

# 6. Camellia sinensis herbal production from Callus and culture suspension

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**Submission date:** 21-Nov-2022 10:33AM (UTC+0700)

**Submission ID:** 1959828566

**File name:** inensis\_herbal\_production\_from\_Callus\_and\_culture\_suspension.pdf (155.16K)

**Word count:** 1439

**Character count:** 7930

( Poster )

## ***Camellia sinensis* herbal production from Callus and culture suspension**

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### Abstract

Herbs in the sense of the technique term is a mixture of leaves, bark, flowers and roots of plants which can be used as an alternative treatment. *Camellia sinensis* plant leaf is one of kind leaves that can also be used to improve the health of the body both by drinking the pouring or applied directly to the skin. To obtain *Camellia sinensis* leaf which is got from plantations requires treatment involving no small amount of power. The purpose of this paper is to product herbal from *Camellia sinensis* through callus culture and suspension cultures. The method applied is growing culture by induction through callus or suspension in a special room that can be developed into an industrial scale. The results obtained is in the form of callus or suspension containing secondary metabolites that is useful to complement the existence of herbal industry in Indonesia.

***Key word: Camellia sinensis, a secondary metabolite, callus culture, cell suspension cultures.***

## 1. INTRODUCTION

Leaf of *Cammellia sinensis* plants is one of the leaves that can be used to improve the health of the body by drinking the tea steeping or even applied to the skin. Therefore, the leaves of *Cammellia sinensis* plant can be included in the herbal group. Some examples of the functions of *Cammellia sinensis* plant include: 1. prevent the formation of kidney stones, it is because it is a diuretic (Yuli, 2014). 2. Inhibit skin cancer because it can capture ultra-violet light type B (Setiawan 2014). 3. Polyphenols in *Cammellia sinensis* can be as a deterrent and treat cancer (Tsukamoto et. al 2012).

Obstacles to obtaining *Cammellia sinensis* leaves obtained from plantations is that it requires treatment involving no small amount of power. Besides, the growth demands climate requirements, planting medium and high altitude between 800-1200 m above sea level (Anonymous 2013).

The purpose of this paper is producing one type of herbal from *Cammellia sinensis* leaf by callus culture and suspension culture.

## 2. LITERATURE REVIEW

Tea leaves (*Camellia sinensis* (L) O.Kuntze) as one plantation commodities contribute greatly to the country's foreign exchange earnings of commodities non-oil on plantation sub-sector (Wachjar 2006). Need of *Cammellia sinensis* leaf as a functional beverage can be said as a second need in drinking after consuming plain water. Beside drinking water from tea steeping which is from *Cammellia sinensis* leaf, it can also be as herbal ingredients because of its multifunction as anti-oxidants. According to Setyawan 2010, Green tea includes botanical herbal that are anti oxidants which oxidants are molecules that have an unpaired electron

in its outer orbit. Oxidant molecules are unstable and will react with the cells of the surrounding tissue, usually attracts electrons surrounding cells and the cells creates a compound that is not normal (more reactive free radicals) and start a chain reaction that can damage vital cells. Oxidants as free radicals can cause: premature aging, increased cholesterol, liver disease, disease heart, cancer, also diabetic disease.

Looking at the importance of *Cammellia sinensis* usage as one of the herbal ingredients which can be as prevention or cure of disease, it is necessary to do research related to the production of herbal *Cammellia sinensis*. This script writing is related to a research in producing *Cammellia sinensis* herbal through in vitro culture method with callus culture techniques or even suspension culture, in which this in vitro culture can be developed into an industrial scale.

## 3. MATERIALS AND METHODS

### MATERIALS

Materials that are used include; *Camellia sinensis* leaf explants that are maintained in pots / polybec picked manually. Media that is used is Murashige and Skoog (MS) in the form of soil and liquid / suspension that contains macro and micro nutrients and enriched with plant growth regulators. Materials for activities of sterilization and analysis of secondary metabolites in *Cammellia sinensis* culture include methanol, Na-hypoklorit, sterile distilled water, 3% solution of Dithane. For extraction: chloroform, ethyl acetate, formic acid, for analysis using chromatographic densitometer. For qualitative test, it uses triokuler microscope or *Scanning electrone Microscope / SEM*.

## Research methods

Research method that was done was growing culture by initiating through callus or suspension in a special sterile room that can be developed into an industrial scale. Callus obtained aseptically transferred into a liquid medium in aseptic too. Then callus in liquid medium are incubated in a sterile room also with agitation using a shaker at a speed of 100-150 rpm. After a 10-20 day suspension cultures harvested, extracted and analyzed.

## 4. RESULTS AND DISCUSSION

### Initiation of callus culture

Initiation of callus cultures obtained after incubation for one to three months in a sterile incubator space at temperatures between 16 till 25 degrees Celsius (Figure 1).



Figure 1. Callus culture (A), callus culture in a special incubator room (B)

In Figure 1. Callus formed with MS medium with the addition of growth regulators auxin and cytokinin with a particular concentration as a result of optimization of callus formation. In

accordance with the research from Seran et. al, 1999, initiation of callus with half MS medium and the addition of growth regulators BAP and 2-4D can produce callus as a material for the manufacture of *Cammellia sinensis* embryos.

### Initiation of suspension cultures

Initiation of suspension cultures derived from callus that were transferred to MS liquid medium with or without growth regulators or with the addition of certain precursors (Figure 2, 3).



Figure 2. Initiation of suspension culture (A), suspension culture in a special incubator space with agitation (B)



Figure 3. Aggregate suspension culture cell

In Figure 2,3. Suspension cultures are formed macroscopically in the form of regular cell aggregates in the form of a single cell or set of cells that are separated. This research is consistent with the results of research from Ke-jiu et. al in 1997 that the use of semi-liquid MS medium enriched with BAP and 2,4-D resulted in embryos *Cammellia sinensis*.

#### Extraction of suspension cultures

Extraction suspension cultures successfully separate the part of secondary metabolites that is soluble in ethyl acetate solvent then performed separation by thin layer chromatography in the form of spots observed with UV light. (Figure 4)

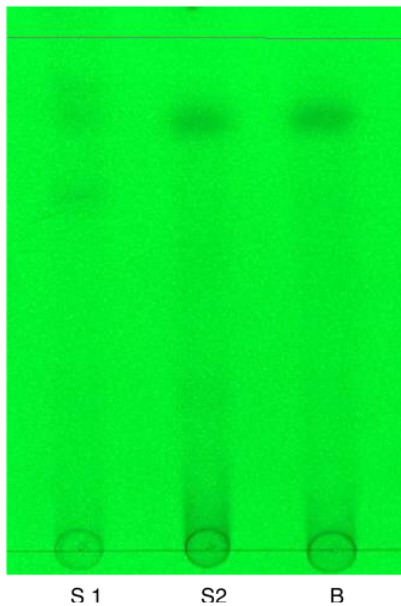


Figure 4. Spots of secondary metabolites on suspension culture *Cammellia sinensis*.

#### Qualitative test of suspension cultures

Qualitative test of suspension culture uses SEM obtained standard particle of

secondary metabolites *Cammellia sinensis* which is similar with particle shape of *Cammellia sinensis* samples (Figure 5).

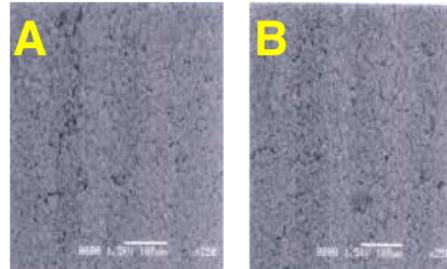


Figure 5. Standard particle Form secondary metabolites *Cammellia sinensis* which is similar to the shape of the particle sample of secondary metabolites *Cammellia sinensis*.

Qualitative test is relevant to study Jin 2007, that the extraction of catechins from green tea can separate the particles, which is similar to the standard used.

## 5. CONCLUSION

Herbal ingredients derived from the leaves of *Camellia sinensis* can be produced by culturing callus and even *Camellia sinensis* suspension that can be harvested in a relatively short time of about one month after the induction culture / cultivation.

The results are expected in the form of callus or suspension that contain secondary metabolites that are useful to complement the herbal industry in Indonesia.

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