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by Sutini Sutini

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THE EXTRACTION PROCESS OF *TRIMETHYL XANTHINA* IN VITRO CULTURE OF CALLUS *CAMELLIA SINENSIS* WITH ETHYL ACETATE SOLVENT

Sutini [1], Susilowati [2], Djoko Agus Purwanto [3], M. Rasjad Indra[4]
[1]Agrotechnology Department of Agriculture Faculty UPN "Veteran" Jawa Timur, Indonesia
[2]Chemical Engineering Department of Industrial Technology Faculty UPN "Veteran" Jawa Timur, Indonesia
[3]Department of Pharmaceutical , Faculty of Pharmacy, Airlangga University, Jawa Timur, Indonesia
[4]Department of the school physiology Faculty of Medical Brawijaya University, Malang, Indonesia
E-mail: tien_basuki@yahoo.com

ABSTRACT

Trimethyl xanthina is one of the compounds contained bioactive culture in vitro *Cammelia sinensis* callus which is widely used in the field of food, beverage, agriculture and health industries. The presence of *trimethyl xanthina* on food, beverages and health is needed in a certain amount depending on the use which is achieved by the user. To get a certain amount of *trimethyl xanthina* from callus culture of *Cammelia sinensis*, the extraction process is performed on the water solvent, as well as non-solvent water / organic solvent such as ethyl acetate. The purpose of this study was to obtain profile of *trimethyl xanthina* in the extraction of *Cammelia sinensis* callus. The experimental methods used consisted of dissolution, filtration, extraction with water solvent and ethyl acetate, then followed by identification of *trimethyl xanthina* using HPLC. The results shows the profile form of *trimethyl xanthina* of *Cammelia sinensis* callus have similarities with the standard form of *trimethyl xanthina*.

Keywords: *cultured in vitro, Cammelia sinensis* callus, *trimethyl xanthina.*

INTRODUCTION

Trimethyl xanthina is one of the bioactive compounds found in vitro culture of callus *Cammelia sinensis* (Shervington et.al., 1998). Trimethyl xanthine, according to a study of Wei et.al. (2008), could be raised in production by culture in vitro by addition of precursors of purine. Research conducted by Li et.al.., (2008) shows that gene *trimethyl xanthina* could expressed on new *Cammelia sinensis* leaves. According to Shane et.al. (2013) on new *Cammelia sinensis* leaves, *trimethyl xanthina* is synthesized in the chloroplasts of cell during photosynthesis than subsequently transported to vascular tissue for plant defense of against pathogens and predators (Ministry for primary Industries Manato Ahu matua New Zealand Government. 2012). *Trimethyl xanthina* is widely used in many fields of food and beverage industry, agriculture, and health.

For the health, *trimethyl xanthina* plays a role in physiological effect on increasing the freshness of the human body (Gramza-Michałowska 2014). According to Rebecca et.al. (2013), *trimethyl xanthina* is classified as food and medicine with a particular dose. Toxic dose is 100 mg for an average adult per day. But according to Krueger and Howard (2011), dose of *trimethyl xanthina* most likely to be effective without causing undesirable side effects is between 100-600 mg.

In the field of food-beverage industry, bioactive *Trimethyl xanthina* increase levels of dopamine, which will be activated the body's metabolism (Susita 2014). In the beverage industry, *trimethyl xanthina* of *Cammelia sinensis* leaves presence is expected and loved by the British people (Kato 1989).

In the agricultural industry, *trimethyl xanthina* is used to eradicate the beetle (Hewavitharanage *et.al..*, 1999). Almost all varieties of tea plants containing *trimethyl xanthina*, but the content of this *trimethyl xanthina* varies depending on the age of the plant, harvesting techniques, the particle size of the harvested leaves, (Hyong et.al.., 2007) and the equipment used in the manufacture of liquid extraction (Astill et.al.., 2001). According Komes et.al.., (2009) the content of *trimethyl xanthina* has been associated with the origin of plants and plant growth conditions.

The use of liquid in liquid-liquid extraction method includes the separation of *trimethyl xanthina* compound that soluble in chloroform solvent, is partially soluble in the solvent ethyl acetate, but in this study in addition to extraction using chloroform also using ethyl acetate solvent. The use of another solvent is ethanol at the optimum temperature (temperature of 60° C) extraction time of 240 minutes, resulting in the highest *trimethyl xanthina* biomass. While the extraction temperature of 40 ° C, 15 min extraction time decreased biomass (Setyo Pratomo 2014).

The purpose of this study was to obtain profile of *trimethyl xanthina* found in in vitro callus cultures of *Camellia sinensis* with the method of callus extraction using ethyl acetate solvent and using HPLC method for the analysis.

METHODOLOGY

Callus *Cammelia sinensis* was made from leaf explants of tea plucked from PT Perkebunan Nusantara (PTPN) XII Lawang, Malang, East Java. Distilled water, chloroform, ethyl acetate, and *trimethyl xanthina* raw material supplies from specialized sales agents from Sigma.

The experimental method was performed as follows: (1) in vitro callus induction by growing tea leaf explants on media with growth regulating substances and the maintenance (2) harvesting callus followed by weighing callus and callus morphological observation. (3) Isolation and *trimethyl xanthina* bioactive extraction (4) qualitative identification of *trimethyl xanthina* of tea callus by HPLC.

Tools for analysis included High-performance liquid chromatography (HPLC) Agilent 1100 with the specification: detector spectrophotometer UV-ST diode array, with the column RP 18 Waters µ Bondapak 10 µm, 3.3 x 300mm. (2) filter "Nylon membrane f ilter "0.2 µm (3) analytical balance (Shimadzu) with 0.001 mg sensitivity (3) pumpkin separating funnel, 5 ml volumetric flask (4) rotavapour.

Callus induction *in vitro*

The results of in vitro callus induction was obtained callus growth starts from explant changes from inflated shape of tea leaf tip, morphological changes such as the formation of callus from the cut edge of the wound leaves until the entire surface filled with callus.

Harvesting callus

Formed callus was harvested then weighed and the callus morphology is observed in micro and macroscopicly (Figure 1).

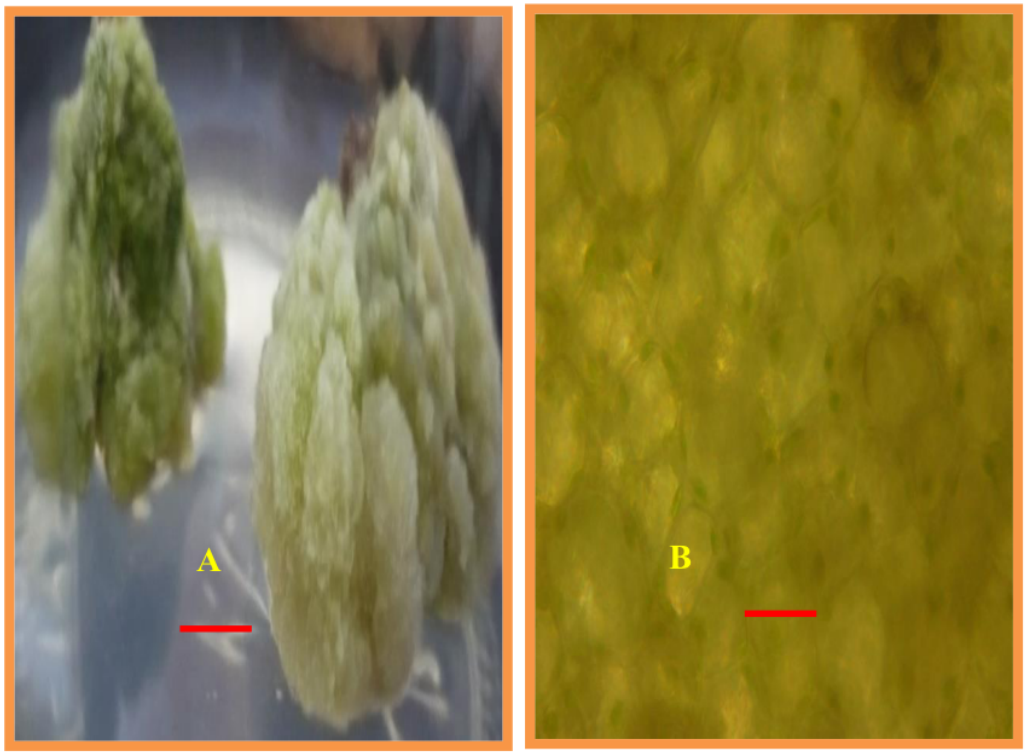

Figure-1. The shape of macroscopis callus (A). Microscopic callus with microscope triokuler with 400x magnification (B). (Bars = 1cm)

Isolation and extraction

Isolation and extraction of *trimethyl xanthina* was done by carrying out the extraction of tea callus by smoothing callus then carefully weighed with ± 415 mg (predetermined water content by gravimetric method) and adding hot distilled water with temperature of 70-80 ° C 25.0 m L, settling for ± 30 minutes (Shirai et.al.., 1994). After that filtered, and put into a 50.0 mL volumetric flask. The dregs of tea callus was rinsed with 10 mL of hot water 2 times, settling ± 30 minutes and then filtered. The second and the third extract results were subsequenly collected in the same flask, then add distilled water up to 50.0 m L. Put 25.0 mL

Tea extract solution was then gently shaken with 25 mL of chloroform in a separating funnel, it will form two layers, the bottom and the top. Bottom layer is the chloroform phase while the upper layer is the water phase. Mixing with chloroform was repeated twice. From obtained water phase, take 25.0 mL then extract with 25 mL of ethyl acetate 3 times to form two layers. Bottom layer is the water phase and the upper layer is the ethyl acetate phase. Ethyl acetate phase is accommodated and rotavapored to obtain a dry extract.

Identification by HPLC

The obtained dry extract was dissolved in methanol and then injected into the HPLC (Karlina 2006), then it will obtain the form of *trimethyl xanthina* profile with retention time (RT) which is approximately equal to the standard (Figure 2).

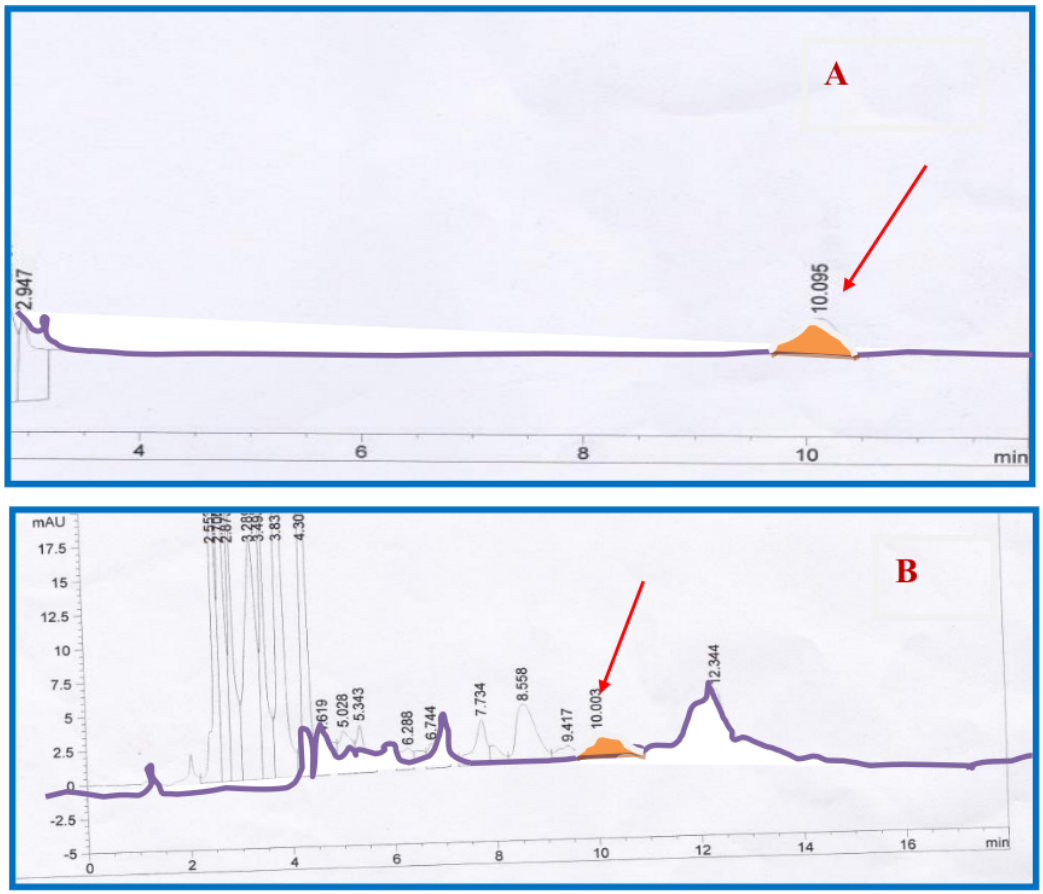

Figure-2. Chromatogram *trimethyl xanthina standard* (A) Chromatogram tri *trimethyl xanthina* methyl xanthina callus (B) with R is approximately equal.

RESULTS AND DISCUSSIONS

Greenish white callus induction, along with greenish white growth on the wound surface is response to damage. According to Sutini (2010) when the tea leaf is cut, there will be thickening wound called callus.

According to research by Borzabad et.al. (2010) on new leaves of the Artemisia vulgaris L plant could induce callus and regenerated with MS medium with growth regulators containing optimum concentrations of 1.0 mg^{-1} BAP and 3.0 mg^{-1} NAA.

Morphology of callus is microscopically obtained irregular shapes since the cells have not differentiated. Final extraction uses ethyl acetate to get *trimethyl xanthina* in a certain amount as research need.

Results of research conducted by Amra et.al. (2006) says that the solvent extraction using water, acetone, ethanol, methanol, acetonitrile at a temperature of 60 to 100 C for 240 minutes produces 36 grams of *trimethyl xanthina* / caffeine / kg dry component.

Identification using HPLC obtained chromatogram with the standard retention time is the same with sample retention time. This study using in *vitro culture* techniques which is relevant to Maria (2013) that *in vitro culture* can be as an alternative for the production of secondary metabolites *Camellia sinensis.*

CONCLUSIONS

Trimethyl xanthine compound is secondary metabolite compounds that are bioactive which can be produced through in vitro culture**.**

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