19. CHARACTERIZATION OF EGCG COMPOUND USE 1 H NMR SPECTRUM ON CAMELLIA SINENSIS (L.) CALLUS

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CHARACTERIZATION OF EGCG COMPOUND USE ^{1}H NMR SPECTRUM ON CAMELLIA SINENSIS (L.) CALLUS

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ABSTRACT

Epigallocatechin gallate (EGCG) are secondary metabolite on Camellia sinensis L as obesity preventing agent. The characterisation of this plant use ^{1}H NMR spectroscopy often have been done, however characterisation on callus both drying with open air and without drying is rare. The purpose of this research is characterize EGCG of tea callus via process both drying with open air and vacuum. Tis method use ^{1}H NMR spectroscopy. The result show that EGCG character of tea callus via process both drying with open air and vacuum are significantly different.

Key note: Epigallocatechin gallate, ^{1}H NMR, Camellia sinensis L callus

INTRODUCTION

Epigallocatechin gallate (EGCG) bioactive is available on tea (*Camellia sinensis L*). The advantage of this are anti obesity, anti cancer, anti diabetic, anti cholesterol, anti bakterial, cardiovaskuler disease and osteoporosis prevention agent. Many function of tea on industry that is beverage, cosmetic, pharmacy, and food (Hartoyo, 2003). EGCG bioactive compound structure as figure 1, is one of flavonoid derivat of phenol on tea (*Camellia sinensis L.)*. Structure that have many hydroxi/ OH^- easy to bond free radical so EGCG identified have multi function in health.

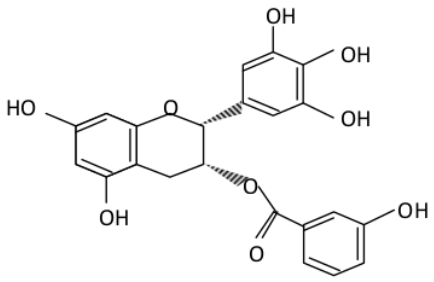

Figure 1. EGCG structure (Thomson, 2004).

Peter W.L. (2000) say that EGCG harvested on winter and summer season different on EGCG value. This is relevan with Caffin, N., D'Arcy, B.,Yao L.,Rintou, N. (2004) note that EGCG amount of tea leaves is increasing harvested on summer (May), however decreasing on winter (November).

In general, this research is aimed at developing production of EGCG technology in vitro by callus culture technique. Characteristics of EGCG are: binding with several biologic matrix and heavy metals, catalyzing electron transportation, and trapping free radicals. Four characteristics above made it a bioactive agent. Hence secondary metabolite of EGCG must be characterized with ^{1}H NMR both on drying and undrying process to improve the product quality. :

Purpose :

Characterize EGCG of tea callus with process both drying in open air and with vacum.

MATERIALS AND METHOD

^{1}H NMR spectroscopy 500 MHz (Bruker,Jerman), 1.5 mL-ependorff tube mL -2 mL, 5 mm NMR tube, centrifuge, Ultrasonic, vortex, vacum dry, metanol-deuterium (CH_3OH-d_4), buffer KH_2PO_4 in D_2O (pH 6,0) containing 0,01% (b / b) TSP, aqua bidestilata.

Extraction

Preparing metanol-deuterium (CH_3OH-d_4) without add standar internal, buffer KH_2PO_4 in D_2O (pH 6,0) containing 0,01% (b / b) TSP**.** Measure gentle powder of 25-50 mg tea callus that both with drying in open air and vacum. Then add CH_3OH-d_4 (without any internal standard), KH_2PO_4 buffer in D_2O (pH 6.0) containing 0.1% (w/w) TSP, in to 2 mL-ependorff tube. This solution vortex for 1 minute at room temperature and then ultrasonication for 5-20 minute at room temperature. This solution centrifuge at room temperature for 5 – 20 minute using microtube centrifugator (13000 rpm, room temperature). Transfer supernatant (more than 1 mL) to 1.5 mL-ependorff tube.

If more centrifugation is necessary centrifugator using microtube centrifugator (13000 rpm, 1 minute, room temperature). Then transfer 800 mL of supernatant to 5 mm NMR tube.

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Characterisation Use ^{1}H Nmr

The study done use 500 spectrometer MHz 1H NMR (Bruker,Jerman) completed by cryoprobes. Chemical shift(δ) is measured on ppm, with standart referency use tetrametil silen zero ppm, with chemical shift range between 4.52-7.08.

RESULTS AND DISCUSSION

The liquid of green browny pure extraction, then spectrum observed. EGCG spectra of tea callus proceed both with drying in open air and wet tea callus in vacumand standar as Figure 1.

Spectrum ^{1}H NMR 500 MHz on methanol deuteurium solvent, (Table 1) showed that chemical shift (δ ppm) and space between two spin/kopling constanta (J in Hz). Proton position are structure from EGCG resonance on H-6, H-8, H-2', H-5',H-6' (Markam, et al, 1994). Chemical shift and coupling constanta on proton position resonance for EGCG show that tea callus with drying in vacum almostly same with standart. This show that tea callus characterization with drying vacum can identify EGCG character, that not happen in tea callus with drying in open air. Proton position of EGCG resonance on chemical shift (δ) and coupling constanta (J), tea callus with open air drying can not show the character because that compound oxydated by air.

Based on Nathalie V, G. research (2001) that flavonoid oxydation caused by temperatur, UV light, and ion Cu^{2+} then change to be unstabile quinon into sulfonat. Using ^{1}H NMR spectroscopy, can characterize proton of EGCG. This is relevant to Moco research (2007) show that ^{1}H NMR can identify flavonoid compound on tomato plant. Then, Tarachiwin L. Et al., 2007 note that ^{1}H NMR spectroscopy combined with multivariat analysis can descript secondary metabolit profile.

However using ^{1}H NMR spectroscopy, have disavantage that is: 1). Relatively low sensitivity than using other analysis technique such as MS, 2). Can produce more than one ambiguous spectra, 3). Chemical shift influenced by the surrounding chemical environment. There is many ways to solve that is: 1). Combine 2D spectrum (two dimension) NMR, 2). This research use standart data comparation refer to the same sovent material.

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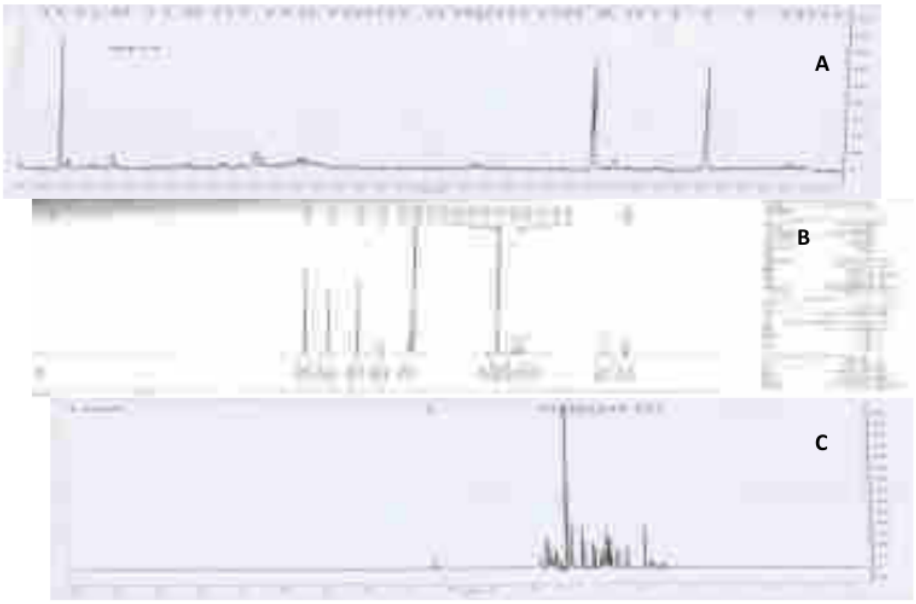

Figure 1. Spektrum ^{1}H NMR 500 MHz on CH_3OH-d_4 solvent from: (A) tea callus with vacum drying, (B), standart , (C) tea callus with drying in open air.

Table 1. Proton position δ and J EGCG , tea callus drying in open air, tea callus Drying with vacuum and standart

Proton Position	δEGCG tea callus drying in open air, (J in Hz)	δEGCG tea callusdrying with vacum, (J in Hz)	δEGCG with standart (J in Hz)
H-2	-	4.90 (s)	4.90 (s)
H-3	-	5.50 (s)	5.51 (s)
H-4α	-	2.97 (dd)	2.97 (dd)
H-4β	-	2.83 (dd)	2.83 (dd)
H-6	-	5.94(s,1.79)	5.93(s,1.79)
H-8	-	5.94(s,1.79)	5.93(s,1.79)
H-2’	6.34(s)	6.49(s,1.80)	6.48(s,1.80)
H-5’	-	-	-
H-6’	6.40(s,)	6.49(s,1.80)	6.48(s,1.80)
H-2”	-	6.95(s)	6.93 (s)

CONCLUSION

Achieve character from EGCG body tat is ^{1}H NMR as H-6, H-8, H-2', H-5', H-6' (Markam,et al, 1994). Observation on chemical shift located between 5,94 – 6,49, this value based on existence range of EGCG compound. This relevant with study by McLeod (2010) that aromatic bonding area located on 5,8 – 8,8

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Question :

1. What the structure resonance in EGCG ?

Answer :

1. Aquired characteristic from EGCG structure resonance on H that H – 6, H – 8, H - 2, H – 5, H – 7, etc.

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