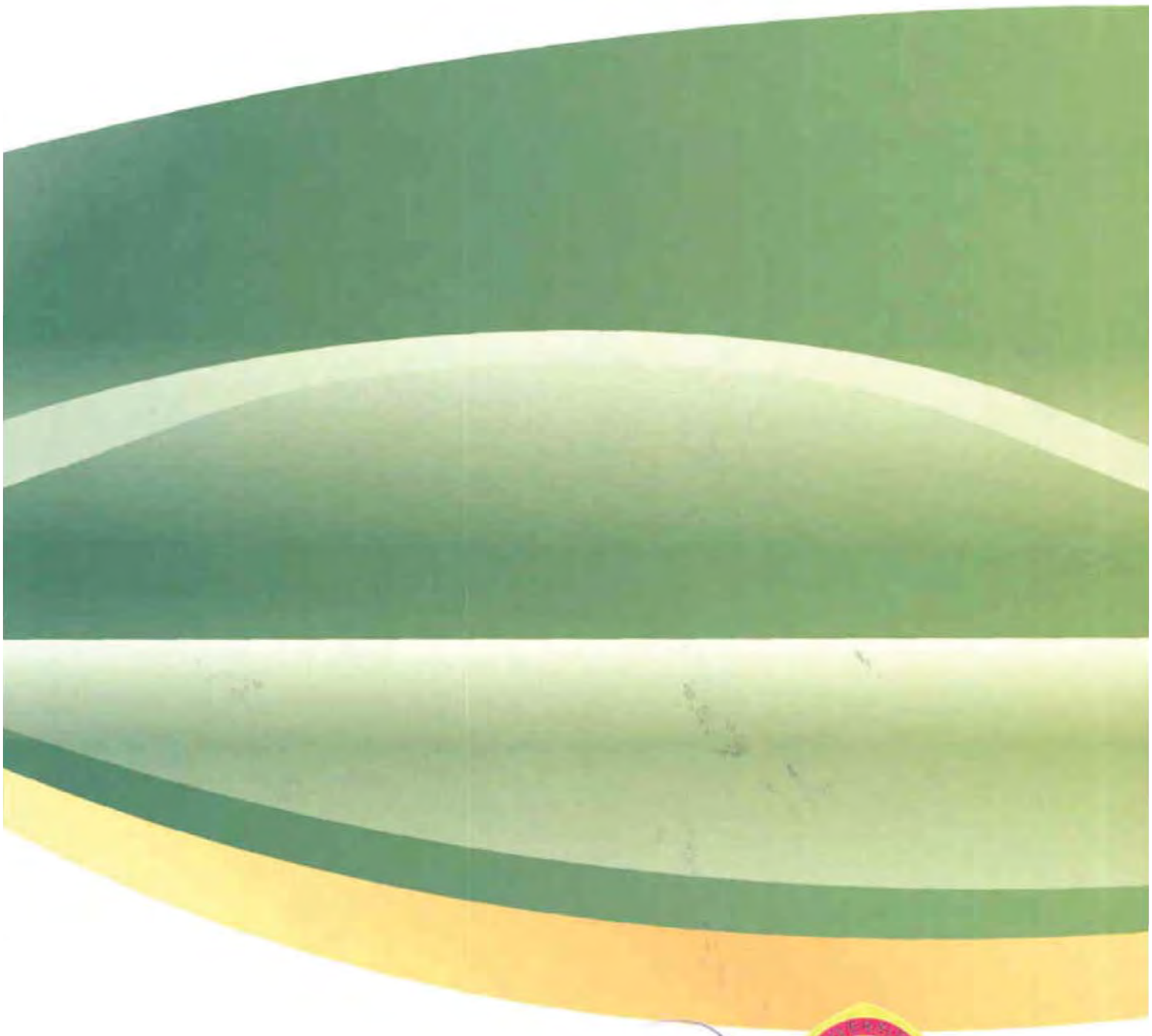

INTERNATIONAL CONFERENCE
NAME 2015
NATURAL, MATHEMATICAL AND ENVIRONMENTAL SCIENCES

FACULTY OF MATHEMATICS AND NATURAL SCIENCES
LAMBUNG MANGKURAT UNIVERSITY

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PREFACE

Assalamu'alaikum Wr. Wb.

All praises are due to ALLAH SWT, God Almighty, Who made this International Conference of successful. The International Conference on Natural, Mathematical and Environmental Sciences (NAMES) 2015 is organized by the Faculty of Mathematics and Natural Sciences (FMIPA), Lambung Mangkurat University, Banjarbaru, South Kalimantan. This conference covered a wide range of topics, including biology, chemistry, physics, mathematics, pharmacy, computer science, material Science, and environmental science.

All papers were compiled into the proceedings book which had six sections, namely Biology, Pharmacy, Chemistry, Mathematics, Physics, and Computer Sciences. This book was also publised in the NAMES Website http://names.fmipa.unlam.ac.id. I am glad that for the first time both types of books can be realized.

The seminar took a theme of "Sustainable Development" as a hot issue in Banjarbaru. Banjarbaru, is a fast growing city in the province of South Kalimantan, Indonesia and famously known as an urban city with a unique natural landscape, a cultural diversity, and a friendly welcoming citizen. Moreover, Banjarbaru becomes the centre of provincial government that its government is located in Banjarmasin today. The conference provided an ideal platform to share information and discuss their scientific results and experiences, with particular references to sustain development.

I was fully satisfied to all the members of the program committee who contributed for the success in framing the program and the books. My appreciation was especially from all delegates who contributed to the success of this conference by accepting our invitation and submitting articles for presentation in the scientific program.

I can guarantee you that this book provides a full of intellectual scientific research activities. I do hope the next conference will pick up similar success, and even better.

Wassalamualaikum Wr. Wb.
Banjarbaru, March 2016

Dr. Krisdianto
Chief of Executive

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The Profile of Volatile Oil on *Blumea balsamifera* L. Calli

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ABSTRACT

This research was aimed to examine the contain of volatile oil on *Blumea balsamifera* calli and compare it with the leaves of the plant. The calli induction from the leaves was done on the media of Murashige-Skoog (MS) by adding Naphtalene Acetid Acid (NAA) 1.0 and 1.5 mg/L and combining it with Benzyl Adenin (BA) 0.05 mg/L. Furthermore, it was sub-cultured into the same media. The growth of calli on NAA 1.5 mg/L was better than NAA 1.0 mg/L. 28 days old calli was extracted by using hexane and the contain of volatile oil was analyzed by using the thin layer of chromatography (TLC). The comparison of TLC profile in volatile oil between calli and leaves of *Blumea balsamifera* showed the difference on amount of spot and Rf value.

Key words: *volatile oil, calli, Blumea balsamifera L.*

INTRODUCTION

The whole plant and its crude extracts of sembung (*Blumea balsamifera* L.) has numerous biological activities, such as antitumor, antioxidant, antimicrobial and anti-inflammation. The content in sembung are volatile oil, borneol (Bhuiyan *et al.*, 2009), sineol, limonene, myristin, sesquiterpenoids (Jiang *et al.*, 2014), tanin and glikosida (Jahan *et al.*, 2014). Volatile oil can be found mostly in the leaves (0.5%), where the main component is borneol (25%) (Oyen, 1999).

The method of plant tissue culture gives possibility to do synthesis of natural chemical compound in the form of secondary metabolites (Zhou *et al.*, 2006). Organ or plant tissue, which is cultured using tissue culture, may produce identical secondary metabolites compared to the original plant or it can produce compound which is totally different from the original plant, or it may not even produce specific compound as the original plant (Indrayanto, 1987).

Naturally, the leaves of *Blumea balsamifera* (L.) DC contain the secondary metabolites in the form of volatile oil, however, if it is bred with tissue culture using callus culture, it does not necessarily contain volatile oil, so the existance of volatile oil should have been futher analyzed. Callus induction of the leaves is using auxin as growth regulator or the combination of auxin and cytokinin. Auxin has bigger impact on biosynthesis of secondary metabolites compared to cytokinins (Prins *et al.*, 2010).

This research aimed to compare the TLC profile of volatile oil on *Blumea balsamifera* L. planted in MS media with BA 0.05 mg/L, treated with NAA 1 mg/L and 1.5 mg/L, with the plant.

MATERIALS AND METHODS

This research was conducted at the biotechnology laboratory, Faculty of Pharmacy, University of Surabaya. The leaves of *Blumea balsamifera* was sterilized in laminar air flow (LAF), using bleach (NaOCl), then it was planted in MS media with BA 0.05 mg/L and treated with NAA 1.0 mg/L and 1.5 mg/L, to do induction for forming calli. The calli then was sub cultured in the same media. The measurement of calli growth index was done on every 7 days by comparing the last weight with the beginning weight of fresh calli. Then, the result of the harvest was dried up. The process of drying was done in the room temperature, and then it was weighing until it was constant. The dried powder (500 mg) callus or leaves of *Blumea balsamifera* L was extracted using 3 ml hexane solvent for 15 minutes by using vortex, then it was filtered and the filtrate is separated. Furthermore, the extract was put a drop on 60_{F254} silica gel for 1 capillary 2 µL (Oyen, 1999).

The identification of volatile oil was done in qualitative by comparing TLC profile of *Blumea balsamifera* L. volatile oil planted on MS media with BA 0.05 mg/L and NAA 1.0 mg/L with MS of BA 0.05 mg/L and NAA 1.5 mg/L of the natural *Blumea balsamifera* L. leaves; it was including the amount, color and the Rf value of the spots. The observed calli was 28 days old calli. Dry extracted (0.5 g) calli or leaves of *Blumea balsamifera* L. plant was dissolved in hexane p.a. The volatile oil TLC analysis of *Blumea balsamifera* L. plant and calli used silica gel 60_{F254} as stationary phase, mobile phase toluene : ethyl acetate (93 : 7), and H_2SO_4p Anisaldehide as visualization spot. Anisaldehide 0.5 mL was mixed with 10 mL glacial acetate acid and add it up with 85 mL methanol and 5 mL concentrated sulfuric acid. After being sprayed with anisaldehide, TLC plate was heated in oven with 100°C for 5 – 10 minutes. If the result of TLC is blue-purple, blue, red-orange, yellow-brown, red-brown, red-purple, then it can be concluded that the extract contains the component of volatile oil (Wagner, 1996).

RESULTS

The visual appearance of *Blumea balsamifera* on 7, 14, 21, 28 and 35 days which was cultured on MS media with NAA 1.0 mg/L was shown in Figure 1, and Figure 2 showed calli which was cultured with NAA 1.5 mg/L.

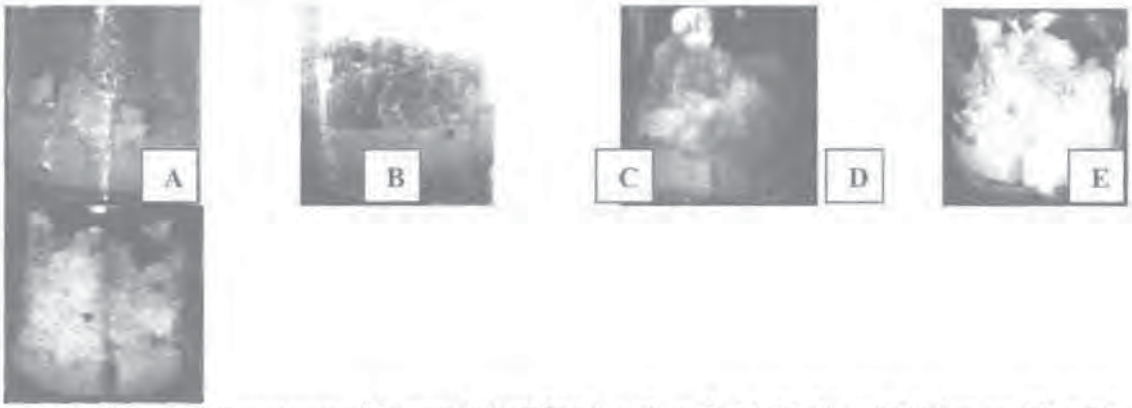

Figure 1. The visual appearance of *Blumea balsamifera* L. calli on 7, 14, 21, 28 and 35 days on MS media containing BA 0.05 mg/L with the treatment of NAA 1.0 mg/L; (A) 7-days calli (B) 14-days calli (C) 21-days calli (D) 28-days calli (E) 35-days calli

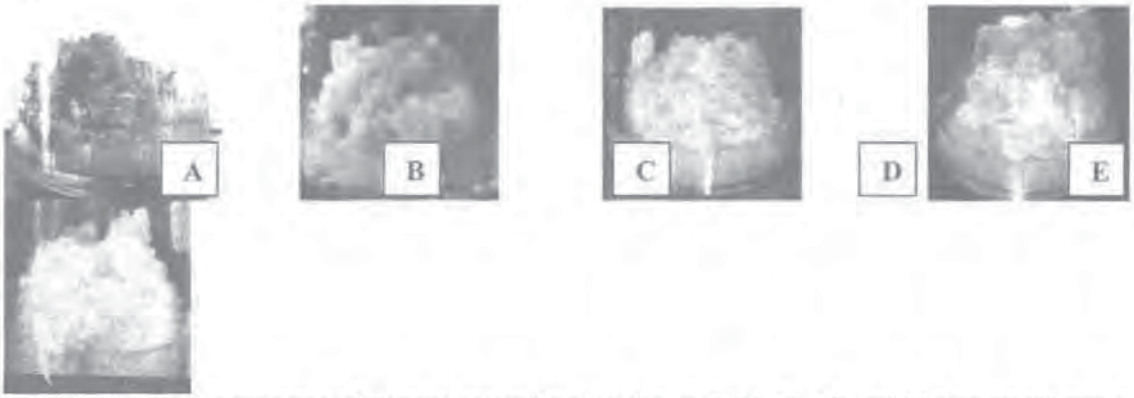

Figure 2. The visual appearance of *Blumea balsamifera* L. callus on 7, 14, 21, 28, and 35 days on MS media containing BA 0.05 mg/L with the treatment of NAA 1.5 mg/L; (A) 7-days calli, (B) 14-days calli, (C) 21-days calli, (D) 28-days calli, (E) 35-days calli

From Figure 3 and Table 1, based on growth index, *Blumea balsamifera* was grown better on MS media + BA 0.05 mg/L with the treatment of NAA 1.5 mg/L compared to the treatment of NAA 1.0 mg/L. NAA auxin has important role in cell division, and the increase on NAA concentration

can increase the speed of cell division, however, callus will be white. High concentration auxin causes cell unable to form chlorophyl.

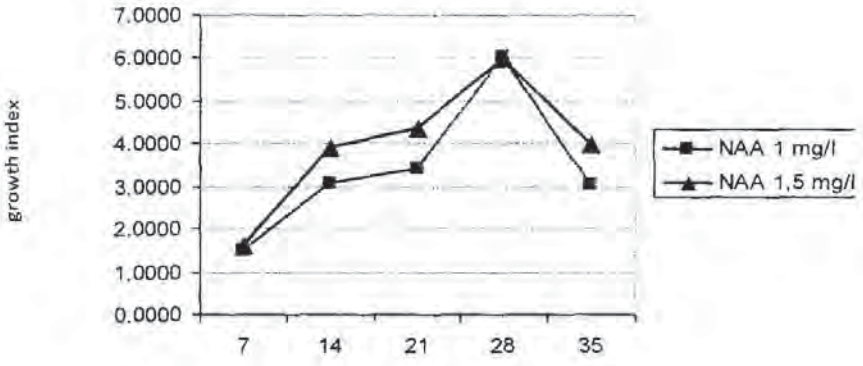

Figure 3. The profile of *Blumea bals* : on MS media containing BA 0.05 mg/L with the treatment of NAA 1.0 mg and NAA 1.5 mg/L.

Table 1. The area under the curve (AUC) from growth index of *Blumea balsamifera* L. calli for the 7th day until the 35th day.

Replication	AUC growth index	
	NAA 1,0 mg/l	NAA 1,5 mg/l
1	111.2528	127.4945
2	111.5496	127.7435
3	112.4474	128.8992
∑	335.2498	384.1372
$\bar{X}$	111.7499 a	128.0457 b

*Numbers followed by letters are those ones totally different from t-test at α=5%

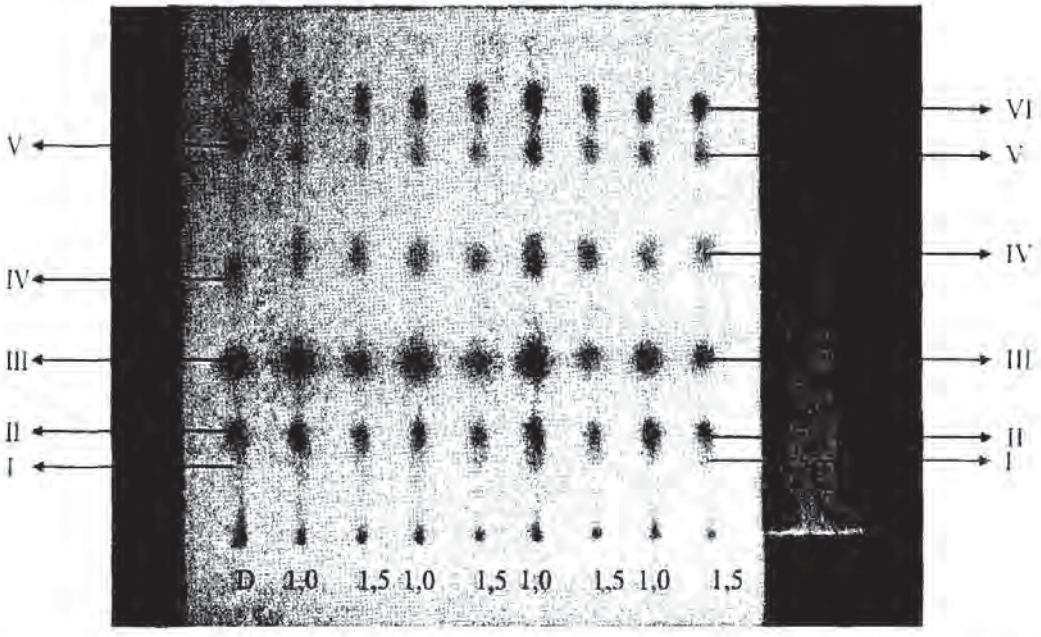

Figure 4. Volatile oil chromatogram of *Blumea balsamifera* calli extract grows in MS media with NAA 1.0 mg/L and BA 0.05 mg/L, and MS media with NAA 1.5 mg/L and BA 0.05 mg/L and with the leaves of *Blumea balsamifera* L.

D : Extract of *Blumea balsamifera* L. leaves
1 : extract calli in MS media +NAA 1.0 mg/L + BA 0.05 mg/L
1.5 : extract calli in MS media + NAA 1.5 mg/L + BA 0.05 mg/L
Stationary phase : silica gel 60_{F254}
Mobile phase : Toluen : ethyl acetate (93 : 7)
Visualization spot : Anisaldehide - H_2SO_4p
Solvent : Hexane p.a.

Table 2. The comparison of volatile oil KLT profile in callus and in the leaves of *Blumea balsamifera* L.

	Σ	Spot I		Spot II		Spot III		Spot IV		Spot V		Spot VI	
	Spot	Colour	Rf	Colour	Rf	Colour	Rf	Colour	Rf	Colour	Rf	Colour	Rf
Leaves	5	Light green	0,13	Blue violet	0,20	Green	0,33	Red purple	0,51	Green	0,78	-	-
Callion NAA 1.0 mg/L	6	Light green	0,16	Blue violet	0,20	Green	0,33	Red purple	0,56	Green	0,76	Blue purple	0,85
Callion NAA 1.5 mg/L	6	Light green	0,16	Blue violet	0,20	Green	0,33	Red purple	0,56	Green	0,76	Blue purple	0,85

DISCUSSION

The results showed that 6 kinds of compounds were separated from volatile oil of *Blumea balsamifera* calli and 5 kinds of compounds on leaves (Figure 4 and Table 2). It was known the spots which could not be found in plant but it was found in calli, the spots were light green with Rf 0.16; red purple spots with Rf = 0.56, green spots with Rf = 0.76; blue purple spots with Rf 0.85. Besides that, there was also some spots that could not be found in calli, such as light green spots with Rf 0.13, red purple spots with Rf 0.51; green spots with Rf 0.78. It showed that on *Blumea balsamifera* L. calli, the same volatile oil was formed in the plant, and also there was component of volatile oil found was not the same with the plant. The possible cause could be the enzime which involve in the process of secondary metabolite biosynthesis, is repressive, or because there is no capability of storing the product, so there is product degradation. On the contrary, there is the form of volatile oil component in calli but it does not exist in the plant, it is possible because of the influence of culture medium component (Indrayanto, 1987). The increase of NAA auxin (1.0 and 1.5 mg/L) does not affect the changing of volatile oil component in *Blumea balsamifera* L. calli.

CONCLUSION

Blumea balsamifera L calli grew better in MS media with BA 0.05 mg/L with the treatment NAA 1.5 mg/L compared to NAA 1.0 mg/L. The difference concentration of NAA does not affect the TLC profile of volatile oil on *Blumea balsamifera* L. calli, but there was difference when it was compared with the leaves of *Blumea balsamifera* L., which was planted conventionally.

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