

The Pharmaceutical Availability of Gambier Leaves Bioactive Fraction Coated Tablet in Simulated Human Body Fluids

Ketersediaan Farmasi Tablet Salut Selaput Fraksi Bioaktif Daun Gambir di Simulasi Cairan Tubuh Manusia

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Abstract

The widespread usage of traditional medicine either in the developed or developing countries, makes traditional medicine requirements become a major concern in terms of assuring the safety and effectiveness of treatment. WHO suggested dissolution study to support traditional medicine clinical trials. The catechins from gambier could reduce atherosclerotic lesions case caused by elevated levels of cholesterol, LDL, and triglycerides. Catechin is hygroscopic that becoming unstable. Raising the stability, gambier is made to a coated tablet. This study aimed to determine the condition of gambier leaves bioactive fraction coated tablet in simulated human body fluids through an *in vitro* testing using dissolution tester. Three formulations coated tablet with different coating percentage had been tested using dissolution tester apparatus. The test was conducted in water, acid, and buffer as dissolution medium to generate the dissolution profile. Tablet evaluation showed that the three formulations dissolved $71.25\% \pm 6.26$ to $91.05\% \pm 3.05$ in the water, acid, and buffer dissolution medium. The gambier leaves bioactive fraction coated tablet had more than 70% pharmaceutical availability in simulated human body fluids.

Keywords: Catechin; Coated tablet; Dissolution test; Gambier leaves bioactive fraction.

Abstrak

Penggunaan obat tradisional secara luas di negara-negara maju maupun berkembang menjadikan pentingnya penetapan syarat obat tradisional untuk keamanan dan keefektifan pengobatan. WHO memasukkan parameter uji disolusi sebagai data yang diperlukan untuk menunjang uji klinis obat tradisional. Katekin dari gambir berkhasiat mengurangi lesi aterosklerosis pada kasus peningkatan kadar kolesterol, LDL, dan trigliserida. Katekin bersifat higroskopis sehingga untuk meningkatkan kestabilannya dibuat tablet salut selaput. Penelitian ini bertujuan untuk mengetahui kelarutan tablet salut selaput fraksi bioaktif daun gambir di dalam cairan tubuh manusia dengan alat uji disolusi. Tiga tablet dengan variasi prosentase penyalut diuji kelarutannya dengan menggunakan alat uji disolusi. Uji disolusi dilakukan dalam media disolusi air, asam, dan buffer untuk mengetahui profil disolusinya pada interval waktu tertentu. Hasil uji disolusi menunjukkan kelarutan katekin sebesar $71,25\% \pm 6,26$ sampai $91,05\% \pm 3,05$ untuk semua variasi tablet di media disolusi air, asam, dan buffer. Tablet salut selaput fraksi bioaktif daun gambir mempunyai nilai ketersediaan farmasi lebih dari 70% di dalam simulasi cairan tubuh manusia.

Kata kunci: Fraksi bioaktif daun gambir ; Katekin; Tablet salut selaput; Uji disolusi.

INTRODUCTION

According to World Health Organization, more than 50% population of a country use traditional medicine as medicaments for threatening short and chronic ailments, termed as complementary medicine.¹ The acceptability of traditional medicine is increasing now a day due to their affordability, effectiveness, and less toxicities.² The medicinal plant's overflow availability and affordable price underlie studies of medicine raw material.³

Recent studies showed Indonesian pharmaceutical industries imported more than 90% medicine raw material for chemical medicine, worth USD 959 million.^{4,5} Optimizing the potential of medicinal plants is expected to reduce the raw material import dependency also stabilizing the price and availability.³

Indonesia as one of developing countries has very potential biodiversity to be developed, among others medicinal plants. Indonesian Ministry of Trade reported in 2013, Indonesia exported traditional medicine worth \$23,44 million.⁶ Gambier is one of the medicinal plants that can be found in Indonesia and become west Sumatra top export commodities. It dominates almost 90% gambier source in Indonesia.⁷

Gambier has 35-95% catechin as the main component that can reduce levels of cholesterol, LDL, triglycerides and increases levels of HDL with 20 mg dose/200 g mice weight.^{8,9} Besides catechin, there are epicatechin, procyanidin, gambierin, red catechu, quercetin, and also fat, wax, and alkaloids.^{10,11}

Due to the widespread usage of traditional medicine, many pharmaceutical companies produce various traditional medicine formulations inclusive of a tablet. Ensuring the quality, The National Agency of Drug and Food Control requires some physical and chemical parameters to be fulfilled. WHO suggested dissolution studies to support clinical trials of herbal products.¹²

Bioavailability is one of the tablet quality requirement to assure the tablet gives therapeutic effects. The bioavailability test as an *in vivo* performance can be predicted through *in vitro* testing using dissolution tester. The dissolution test will show the tablet pharmaceutical availability that represents the amount of dissolved active substances. The higher active substances dissolved, the higher active substances absorbed in stomach and intestines to give therapeutic effects.¹³

Catechin is hygroscopic due to its many hydroxyls.¹⁴ Furthermore, excessive ultraviolet radiation including high influence rate and long period radiation decreased the level of total catechin.¹⁵ The instability of catechin is proven that after six weeks, catechin in gambier mouthwash decreased 5,06%.¹⁶ Increasing the stability, gambier requires to be fractionated and the tablets require to be coated. While to ascertain the efficacy, the tablets require dissolution test.

Gambier leaves extract was fractionated with ethyl acetate due to Kassim found that the highest catechin was in ethyl acetate than either in methanol or in hot water.¹⁷ Gambier tablet was formulated into 4%, 6%, and 8% weight gain accordance to Subburayalu, that opadry AMB as a protective layer found to be stable at 4%, 6%, and 8% of weight gain.¹⁸

Dissolution profile is the time required for tablets to dissolve in the dissolution medium. The dissolution profile differences occur due to the formulas. The aim of this study is to determine the dissolution profile of three different coating percentage tablets in simulated human body fluids: water, acid, and buffer.

The dissolution test was carried out in three dissolution medium that represents a place of drugs absorption. Water represents major human body fluids, acid medium pH 1.2 represents gastric fluids, and buffer medium pH 6.8 represents intestinal fluids.¹⁹ The tablets were tested in a wide

physiological pH range from 1.2 to 7.8 to determine the solubility characteristic.²

METHOD

This study was conducted in Pharmacy Laboratory, Center for Research on Biomedic and Basic Health Technology, National Institute of Health Research and Development. Three different coating percentage tablets of gambier leave bioactive fraction had been tested using dissolution tester apparatus. The test was conducted in three simulated human body fluids (without enzyme): water, acid, and buffer. Dissolved catechin in dissolution medium was analyzed using high performance liquid chromatography (HPLC).

Equipment and materials

The equipment were: analytical balance (Mettler Toledo), dissolution tester (Hanson Vision G2 Elite), high performance liquid chromatography (Waters Alliance 2695).

The materials were: catechin reference (Sigma Aldrich), methanol LC grade (Merck), trifluoroacetic acid (Merck), acetonitrile LC grade (Merck), formic acid (Merck), universal pH tape measurement (Merck), chloride acid 32% (Merck), potassium dihydrogen phosphate (Merck), sodium chloride (Merck), sodium hydroxide (Merck), distilled water, microcrystallin cellulose (Avicel PH 102), polyvinyl pyrrolidone (PVP Kollidon K30), Magnesium stearate, sodium starch glycolate (Primojel), talc, aerosil, and opadry AMB white (Colorcon).

Samples

The samples were: core tablet of gambier bioactive fraction, tablet A: 4% weight gain coated tablet of gambier leaves bioactive fraction, tablet B: 6% weight gain coated tablet of gambier leaves bioactive fraction, and tablet C: 8% weight gain coated tablet of gambier leaves bioactive fraction.

Gambier leaves extract fractionation

Amount of gambier leaves extract was fractionated with ethyl acetate. The fractionation was preceded by 6-hour maceration. Percolation is afterward. The fractionation was done up to limpid fraction.¹⁷

Tablet coating

The core tablet of gambier bioactive fraction was coated with opadry AMB white suspension. The tablet coating was conducted with a round coating pan that rolling stable. The opadry AMB suspension was sprayed with spray gun into the pan contained the core tablet. Whereas in front of the pan, there is a hot air blower that blown a hot air into the pan to dry up the tablet. The air inside the pan was suctioned out with vacuum exhaust. The coating was done up to 4%, 6%, and 8% of weight gain.²⁰

Simulated gastric fluid pH 1.2

2.0 g of sodium chloride dissolved in 7.0 ml of chloride acid 32%, diluted with distilled water up to 1000 ml.¹⁹

Simulated intestinal fluid pH 6.8

8.96 g of sodium hydroxide was added into 68.05 g potassium dihydrogen phosphate then dilute with distilled water up to 1000 ml.¹⁹

Dissolution studies

Dissolution studies were carried out in 900 ml, in each of distilled water, simulated gastric fluid pH 1.2, and simulated intestinal fluid pH 6.8 using Hanson Vision G2 Elite type II (paddle) dissolution test apparatus. The dissolution medium was maintained at $37 \pm 0.5^\circ\text{C}$ and a speed of 100 rpm paddle stirrer. A 10 ml aliquot was withdrawn at different time intervals (15, 30, 45, and 60 minutes), filtered, and replaced with 10 ml of fresh dissolution medium to maintain a constant volume. The aliquot was analyzed using high performance liquid chromatography.²¹

Catechin assay

The aliquot was analyzed by high performance liquid chromatography that equipped with a 4.6 x 150 mm Sun Fire column and PDA detector. Catechin was detected at 280 nm with 0.45 ml/min flow rate and 1.0 µL volume of injection. The method is using 0.03% trifluoroacetic acid in acetonitrile:distilled water (5:95) as mobile phase A and 0.1% trifluoroacetic acid in acetonitrile as mobile phase B with gradient condition: 0-4 minutes (100% A), 4-20 minutes (71.5 A; 28.5 B) and 20-30 minutes (100% B).⁹

RESULT AND DISCUSSION

Dissolution is a movement of particles from a solid into a solvent (medium) where the particles become a solution. The dissolution studies show the tablets ability to release active substances inside that dissolve in gastric or intestinal fluids. The dissolution test will confirm disintegration time due to disintegration time simply indicates the time it takes for the tablet to

disintegrate into small particles, without giving assurance that the tablet is able to release the active substances inside.²² Tablet with fast disintegration time is not necessarily high soluble that the dissolution test is required. The dissolution test describes the release of the drug into a bioavailability form.²³

The dissolution results as the means of percents dissolved versus time for core tablet of gambier bioactive fraction, tablet A, tablet B, and tablet C coated tablet of gambier leaves bioactive fraction are given in Table 1. The dissolution profile of all samples in water, acid, and buffer dissolution medium is given in figure 2, 3, and 4 respectively.

The chromatogram in figure 1, showed catechin reference and dissolved catechin peak at thirteenth minutes of retention time. The retention time similarity between catechin reference and dissolved substances in dissolution medium confirmed that the dissolved substance is catechin.

Table 1. Dissolution Test Result

| Medium and Time | Dissolution Test Result (%) | | | | |
|-----------------|-----------------------------|--------------|--------------|--------------|--------------|
| | Core Tablet | Tablet A | Tablet B | Tablet C | |
| Water | 15' | 84.74 ± 3.97 | 87.43 ± 4.36 | 91.05 ± 3.05 | 90.14 ± 2.25 |
| | 30' | 86.93 ± 3.92 | 89.92 ± 2.35 | 87.97 ± 5.42 | 87.51 ± 2.43 |
| | 45' | 87.49 ± 5.28 | 89.25 ± 2.53 | 90.71 ± 0.83 | 86.76 ± 4.40 |
| | 60' | 88.15 ± 2.85 | 86.96 ± 1.19 | 88.27 ± 0.80 | 85.36 ± 4.48 |
| Acid pH 1.2 | 15' | 86.95 ± 6.49 | 86.08 ± 3.61 | 90.68 ± 8.58 | 89.69 ± 8.23 |
| | 30' | 84.09 ± 4.66 | 92.54 ± 2.02 | 93.03 ± 2.39 | 86.73 ± 5.25 |
| | 45' | 83.47 ± 5.78 | 80.69 ± 2.95 | 88.55 ± 5.84 | 97.39 ± 2.28 |
| | 60' | 94.42 ± 2.42 | 91.65 ± 6.62 | 92.68 ± 3.42 | 93.37 ± 2.94 |
| Buffer pH 6.8 | 15' | 83.62 ± 5.15 | 82.16 ± 4.50 | 79.66 ± 2.77 | 71.25 ± 6.26 |
| | 30' | 73.43 ± 2.40 | 67.39 ± 2.66 | 70.96 ± 1.32 | 65.57 ± 3.31 |
| | 45' | 60.74 ± 2.34 | 55.97 ± 1.61 | 55.46 ± 3.65 | 54.26 ± 3.01 |
| | 60' | 56.79 ± 1.25 | 48.77 ± 2.53 | 50.31 ± 3.94 | 49.66 ± 1.30 |

Catechin is poorly soluble in water but dissolves easily in hot water.^{17,24} Dissolution medium with 37°C temperature has enhanced catechin solubility in water. Catechin in the core and coated tablets dissolved 84.74% ± 3.97 to 91.05% ± 3.05 (Table 1) in water at the first fifteen minutes.

In water dissolution medium, tablet A, B, and C had nearly the same profile dissolution with core tablet of gambier leaves bioactive fraction (Figure 2). All samples dissolved significantly at the first fifteen minutes and dissolved stably up to

the sixtieth minute. According to the data, tablet coating is not affecting drugs release accordance to Patel.²⁵

Dissolved catechin in water dissolution medium showed in the first fifteen minutes, the core tablet and tablet A, B, and C dissolved more than 80% but decreased slightly in the last sixty minutes (figure 2). The reduction of catechin in the dissolution medium is due to there had been a maximum dissolution in the two first time interval. The dissolution medium addition will cause dilution.²⁶

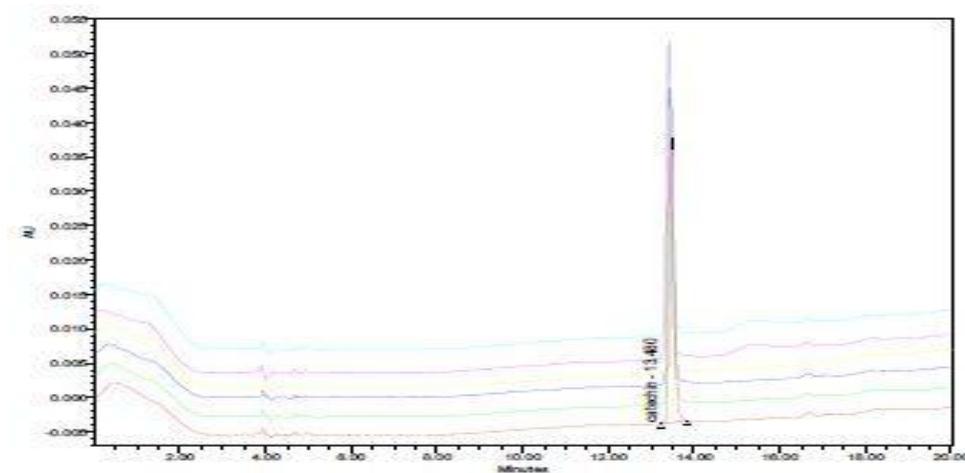


Figure 1. HPLC chromatogram of catechin reference and dissolution sample

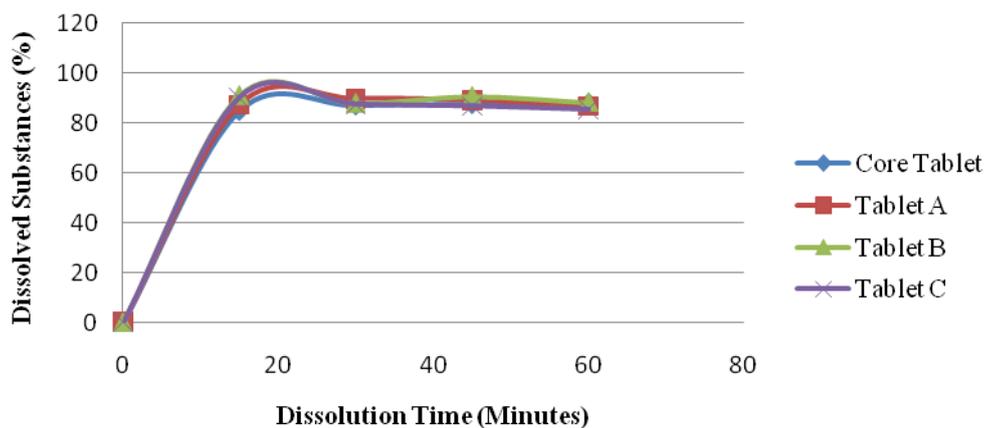


Figure 2. Dissolution profile in water

Active substances in acid dissolution medium produce fluctuate dissolution profile chart (Figure 3). The fluctuation is affected by type of apparatus (paddle or basket), stirring rate, dissolution medium temperature tolerance, as well as withdrawing and substituting dissolution medium.²

Core tablet, tablet A, B, and C dissolved more than 80% (table 1) in the first fifteen minutes due to catechin has a high reactivity in acid physiological pH.²⁵ Percentage of coating had not shown containment of active substance, precisely because tablet B with 6% of coating

resulted dissolved catechin greater than core tablet. Coating only affects the physical and chemical stability of the tablet.²⁵

Core tablet, Tablet A, B, and C in buffer medium gave different dissolution profiles (Figure 4) than in water and acid medium. In the first fifteen minutes, dissolution occurs in a range of $71.25\% \pm 6.26$ to $83.62\% \pm 5.15$ (Table 1). In the next three interval times: 30, 45, and 60 minutes the dissolution decreased consecutively, in a range $56.79\% \pm 1.25$ to $49.66\% \pm 1.30$ (Table 1).

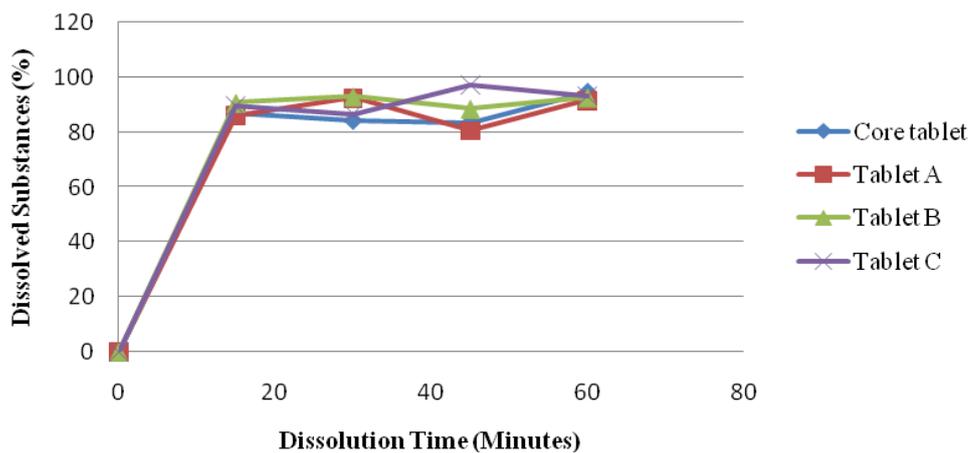


Figure 3. Dissolution profile in acid

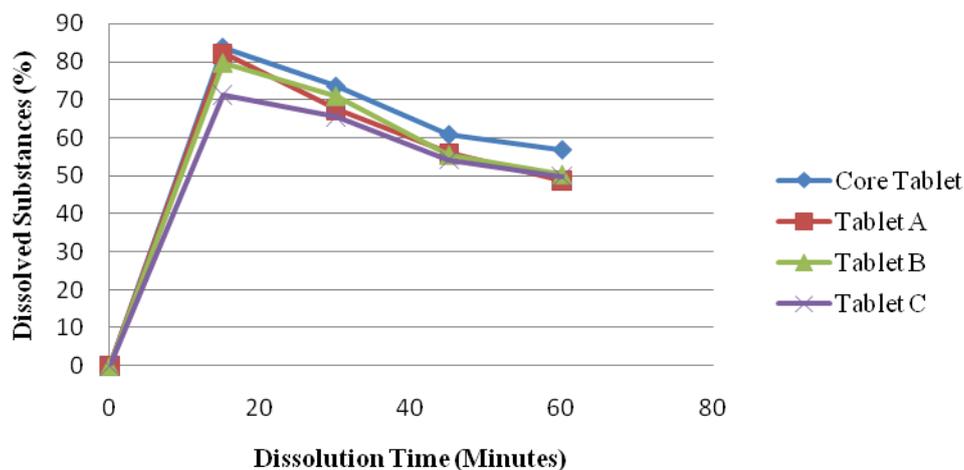


Figure 4. Dissolution profile in buffer

The decreased level of dissolved catechin is affected by the tablet excipient, polyvinyl pyrrolidone that can form high viscosity layer around the tablet if it reacts to water (part of buffer dissolution medium). The layer could inhibit releasement of catechin as the active substance into dissolution medium. The containment gave a level decrease. Whereas, catechin in pH 6.9 easily turns into tannic catechu acid.²⁷

The lessening of catechin in buffer medium is expected not to affect the effectiveness of treatment because the tablet had already dissolved more than 70% in water and acid that represent stomach, a place of absorption before intestines. Accordance with the function of the coated tablet that expected to disintegrate in stomach.²⁸

The result of dissolution test showed all the tablets had 71.25% \pm 6.26 to 91.05% \pm 3.05 (table 1) pharmaceutical availability in water that represents a major human body fluid, in acid that represents the stomach, and in buffer that represents the intestine.

CONCLUSION

The gambier leaves bioactive fraction coated tablet had more than 70% pharmaceutical availability in simulated human body fluids: water, acid, and buffer. A large amount of dissolved catechin is expected to generate effective treatment.

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