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Anticancer activity of Lampung Robusta Coffee extract fractions

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Abstract. Coffee is a popular beverage that is consumed worldwide. Habitual coffee drinking is associated with cancer risk reduction including pre- and post-menopausal breast cancer. The invert correlation between coffee intake and breast cancer risk is weak, therefore coffee is potential for functional beverage. Coffee should be taken for several times per day to get its benefit. Compounds which give the cancer reduction effect are still debatable, they can be compounds with antioxidant or non-antioxidant properties. Robusta coffee from Lampung has a special meaning for Indonesian coffee lovers due to its strong aroma and flavour with affordable price. In order to observe the anticancer activity of Lampung Robusta coffee, its aqueous methanol extract was passed through a chromatography column containing polyamide with aqueous methanol as the eluent with a gradient of 10%. A total of 54 fractions were obtained and labelled as A to K fractions. Chlorogenic acid was detected in fractions A and B. Meanwhile, it was not detected in other fractions including G fraction. This G fraction, however, was the only one to show cytotoxicity against MCF-7 breast cancer cell line. This result suggests that this fraction contains potential compounds with anticancer activity.

1. Introduction

One of the main plantation commodities in Indonesian is coffee. Lampung is the second biggest coffee producer province in Indonesia after South Sumatera. Lampung has a very potential role in national coffee production, in 2017 it produced 107,219 ton of total 716,089 ton Indonesia coffee production for the year [1].

Coffee contains various compounds such as caffeine, chlorogenic acid, protein, and aromatic volatile compounds [2]. Most of the research and scientific publications only focus on caffeine in coffee, while characteristics and benefit of other compounds such as the chlorogenic acid (CGA) was not widely known and studied. CGA is an ester formed from caffeic acid and quinic acid, it is one of the most abundant polyphenol compounds, consists of a group of phenolic secondary metabolites, it isolated from the leaves and fruits of dicotyledonous plants [3]. CGA soluble phenolic compounds are conjugated forms of hydroxycinnamic acids with quinic acid, which accumulate in coffee plants [4].

There are several isomers of CGA for each subgroup of coffee plant varieties and each coffee extract contains different CGA [5]. Qualitative and quantitative differences of CGA are not only

among species but also within species, in the coffee berries [4]. The CGA composition of the green coffee bean is also specifically affected by the way of processing [6].

CGA is an important and biologically active dietary polyphenol, playing several important and therapeutic roles such as antioxidant activity, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, antiobesity, antiviral, antimicrobial, antihypertension, free radicals scavenger and a central nervous system (CNS) stimulator [4], The CGA extract from coffee bean has a potential activity as an anticancer and antioxidant [7-11] .

Previous research on human liver hepatocellular cancer cell, HepG2 and HepG3 cell concluded that CGA is activating and inhibiting some important pathways in cancer metabolism [12,13]. CGA as cancer treatment on Hep G2 cell indicated that it is less toxic then doxorubicin, the commercial chemotherapy medication for cancer treatment [13]. The IC_{50} value of CGA on Hep-G2 cell is 727μ M (μ mol/L) [14].

Previous studies on the effect of CGA on colon cancer concluded that CGA reduced colon cancer-cell proliferation [15-16]. Previous research was using CGA complex named CGA7, a decaffeinated water-soluble green coffee bean extract for human and mouse cancer line, colon cancer HCT-116 cells treatment, the overall findings indicated that CGA7 complex, a potential anticancer molecule found in green coffee beans, is most likely safe [17].

This research focuses on the variation of mobile phase solvent composition in product purification of CGA and coffee bean extract fractions and anticancer activity of extracts. This research applied fractionation based on mobile phase polarity. The variation of mobile phase composition is composed from polar to nonpolar organic solvent with gradual addition of nonpolar solvent. The objective of this variation is to select and separate compounds based on compound polarity, from high polar compound to less polar compound. This research studies correlation of the solvent to the extraction products characteristics. The different solvent composition used in chromatographic separation process. Each extract is tested for CGA contents and anticancer activity on MCF-7 cell. MCF-7 breast cancer cell line was chosen for anticancer activity assay. This cell line was available at Research Center for Chemistry (LIPI) and displayed good response toward various botanical extracts. MCF-7 cell is a human breast cancer cell line with estrogenic, progesterone and glucocorticoid receptors [18].

2. Material and Method

2.1. Materials

The material in this research is organic green Robusta coffee beans from Padang Cermin, Pesawaran, a city in Lampung province Indonesia. The coffee beans were determined by Herbarium Bogoriense, Research center for Biology-LIPI Bogor, as *Coffea canephora Pierre ex A. Frochne*r. The solvent for extraction and chromatographic separation process was methanol distillated technical/industrial grade solvents, while for spectrophotometric analysis used pro-analysis grade solvents. Silica Thin Layer Chromatography (TLC) for separation process.

MCF-7 breast cancer cell from Research Center for Chemistry (LIPI) for testing anticancer activity.

2.2. Methods

2.2.1. Extractions. Ground coffee was extracted with methanol [18]. The ratio of ground coffee and solvent was 1:4. The extract was concentrated with rotary vacuum evaporator to obtain the methanol extract. The methanol extract was later purified by nonpolar solvent n-hexane using separation device. During this process nonpolar compounds were removed from the extract. The process continued with purification using semi polar solvent ethyl acetate, which removed the semi polar compounds from extract. The remaining compounds in extract were polar compounds. CGA was possibly one of them.

The separation process continued with the polar mobile phase in film chromatographic column. The different compositions of polar mobile phase were applied in this experiment in order to obtain the different active compounds. The mobile phase were water methanol solutions with different

volume ratio (Table 1) start from 100% water without methanol. The methanol addition reduced water polarity. This experiment started from 100% water because the main objective was to get the CGA extract in early the beginning or early fraction. The chromatographic method was performed in a column with polyamide as the stationary phase while demineralized water and methanol mixture was the mobile phase with 10% gradient (Table 1).

Table 1. Mobile phase composition.

Sample Fraction (Code)	Water (%)	Methanol (%)
A	100	0
B	90	10
C	80	20
D	70	30
E	60	40
F	50	50
G	40	60
H	30	70
I	20	80
J	10	90
K	0	100

2.2.2. Detection of CGA. Each fraction from chromatographic column was coded according to Table 1 and analysed using thin layer chromatography (TLC) for CGA qualitative testing. The later test was performed using LAAN-A-LC-E008 manual Shimadzu Application NN. L.3016 and Agilent Application Note 2016.

2.2.3. Test for anticancer activity. Anticancer activity was performed using MCF-7 breast cancer cells in Roswell Park Memorial Institute (RPMI) solution ($1x10^4$ cells/ml) [19]. The cells were transferred into a 96-well plate (100 μL per well) and incubated for 4 h at 37 °C to let the cells grew. Samples (10 μL each) with above mentioned codes (Table 1) were added. The cells were incubated for 24 h and then analyzed under microscope. Solvent control (positive cell growth) was prepared by transferring and incubating 100 μL cancer cells in RPMI, in solvent control 10 μL of solvent only was added into the cells without sample addition.

3. Result

The presence of CGA and anticancer activity in each sample fraction is presented in Table 2. This table also mentioned about the colour of the fractions as determined visually. For better visualisation, the compiled pictures of all fractions are displayed as Figure 1.

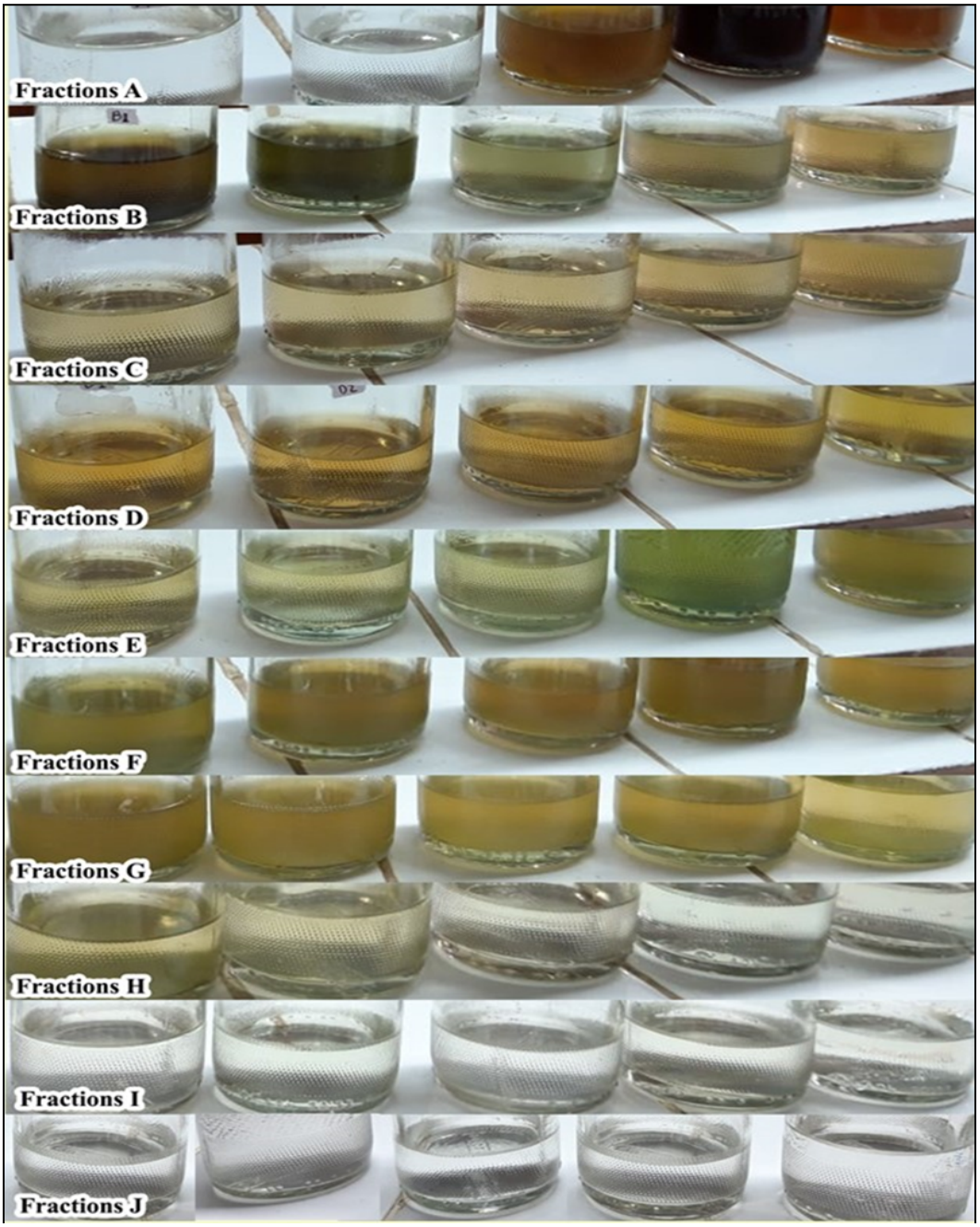

Figure 1. Different fraction coffee extracts, coded according to Table 1.

Table 2. The presence of CGA and anticancer activity of coffee sample fractions, along with their color as perceived by naked eyes.

Sample Fraction (Code)	CGA (+/–)[a]	Anticancer Activity (+/–)[b]	Color
A	+	–	Dark Brown
B	+	–	Brown
C	–	–	Light Brown
D	–	–	Brown Yellow
E	–	–	Green
F	–	–	Brown
G	–	+	Brown Yellow
H	–	–	Light Yellow
I	–	–	Clear (colorless)
J	–	–	Clear (colorless)
K	–	–	Clear (colorless)

Note: [a] (+) =detected, (-) = not detected based on TLC (see example in Figure2)
[b] (+) =detected, (-) = not detected based on anticancer analysis (see example in Figure 3)

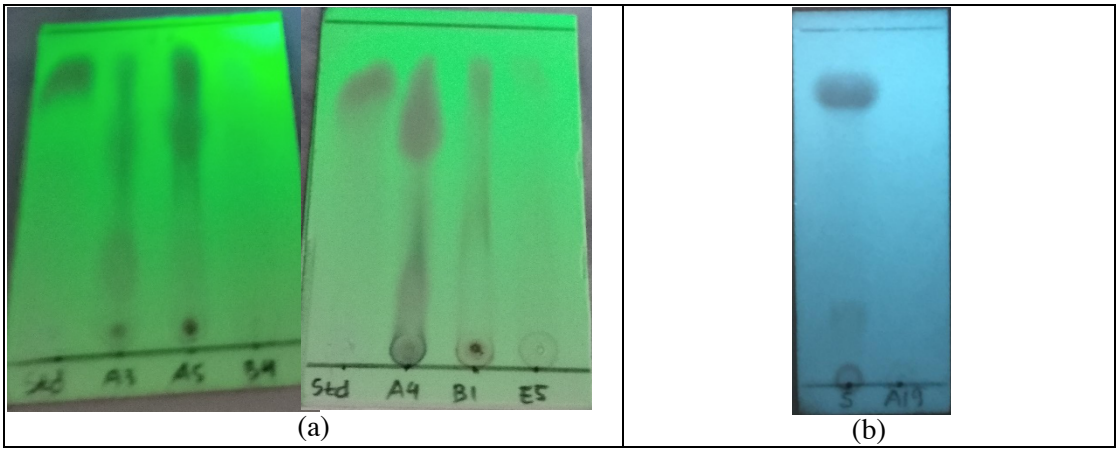

Figure 2. Compounds identification by TLC compare to CGA standard solution. (a) Fraction A-E, (b) fraction G.

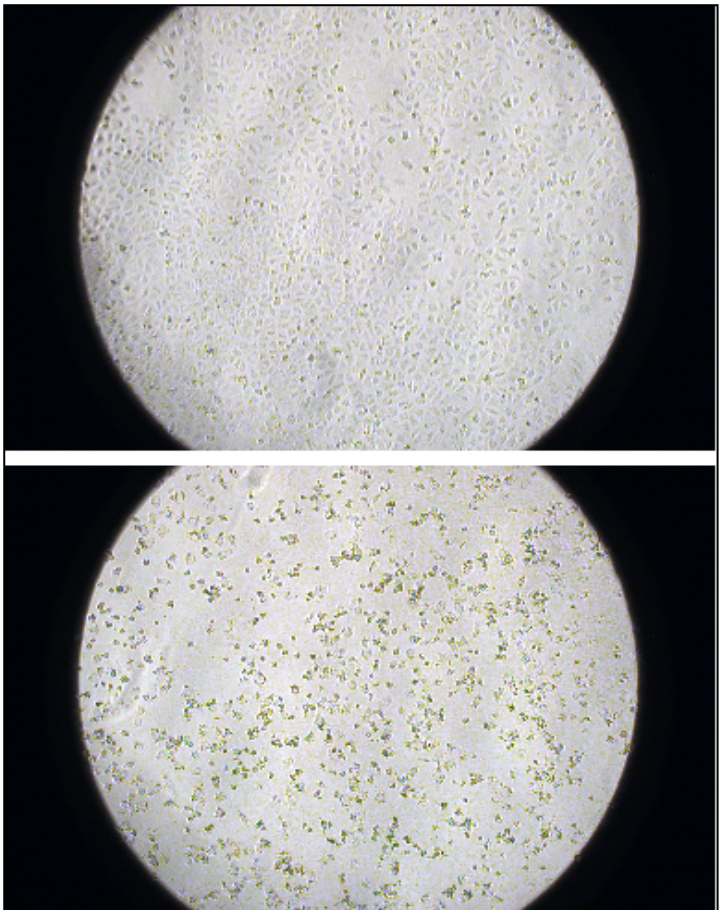

Figure 3. Microscopy images of MCF-7 after 24 h incubation. Without sample (above) and with sample G (below). The below image displays death of most MCF-7 cells.

3. Discussion

This research applied separation based on polarity using chromatographic column. In chromatographic methods, separation process distributes components and separates between two phases, stationary phase and mobile phase which move in a definite direction. The mobile phase leaves the column and this is called effluent. Based on molecule structure theory CGA is a polar compound, therefore the separation process was started polar solvent, followed by removing semi polar and non polar impurities. This was meant to purify the extract from nonpolar and semi polar compounds. The purified extract was fractionated with chromatographic column of water-methanol mixture to obtain 10 different fractions, coded according to Table 1.

The results obtained in accordance with the theory, the CGA was detected in the early fractions, which were A and B fractions. As predicted, CGA was not detected in the later fractions, which referred to C to K fractions (Table 2 and Figure 2).

Qualitative separation based on visual technique is a preliminary process for collecting fraction which dissolved in solvent from polyamide column. The next step is verification compounds by identify chemical compounds and determine compound purity using Silica thin layer chromatography (TLC), visual analysis on TLC spot, it is a quick and simple chemical analysis method [1].

The determination of CGA absence was conducted by visual determination on the spot by TLC analysis compare to CGA standard solution. Picture above (Figure 2) mentioned TLC analysis on CGA standard solution, the TLC analysis of sample from fraction A-E indicates the positively

availability of CGA substance in the sample A3, A4, A5, and B1, as a conclusion all fraction A and B is positively contain CGA, while the test on sample E5 indicates there was no CGA substance in fraction E5. The test on sample also G indicates that there was no CGA substance in sample G.

The separation in this fractionation process is based on polarity produced different samples, in each fraction indicated that there were different chemical compounds in every fraction. These differences were possibly due to different polarity of water-methanol mixtures, which were used as the mobile phase. The most polar compounds were in the beginning fractions, hereinafter were the less polar compounds.

Each fraction was tested against MCF-7 breast cancer cell line in order to study its anticancer activity. The G fraction was the only sample showed cytotoxicity against MCF-7 cell line although it did not show any presence of CGA based on the thin layer chromatography analysis. Previous studies on anticancer activity of CGA are scarce. Lee and Zu (2006) found that human breast cancer cell cultures MCF-7 which were treated with CGA 20 μM displayed inhibited DNA methylation approximately by 80% [22]. Meanwhile Yamagata et al. (2018) observed that human lung cancer cells which were cultured with CGA 50 μM displayed reduced cell proliferation by 40% [23]. Our samples, particularly fractions A and B, which contained CGA, did not indicate any anticancer characteristics. There are some possibilities beyond these negative results. First, anticancer activity of CGA is dose and time dependent [24]. The concentration of CGA in both fractions A and B were too low to show any anticancer effect. Second, there are compounds other than CGA in both fractions A and B with anticancer effects. In addition to CGA, it is suggested that several compounds from coffee such as caffeine, ferulic acid, and kahweol, which have anticancer characteristic as displayed in either in vitro or in vivo studies [25]. The G fraction in this study needs to be investigated in the future research to identify the potential anticancer compound.

4. Conclusion

Separation of coffee extract using a polyamide column chromatography with gradient water methanol solvent produced fractions containing different composition of compounds. TLC analysis showed that fractions A and B contain CGA substances. Fraction A-F and H-K did not show anticancer activity. Fraction G showed no CGA present was the only sample that showed anticancer activity against MCF-7 breast cancer cell line.

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