13. Production of secondary metabolites trimethyl xanthina by Camellia sinensis L suspension culture

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PRODUCTION OF SECONDARY METABOLITES *TRIME THYL XANTHINA* BY *CAMELLIA SINENSIS L* SUSPENSION CULTURE

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Abstract. Bioactive *trimethyl xanthina* can be obtained from the plant *Camellia sinensis L.* To obtain bioactive plant of which there are several hurdles for instance to wait up to five years to be harvested, also it needs land at a certain height from the sea level. Therefore, the production of secondary metabolites *trimethyl xanthina* need to be developed with suspense culture techniques. The purpose of this study obtained the production of bioactive *trimethyl xanthina* way culturally suspense in large scale with a relatively short time, potentially as anti-oxidants. Research methods include: (1) initiation of callus from pieces of leaves, shoots the youngest of the plant *Camellia sinensis L* in the media MS with the optimization of the addition of growth regulators, (2) the subculture of callus on media and plant growth regulator that is equal to the stage of initiation, (3) initiation of suspension culture using explants of callus *Camellia sinensis L*, (4) Analysis of secondary metabolites *trimethyl xanthina* growth in suspension culture, (5) the isolation and identification *of trimethyl xanthina* qualitatively and quantitatively using thin layer chromatography / high performance chromatography column. The results of the study suspension cultures containing bioactive *trimethyl xanthina* candidates that can be used as an antioxidant.

Keywords: *Suspension Culture, Cammelia sinensis L,* bioactive *trimethyl xanthina.*

INTRODUCTION

Bioactive *Trimethyl xanthina* can be obtained from the plant *Camellia sinensis L.* Relevant to research Amra et al. [1] *Trimethyl xanthina* on young leaves of the plant Camellia sinensis L reached 2.4% and the content of old leaves *Trimethyl xanthina* declined by 50% [2]. As the bioactive ingredient has anti-diabetic effect [3], on agriculture can be as alelopaty [4], and anti-bacterial in the area of food - beverages, such as in soy juice beverage storage [5].

Some of the obstacles are, the availability of the plant, including waiting time of up to five years to be harvested, but it requires land at an altitude of 800-1200 m above sea level, Shane *et al.* [6,7]. Therefore, the production of secondary metabolites *Trimethyl xanthina* need to be developed, namely the suspension culture technique.

Suspension technique is a method of *in vitro* culture are important, which can be used as a basis for the study of plants that includes: biochemistry, biomolecular, biosynthetic, production of secondary metabolites with a large scale in a bioreactor. Besides, the suspension culture has several advantages for i.e: able to study the model of a plant cell in a rapid, uniform manner to optimize nutrition, plant growth regulators, also elicitor [8]. Associated with the production of secondary metabolites, these techniques act as rapid chemicals exploitation, simultaneously, so that it will support the exploitation of agribusiness than have highly commercialization competitive.

Trimethyl xanthina biosynthesis of secondary metabolites in plant *Camellia sinensis L* by Ashihara et al. [9] influenced by the presence of the enzyme *xanthosine* experiencing gradual methylation. The existence of this enzyme *in vitro* culture Callus will increase metabolite, if the added by precursor adenosine, guanosine and hypoxanthine [10]. This study aims to obtain a secondary metabolite production methods *Trimethyl xanthina* with *in vitro* suspension culture techniques in an attempt to obtain bioactive materials on a large scale.

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MATERIALS AND METHODS

Production Research of secondary metabolites *Trimethyl xanthina* through suspension cultures of plant *Camellia sinensis L*, made through several processes, namely: (1) initiation of callus from pieces of leaves, shoots the youngest of the plant *Camellia sinensis L* on Murashige and Skoog (MS), with the optimization of the addition of regulators grow, 6-Benzylaminopurine (BAP) 1 ppm, (2) the subculture of callus on media and growth regulator that is equal to the stage of initiation, (3) initiation of suspension culture using explants callus initiation *Camellia sinensis L* results 1 and 2 [11]. Callus was cut by approximately 1 mm then weighed as much as 100 mg in LAFC (Laminair Air Flow Cabinet). Callus was weighed, and then dissolved in liquid MS medium supplemented precursor phenyl alanine 400 ppm, incubated in incubation room, shake with a speed of 100 rpm. (4) Analysis of secondary metabolites *Trimethyl xanthina* growth in suspension culture. (5) Isolation and identification of *Trimethyl xanthina* qualitatively and quantitatively using thin layer chromatography / high performance chromatography column. After 30th day suspension culture *Camellia sinensis L* harvested weighed, and then extracted using warm distilled water as solvent, chloroform. Dirotavapour chloroform phase until visible stain dries. Noda dried chloroform phase was dissolved in methanol for analysis menggunakaan thin layer chromatography / TLC, others observed the morphology and shape of cell suspension, using light mikroskup triokuler brand Olympus BX 41.

RESULTS AND DISCUSSIONS

At 40 days after the initiation of callus from *Camellia sinensis L* leaf explants on MS medium with BAP 1ppm obtained successive morphological changes occur bubbling - twist, edge sprung up to spread over the surfaces of the callus explants formed callus (Fig.1)

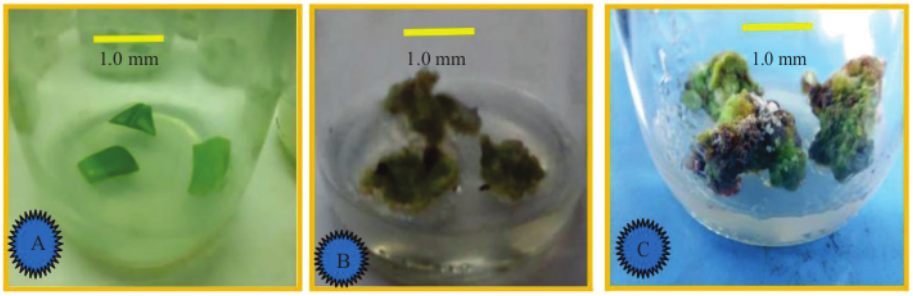

FIGURE 1. Initiation of callus from *Camellia sinensis L* leaf explants on MS medium with BAP 1ppm obtained successive morphological changes occur : (A) bubbling - twist (B) edge sprung (C) spread over the surfaces of the callus explants formed callus

The initiation of suspension culture using callus explants of *Camellia sinensis L* in liquid MS medium with the use of growth regulators BAP 1 ppm and 400 ppm precursor phenyl alanine suspense culture growth can be observed by watching wet weight suspension culture as listed in Table 1.

Table 1.Wet weight suspension culture *Camellia sinensis L*

Use	Wet weight suspension culture *Camellia sinensis L* (mg) Age (weeks)				
	weeks				
	0	1	2	3	4
benzylaminopurine 1 ppm	100	150	200	200	250
Phenylalanine 400 ppm	100	200	250	300	250

On Table 1, wet weight suspension culture *Camellia sinensis L* at 3th week there are growth with an increase of almost 2 times, with the use of BAP. The use of BAP according Hartini and Semiarti [12] is a growth regulator that can be used to grow the buds on the plants *Santalum album Linnaeus*, who first begins with the formation of callus. While the use of phenyl alanine 400 ppm, increased approximately 3 times. Found that the use of phenyl alanine 400 ppm at the age of three weeks obtained the highest wet weight, this is match to research by Sutini [13] that the use of phenyl alanine in callus culture can be increased callus growth *Camellia sinensis L*. According Lepelley *et al.* [14] phenyl alanine is part of *phenylalanine ammonia lyase* enzyme in the biosynthesis of phenolic acids and enzymes also contribute to the formation of *Trimethyl xanthina*

Identification of cell suspension cultures *Trimethyl xanthina* qualitatively observed by light microscopy obtained standard cell color similar to the color of the cell suspension cultures such as in Figure 2.

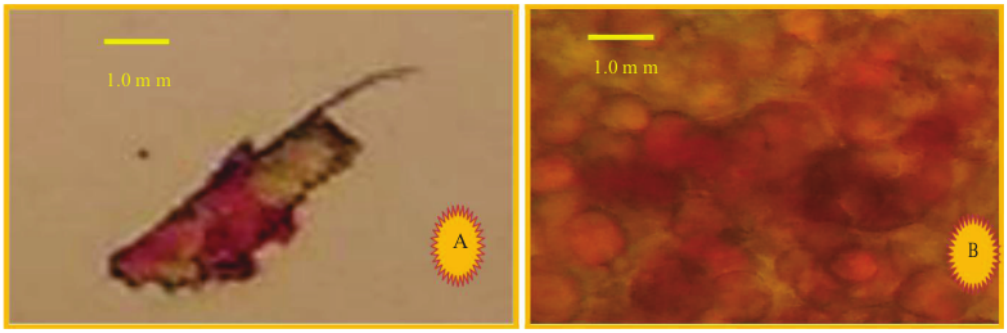

FIGURE 2. *Trimethyl xanthina* cell color on a light microscope magnification of 400 X. (A) Standard, (B) sample suspension

Isolation - identification by HPLC (High-performance liquid chromatography) [15], the profile obtained in the form of retention time on a chromatogram *Trimethyl xanthina* of culture suspense same retention time on a standard chromatogran Figure 3.

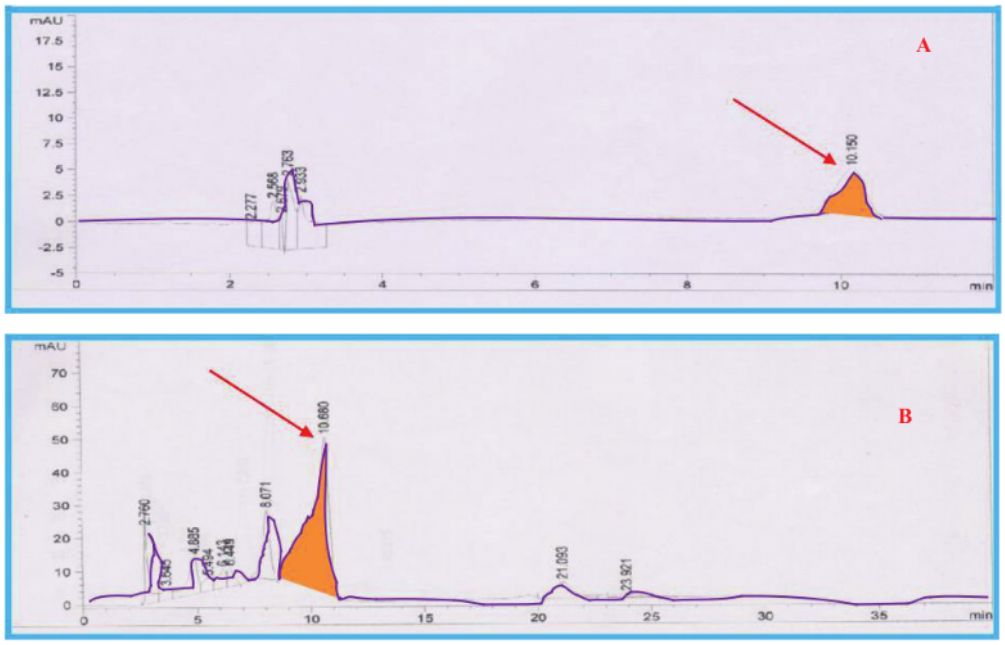

FIGURE 3. Retention time *Trimethyl xanthina* on the chromatogram. (A) Standard, (B) sample suspension

From figure 2 found that the color of the same cells with a light microscope, it shows that in suspension cultures of *Camellia sinensis L* contains bioactive *Trimethyl xanthina*. To reinforce the data identification *Trimethyl xanthina* performed well on the chromatogram obtained chromatogram retention time at 10 minutes it does show that suspension cultures of *Camellia sinensis L* contains bioactive *Trimethyl xanthina* Levels of suspension cultures *Trimethyl xanthina* on HPLC using a comparison area, between the sample and standard areas are determined according to Farmacope Indonesia [12]. Levels of suspense *Trimethyl xanthina* culture harvested at three weeks with a comparison method are obtained at 28.07 ppm. Relevant to the research conducted by Sutini *et al.* [17] that kalaus from the plant *Camellia sinensis L* extracted using solvents consecutive distilled water, chloroform and ethyl acetate, then dirotavapour. Dry extract at rotavapour then dissolved in methanol which is then injected on High-performance liquid chromatography (HPLC) chromatogram obtained *Trimethyl xanthina*. Research conducted by Deng *et al.* [18] of the *Camellia sinensis L* plant callus L harvested in three months gained hypoxanthine 6 mol / g fresh weight of callus.

CONCLUSION

Trimethyl xanthina secondary metabolite production by in vitro culture techniques suspension can be produced with the levels reached 28.07 ppm within 3- 4 weeks of explant *Camellia sinensis L* in callus

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