International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 05 Issue 12 December 2022
DOI: 10.47191/ijcsrr/V5-i12-51, Impact Factor: 5.995
IJCSRR @ 2022
www.ijcsrr.org

Formulation of Compressed Lozenges from Decaffeinated Arabica Green Coffee (Coffea arabica L.) Bean Extract as Immune Booster

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ABSTRACT: Global public health issues include emerging and reemerging viruses. The most recent cause of COVID-19, the severe acute respiratory syndrome coronavirus (SARS-CoV-2). The main goal of current medical research is the creation of new, affordable, and effective anti-COVID 19 medications. The immune system regulates coronavirus infection of the human body. The current study explores the relationship between antioxidant and the immune system's ability to fight off infections and the pathogenicity of the coronavirus. Arabica coffee contains chlorogenic acid is efficacious as a contributor to antioxidant and antiviral activity. But arabica coffee also contains caffeine which can cause ulcers that do decaffeination using dichloromethane solvent to reduce levels of caffeine. The objective of this study is to create Arabica green coffee seed extract compressed lozenges as antioxidant to boost immune system. Arabica coffee extraction is done by soxhletasi. Formulation lozenges made in three formulas with varying type of flavored creamer F1 (strawberry), F2 (tiramisu), and F3 (vanilla). Lozenges were analyzed using HPLC with pretreatment SPE to see the effect of the formulation on the content of caffeine and chlorogenic acid. F1 caffeine content 1.324%, 4.484% F2, F3 0.134. F1 chlorogenic acid content 2.996%, 2.834% F2, F3 4.530% .The result granules and lozenges evaluation is required of granules and lozenges requirements. Result of hedonic, respondent can receive a taste of lozenges. Formula lozenges which most preferred is the formula F1 with strawberries creamer diluent with caffeine content of 1.324% and chlorogenic acid content of 2.996%. The conclusion of this study is that green coffee bean extract can be made into lozenges, and caffeine levels in coffee can be reduced by decaffeination. but the level of chlorogenic acid decreased along with the decrease in caffeine.

KEYWORDS: Anti-virus, Arabica coffee, Chlorogenic acid, Covid-19, Immunity, Immune Booster.

INTRODUCTION

The effectiveness and functionality of the immune response is the most important component in the defense against viral infections, especially in the current COVID-19 pandemic situation, where there is neither an ideal vaccine nor a treatment. The immune response is essential to control and eliminate CoV infections. The immune system needs a number of nutrients, particularly vitamins and microelements, to function properly. Furthermore, consuming extra amounts of these nutrients has positive effects on cellular processes,viral replication, and immunological responses to viral infections[1].

Almost all viral infections result in the production of different inflammatory cells, particularly macrophages and neutrophils in certain infections[2]. On the other hand, virus infections typically result in the production of oxygen and nitrogen radicals. These radicals not only function as a physiological pathogen-clearing process but also have a wide range of pathological effects. While high concentrations of radicals can impede viral replication and harm cells due to their mitogenic action, low concentrations of radicals can enhance viral replication[3]. So oxidative stress and inflammatory processes have a profound modulating effect on the immune response[4].

Coffee as a functional food has antioxidant, anti-inflammatory, and anti-virus activity[5]–[7]. These qualities are linked to bioactive compounds, including caffeine, theophylline and theobromine, cafestol, kahweol, tocopherols, and trigonelline, in addition to chlorogenic acids and their derivatives[7]. Coffee beans' antioxidant capacity is dependent on the properties of phenolic chemicals, particularly chlorogenic acids (CGA), which have antioxidant capacity both in vitro and in vivo[8], [9]. CGA is antiviral and effective against several viruses, such as Human immunodeficiency virus[10], hepatitis B virus[11], influenza A virus[12], reduces

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inflammation brought on by viral infection, and also Corona virus[13], [14]. The SARS-CoV-2 receptors ACE2 and its co-expressed proteins are thought to be the main route by which SARS-CoV-2 enters target cells and have been connected to SARS-CoV-2 infection. The findings suggested that CGA in COVID-19 may function via moderating the inflammatory response[15].
Besides CGA, coffee also contains caffeine. Coffee's caffeine inhibits hydrogen peroxide-induced lipid peroxidation products in human skin fibroblasts and tissue lipid peroxidation and ROS. It also decreases oxidative stress and preserves the antioxidant system in hypoxia-induced pulmonary epithelial cells[16], [17]. But in other hand coffee frequently causes gastric release, GERD, and peptic ulcer disease, all of which are dyspeptic conditions[18].

MATERIALS AND METHODS

A. Materials

Plant Material

The Arabica green coffee bean collected from Pangalengan, West Java and authenticated by the Department of Biology, Faculty of Science, Universitas Padjadjaran, Bandung, Indonesia.

Materials

Ethanol 70%, chloroform, dichloromethane, stevia, creamer , aerosil® R200, magnesium stearate

B. Methods

Extraction

Arabica green coffee beans are dried at 40-50^{0}C, then meshed with No. 60. Arabica green coffee beans extracted using 70% ethanol with a soxhlet method. The ethanol was removed by a rotary evaporator (IKA RV 10, IKA Company, Staufen, Germany) at 40°C to obtain the crude extract[19].

Phytochemical Screening

To determine whether secondary metabolites like flavonoids, alkaloids, polyphenols, tannins, saponins, quinones, steroids/triterpenoids, monoterpenes, and sesquiterpenes were present, phytochemical screening was done[20].

Decaffeination

The decaffeination of the extract was carried out with dichloromethane and water in 1:1 ratio and then stirred using a magnetic stirrer for 10 minutes at room temperature, the procedure was repeated three times (Massih et.al., 2010). Caffeine will dissolve better in dichloromethane (140 mg/ml) than water (22 mg/ml)[21].

Chlorogenic Acid and Caffein Measurement with HPLC

Measurement of chlorogenic acid and caffeine levels was carried out on arabica green coffee bean extract before and after the decaffeination process. Dionex Thermo Ultimate 3000 HPLC with an EnduroSphere C18 column was used for both tests. Optimum test conditions for both were carried out using methanol and 1% acetic acid solution (40:60) as diluent, at a wavelength of 277nm with a flow rate of 1ml/min.

Lozenge Formulation

Arabica green coffee beans lozenge were made with all ingredients are mixed until a homogeneous mass is obtained. then sieved on sieve No.12. These were then directly compressed into tablets with a press, filling an average weight of 2,5 grams, with the composition shown in Table 1.

Evaluation of Arabica Green Coffee Extract Granules

The flow properties, defined by angle of repose and compressibility parameters by Carr's index and Hausner ratio, as well as tapped and bulk density, loss on drying (LOD) were used to analyze the granules made up of arabica green coffee bean extract and excipient[19].

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Evaluation of Arabica Green Coffee Extract Lozenges

a. The thickness and weight variation

A Vernier Caliper was used to measure the thickness of 20 lozenges per formula. Additionally, 20 tablets were individually weighed with an electronic balance and the results were compared to the weight of a average tablet. The data were reported as mean ± standard deviation (SD)[22].

b. Hardness

Ten tablets were chosen at random from each batch, and a hardness tester was used to assess the tablets' hardness. Each batch's mean values and standard deviation were determined[22].

c. Friability

Using a friability tester, the friability of tablets was determined. Twenty tablets were put in a plastic friability tester that was linked to a motor and rotated for four minutes at a speed of 25 rpm. The percentage of weight reduction was then measured using the procedure after they were brushed and reweighed[22].

d. Disintegration Time

Tablet disintegration time was carried out on 30 volunteers. mean value and standard deviation are calculated.

Hedonic Test

3 formula lozenges were given to 30 volunteers. Each formula is given a time lag of 2 minutes to neutralize the sense of taste. Hedonictest was assessed using a Likert scale. hedonic test results were then analyzed statistically using one-way analysis of variance (ANOVA) method. However, Kruskal–Wallis analysis method was used on instances where the data were not normally distributed.

Table 1. Formulation of arabica green coffee bean extract lozenges

Ingredients	*Composition of Lozenges (%)*		
	F1	*F2*	*F3*
Coffee extract	*5*	*5*	*5*
Strawberry creamer	*83*	-	-
Tiramisu creamer	-	*83*	-
Vanilla creamer	-	-	*83*
Aerosil R200	*2*	*2*	*2*
Stevia	*8*	*8*	*8*
Magnesium Stearate	*2*	*2*	*2*

RESULT AND DISCUSSION

Extraction

Arabica green coffee beans as much as 3.50 Kg were crushed and then extracted by soxhletation with 70% ethanol solvent. The extraction results are evaporated on the rotary evaporator which is then evaporated on the water bath. The resulting thick extract was 415.45 grams with an rendement of 11.87%.

Phytochemical Screening

The phytochemical screening of the extract aims to determine the compounds contained in the extract. The results of the phytochemical screening of the green coffee bean sample extract compared to the standard showed the same results, which contained alkaloids, flavonoids, polyphenols, monoterpenes and sesquiterpenes, triterpenoids, steroids and quinones.

Decaffeination and Chlorogenic Acid and Caffein Measurement with HPLC

Decaffeination uses dichloromethane as a solvent because it is a less toxic solvent when compared to chloroform. The decaffeination process was carried out three times with a 1: 1 ratio of water and dichloromethane and then analyzed using HPLC. From the results of decaffeination, caffeine and chlorogenic acid decreased. These results indicate that the decaffeination process using dichloromethane solvent can reduce caffeine levels in Arabica coffee beans[23], [24]. Result of concentration of chlorogenic acid and caffein after decaffeinated process shown in Table 2.

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Table 2. Chlorogenic acid and caffein concentration from decaffeinated arabica green coffee extract

Extract	*Concentration (%)*	
	Caffein	*Chlorogenic Acid*
Before Decaffeination	3,380 ± 0,08	4,159 ± 0,158
After Decaffeination	1,029 ± 0,08	3,019 ± 0,138

Evaluation of Arabica Green Coffee Extract Granules

The result of arabica green coffee bean extract granules evaluation is shown in Table 3. The results of the loss on drying granule of green arabica coffee bean extract (Coffea arabica L.) were between 3.90% and 4.15%. This shows that the loss on drying granules meets the requirements because the content is below 10%. The flow rate for all formulations ranged from approximately 14,40 to 15,44 g/s, angle of response 21,66º to 21,99º, Carr's compressibility index of approximately 14,63%–17,56% and Hausner compressibility ratio of approximately 1,171% to 1,206%. These show that the arabica green coffee bean extract and all excipients have good granule flow properties[19].

Evaluation of Arabica Green Coffee Extract Lozenges

Table 4 summarizes the findings from the evaluation of lozenges, including weight variation, diameter, thickness, hardness, friability, and disintegration time. The evaluation show that the lozenges formula produces good lozenges that meet the criteria.

Hedonic Test

The hedonic test was carried out on 30 volunteers. The data obtained is presented on a numerical scale. A hedonic test was conducted to find out whether all the tablet formulas with various flavors were acceptable to the volunteers. The results of the hedonic test were then analyzed for the normality distribution to find out whether the data used was normally distributed or not. The results showed that the data obtained were not normally distributed and continued further testing using the Kruskal-Wallis method. The results of the hedonic test showed no significant difference from all formulas (H0 was accepted). This result is indicated by the value of Assymp sig. 0.651 > 0.05. These results indicate that volunteers rated all formulas with various flavors as acceptable.

Table 3. Evaluation of granules

Formula	*LOD (%)*	*Flow rate (g/s)*	*Angle of response (º)*	*Carr's index (%)*	*Hausner ratio (%)*
F1	3,90 ± 0,08	15,44 ± 0,5	21,66 ± 1,13	14,63 ± 1,5	1,171 ± 0,02
F2	4,06 ± 0,05	14,87 ± 0,27	21,95 ± 0,53	16,10 ± 1,48	1,193 ± 0,02
F3	4,15 ± 0,02	14,40 ± 0,84	21,99 ± 1,4	17,56 ± 0,15	1,206 ± 0,01

All the values were calculated as mean ± standard deviation

Table 4. Evaluation of lozenges

Formula	*Weight variation (g)*	*Diameter (mm)*	*Thickness (mm)*	*Hardness (N)*	*Friability (%)*	*Disintegration time (min)*
F1	2,50 ± 0,008	25,10 ± 0,03	4,22 ± 0,06	79,58 ± 1,39	0,29 ± 0,02	7,04 ± 1,92
F2	2,50 ± 0,009	25,10 ± 0,02	4,21 ± 0,04	80,85 ± 1,26	0,23 ± 0,02	6,97 ± 1,42
F3	2,50 ± 0,01	25,21 ± 0,06	4,22 ± 0,03	81,15 ± 2,12	0,20 ± 0	6,93 ± 1,90

All the values were calculated as mean ± standard deviation

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Cite this Article: Tubagus Akmal, Arif Budiman, Holis Abdul Holik (2022). Formulation of Compressed Lozenges from Decaffeinated Arabica Green Coffee (Coffea arabica L.) Bean Extract as Immune Booster. International Journal of Current Science Research and Review, 5(12), 4942-4947