4. Secondary metabolites production of epigallocatechin gallate through in vitro culture of Camellia sinensis l with cinnamic acid precursors

by Sutini Sutini

Submission date: 21-Nov-2022 10:32AM (UTC+0700)
Submission ID: 1959827169
File name: 4-_18._AIP-Prosid._Secondary_metabolites_production_of.pdf (1.2M)
Word count: 2442
Character count: 13479

Secondary metabolites production of epigallocatechin gallate through in *vitro* culture of *Camellia sinensis* l with cinnamic acid precursors

Cite as: AIP Conference Proceedings **2231**, 040082 (2020); https://doi.org/10.1063/5.0002565
Published Online: 22 April 2020

Sutini, Widiwurjani, Nora Augustien, et al.

Secondary Metabolites Production of Epigallocatechin Gallate Through in *vitro* Culture of *Camellia sinensis* L with Cinnamic Acid Precursors

Sutini [1,a)], Widiwurjani [1,b)], Nora Augustien [1,c)], Djoko Agus Purwanto [2,d)], and Wirdhatul Muslihatin [3,e)]

[1] *Department of Agrotechnology Faculty of Agriculture, UPN Veteran Jawa Timur, Indonesia.*
[2] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.*
[3] *Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Teknologi Sepuluh Nopember, Indonesia*

[a)]Corresponding author: tien_basuki@yahoo.com
[b)]wurjani@gmail.com,
[c)]noraagustien@yahoo.co.id,
[d)]djokoagus@ff.unair.ac.id,
[e)]w_muslih@bio.its.ac.id

Abstract. The secondary metabolite epigallocatechin gallate found in the *Camellia sinensis* L plant, which is bioactive, can be an antioxidant and can prevent cancer. The problem of epigallocatechin gallate presence from plants is dependent on land height, environmental temperature conditions, the need for intensive care, and relatively low production levels. Therefore, we need the technology of epigallocatechin gallate production through in vitro culture techniques. The study aimed to obtain a technique of secondary metabolites production of epigallocatechin gallate through culture in vitro by optimizing the medium and providing precursors. The method used is to initiate callus by adding growth regulators and cinnamic acid precursors. After that, the growth of callus was observed then tested both qualitatively and quantitatively. The result obtained is a compact form of callus containing epigallocatechin gallate. This research suggests that the use of cinnamic acid precursors in vitro culture techniques of *Camellia sinensis* L in a relatively short period can increase wet callus weight.

INTRODUCTION

Introduction. Epigallocatechin gallate is the most dominant secondary metabolite contained in the leaves of the tea plant, which is an ester compound in the form of a polymer of epigallocatechin and gallic acid [1] that is bioactive as an antioxidant and capable of preventing cancer. Epigallocatechin gallate is contained in a variety of vegetables [2], fruits/chocolate fruits [3], and leaves of the *Camellia sinensis* L plant [4]. In *Camellia sinensis* L plant, epigallocatechin gallate is formed from a complex biosynthesis that is through the phenylpropanoid pathway with the pioneering mixture is phenylalanine [5] Epigallocatechin gallate is a nutraceutical that is bioactive in various industrial agro, can be used as an anti-bacterial [6], antioxidant, anti-cancer [7], anti-fatty in the body/cholesterol [8]. In the food industry as an additional ingredient as well as anti-bacterial [9]. In the pharmaceutical industry, as a candidate for anti-UV light exposure to protect the skin [10].

The problem of obtaining epigallocatechin gallate from *Camellia sinensis* L plant depends on land height from sea level [11], environmental conditions, requires serious maintenance, the production level is limited according to the desired standard and have so much variation [12] So as an alternative production technology from

epigallocatechin gallate is done through in vitro culture techniques. This technique is a very controlled environment, useful in cultivation, and only requires a limited area.

International Conference on Life Sciences and Technology (ICoLiST)
AIP Conf. Proc. 2231, 040082-1–040082-5; https://doi.org/10.1063/5.0002565
Published by AIP Publishing. 978-0-7354-1990-2/$30.00

The advantage of this technique is that it only requires laboratory-scale land that can be developed to an industrial scale, the cultivation is relatively fast, and the production results can be similar. Besides having advantages, in vitro culture requires sufficient initial capital, including at the beginning of the preparation it requires the adequate investment of tools and materials, as well as skilled workers, but after it is implemented, capital requirements are not too large. Related to the initial capital in this research is to procure raw materials such as the use of cinnamic acid precursors that must be imported or purchased from distributors abroad. To overcome the problem of in vitro culture, techniques were carried out with media optimization, growth regulators, and with the addition of cinnamic acid precursors. This research is necessary because this technology is very efficient in the use of a relatively short time, that is, the callus can be produced or harvested in about three to four months. The purpose of this study was to obtain secondary metabolites of epigallocatechin gallate producing technique through in vitro culture by optimizing the medium and providing cinnamic acid precursor.

EXPERIMENTAL DETAILS

Materials

The materials used were the shoots of the two-year-old *Camellia sinensis* L plant, which was maintained in a greenhouse that has five to ten branched buds until the young leaves reach about twenty leaves, which were used as explants. Materials for sterile space and explants include 70% ethanol, 5% NaOCl, gelatine in the form of powder, Murashige and Skoog (MS) primary media and standard ingredients for sigma epigallocatechin gallate products, cinnamic acid, growth regulator a-naphthalene acetic acid (NAA) and BAP

Methods

Callus initiation with explants from leaf pieces on the growth medium that were immersed and being added by growth-regulating hormones [13]. (2) callus culture was stimulated with cinnamic acid precursors that given [14]. (3) the test of callus growth. (4) the test of the epigallocatechin gallate morphology from a callus in a qualitative way. (5) the test of partially modified quantitative from callus high-performance liquid chromatography (HPLC) [15].

RESULTS AND DISCUSSION

The results obtained by the stages are initiation of callus, callus growth, callus shape, and epigallocatechin gallate levels. Callus initiation is the most critical stage of this research because it is the initial stage of exploding leaf pieces explants to change regularly to the form of callus. Whereas the growth of callus is a growth process that informs that there is a change in shape and an increase in wet callus weight. In addition to the results of wet weight, callus can also be tested to identify the callus content associated with the content of epigallocatechin gallate. The results in detail can be explained in the following paragraphs.

Initiation of callus

The initiation of callus is the most certain activity for the continuation of research. The initiation activity was carried out from the initial initiation until the callus was 14 weeks old and obtained explant character changes of the *Camellia sinensis* L leaf that is dynamic, and various were displayed in Fig.1.

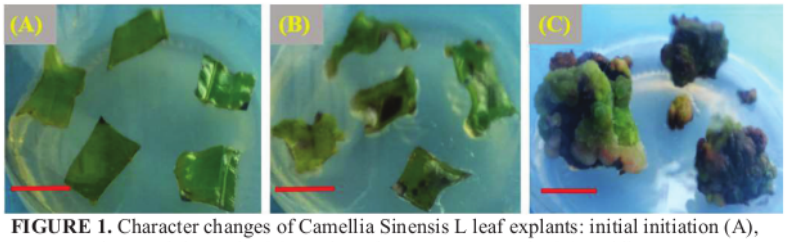

FIGURE 1. Character changes of Camellia Sinensis L leaf explants: initial initiation (A), explant at eight weeks (B), explant character at 14 weeks (C), red line 2 cm

In the initiation of callus, there is a change in the form of explants that are sliced consecutively according to the model form of rolling, twisting until the form of the callus mass that huddles alongside the explants that prove the cell changes. This cell change is an indicator of changes in the composition of the cell wall, cell nucleus, and expansion of storage areas called vacuoles. Changes in the composition of the cell wall involve cellulose, lignin, and pectin. Changes in cell nucleus involve changes in the presence of protein, fat, and starch. While changes in storage are vacuoles associated with increased volumes of epigallocatechin gallate in vacuoles. In in vitro culture, this cell shape changes as a result of cell responses to the environment, nutrients, and the presence of endogenous and exogenous growth regulators which are imposed on MS media. This research is relevant to the research [18] on Arabidopsis thaliana embryos that cell changes/cell expansions until it forms callus because of the growth regulators role that expands to cause extension or expansion of cells that appear as growth to form a callus.

Callus growth

The callus from the initiation was then cut and transferred into the media, which was initiated with cinnamic acid precursors of various concentrations, respectively 0.0, 0.5, 1.0, 1.5. 2,0,2,5 mg. L-1. Callus pieces were dissolved in media containing cinnamic acid cultured with growth observed by seeing an increase in wet weight after thirty days of cinnamic acid administration, shown in Figure 2.

In cinnamic acid plants are compounds involved in the mevalonate pathway or shikimate pathway by involving several enzymes that accelerate the formation of epigallocatechin gallate compounds, so that cinnamic acid acts as a precursor. Cinnamic acid precursors cause real callus growth, and this is because cinnamic acid is a phenolic acid compound as a precursor in the biosynthesis of secondary metabolites by activating several enzymes involved, which mediate the formation of epigallocatechin gallate [19].

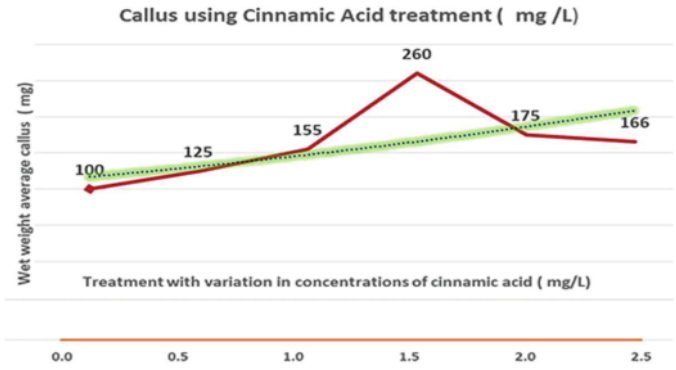

FIGURE 2. Callus with of cinnamic acid treatment

With the presence of cinnamic acid precursors, besides epigallocatechin gallate metabolites is formed, there is also an occurrence of other compounds isoflavone, anthocyanin, and flavanone groups so that the wet biomass weight is increased. This increase in wet weight is relevant to research [21] that the cinnamic acid precursor formed flavan compounds with a shady environment or a light reduction. Research conducted by [20] supports this research, namely, cinnamic acid entering the phenylpropanoid pathway, which increases hydrogen so that lignin cells have increased.

Callus shape

Callus shape at week 16 was observed using a trinocular microscope obtained a standard epigallocatechin gallate cell shape similar to the shape of the callus cell sample in Fig. 3. The morphology of the callus that was displayed on a microscope monitor, it appeared that callus cells had/contained secondary metabolites of epigallocatechin gallate because of their cell shape, which was similar to the standard cell of epigallocatechin gallate. The shape of this cell is similar to the research. Research by [22] shows the same symptoms when Mangifera indica L and Ficus benjamina L leaves are exposed to air pollution, which causes cells to form lesions, that cells in the part of the stomata are raised due to distortion of pollutant gases.

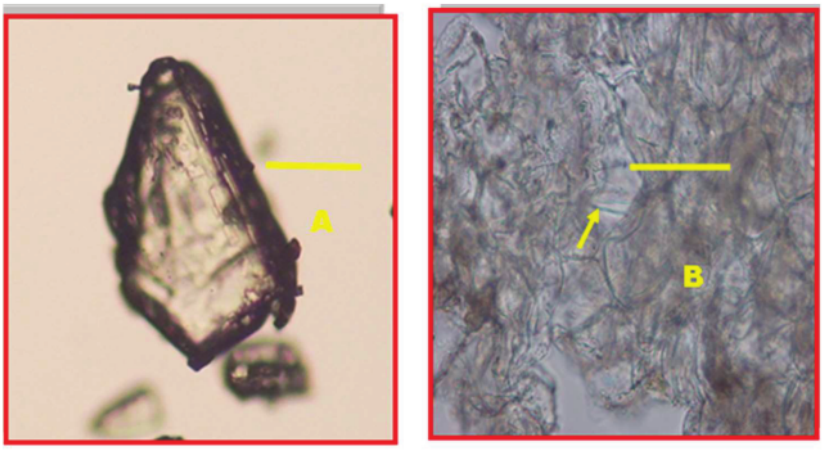

FIGURE 3. Epigallocatechin cells (A), standard, callus cells (B), 5 mm bars (yellow straight line)

Epigallocatechin gallate levels.

Epigallocatechin gallate levels were produced by calculation following the 2008 edition of the Indonesian herbal Pharmacopoeia procedure [17] that is standard epigallocatechin gallate levels were same with broad chromatograms of epigallocatechin gallate (samples) were divided by standard broad chromatograms of epigallocatechin gallate multiplied by standard epigallocatechin gallate concentrations that is used and obtained level of 13.04 %.

Epigallocatechin gallate levels acquisition is 13.04 %. Previous research has obtained epigallocatechin gallate compounds using salicylic acid boosters on patent ID number POO 31047 B [23]. This compound acquisition is in line with indicating that in vitro culture techniques can be an alternative to supporting health materials in Indonesia following Indonesian government policy [24].

SUMMARY

In summary. In vitro culture of *Camellia sinensis* L plant can produce callus biomass with a compact texture and whitish green color. Callus formed after initiation on the thirtieth day by using an inducer in the form of cinnamic acid. The use of cinnamic acid precursors can form secondary metabolites of epigallocatechin gallate. The use of cinnamic acid precursors can form secondary metabolites of epigallocatechin gallate. Secondary metabolites of epigallocatechin gallate are not only obtained from plants but can also be produced through in vitro culture techniques of the Camellia sinensis L plant by optimizing the addition of cinnamic acid precursors. Epigallocatechin gallate secondary metabolites formed in this research were 13.04%, which could be candidates for a mixture of drugs or can be directly used as drugs such as anti-oxidant drugs.

ACKNOWLEDGMENTS

We would like to acknowledge the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia, which has funded the research through competition grants 2017-2019 with the research contract number: 086/SP2H/LT/DRPM/2019.

REFERENCES

1. N. Perera, P. Ambigaipalan and F. Shahidi, Journal of Food Bioactives. **1** (2018).
2. W. Harn Pan, N.Yeh, R.Yang, W. Lin,W.Wu, W.Yeh, M. Sung, H.Lee,S. Chang, C.Huang, B.Lin, M. Chiang, journal of food and drug analysis **26** (2018).
3. ICW. Arts, B.Putte, PCH. Hollman, J. Agric. Food Chem **48** (2000).

4. L. Zhang, K. Wei, H.Cheng. Bot Stud **57** 31 (2016)
5. Z.Wu, X.Li, Z.Liu, Z.Xu, J. Zhuang. J. BMC Plant Biology, **14 (**2014).
6. Y. Zhou, N. Zhang, YK. Andrea, C. Chen. J. Metabolites **9** 96 (2019).
7. Y. Zhong. Dissertation. Department of Biochemistry Memorial University of Newfoundland (201 0)
8. J. Intra and S.Kuo. J. *Chem Biol Interact*.**169** 2 (2007).
9. D. Shi. Dissertations**.** Montclair State University Digital Commons (2018)
10. G. L. Russo. J. *Biochemical Pharmacology*, **74** (2007).
11. J. lee,b. lee, j. Chung, c.lee, j. Hwang,y.hong, s. Lee. J. Agric. Food Chem. **58 (**2010).
12. L.yao, N. caffin, R. Singanusong, B. d'arcy, X.liu, Y. jiang, N. datta, Y. xu, John shi,Y. kakuda, J. Agric. Food Chem, **53** (2005).
13. Sutini, M. Sodiq, W. Muslihatin, and M. R. Indra, AIP Conference Proceedings (Published by the American Institute of Physics) **020036** (2017).
14. Sutini, Widiwurjani, Nora Augustien, Djoko Agus Purwanto, and Wirdhatul Muslihatin , AIP Conference Proceedings **2120** (2019).
15. Sutini, Susilowati, Indra RM, Djoko AP, *Journal of* Biological Researches. **22** 1 (2016).
16. L.Yao, Y. Jiang, N. Datta, R.Singanusong, J. Duan, K. Raymont, A. Lisle, Y. Xu,X Liu, J. Food Chemistry **84** (2004).
17. Herbal Pharmacopoeia Indonesia. Directorate General of Pharmaceutical Services and Health Ministry of Health R.I. (2008)
18. V. Raghavan, American Journal of Botany. **91** 11 (2004).
19. L. Xiong, J.Li, Y. Li, L. Yuan, S. Liu, J. Huang, Z. Liu. J. Plant Physiology and Biochemistry **71** (2013)
20. VH.Salvador, RB. Lima, DS. Wanderley, RS. Anderson, AF. Paulo, R. Marchiosi, LL. Maria, O. FerrareseFilho. J. Plos one **8** 7 (2013).
21. Y. Wang, L. Gao, Y. Shan, Y. Liu, Y. Tian, T. Xia. J. Scientia Horticulturae **141** (2012).
22. MD. Angeles, CB. Serino , and KB. Granada. Journal of Nature Studies **17** 2 (2018).
23. Sutini, M. Rasjad, T. Wardiyati, BS. Sutiman. Patent No. ID. POO 31047 B (2012).
24. M. Nasir. 2017. National Research Master Plan for 2017-2045. Ministry of Research and Technology of Higher Education.

4. Secondary metabolites production of epigallocatechin gallate through in vitro culture of Camellia sinensis l with cinnamic acid precursors

ORIGINALITY REPORT

7%	%	%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universitas Brawijaya Student Paper	2%
2	Submitted to School of Business and Management ITB Student Paper	2%
3	Submitted to University of Anbar Student Paper	1%
4	Submitted to Manchester Metropolitan University Student Paper	1%
5	Submitted to Universitas Diponegoro Student Paper	1%
6	Submitted to Universitas Sebelas Maret Student Paper	1%
7	Submitted to iGroup Student Paper	1%

8	Submitted to Universitas Islam Negeri Sumatera Utara Student Paper	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		