

## ***In vitro* lipase enzyme inhibitory activities of green tea and other herbs**

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### **ABSTRACT**

**Background:** Increased lipase activity can increase the amount of monoglycerides and fatty acids absorbed by the body, this is what affects obesity. If pancreatic lipase activity is inhibited, the production of fatty acids will decrease, consequently the level of fat in the blood will also decrease.

**Objective:** To study anti obesity potential of a single herb or a mixture of green tea and other herbs (*Syzygium polyanthum. Lv*, *Artocarpus communis. Lv*, *Cinnamomum verum. Lv*, *Manilkara zapota. Lv*, *Tectona grandis. Lv*) by the *in vitro* inhibition assay of the lipase enzyme activity

**Method:** A single (8 herbs) or a mixture of three herbs powder (12 formulas) was put into a tea bag with a total weight of 1.5 g to be brewed in 100 mL bottled mineral water at 70°C-90°C for 10 minutes. *In vitro* lipase enzyme inhibition assay were conducted to measure the ability of these samples as lipase inhibitor. Orlistat used as positive lipase inhibitor.

**Results:** There was a difference results of lipase inhibition activity between the sample of a single and a mixture of herbs. Interestingly, the results shows that formula 3 (mixture of green tea, *Manilkara zapota* and cinnamon). has 53.942 % inhibition, formula 9 (a mixture of green tea, *Syzygium polyanthum* and cinnamon) has 67.322 % inhibition and formula 12 (a mixture of green tea, *Tectona grandis* and cinnamon) has 56.612 % inhibition which close to lipase inhibitory activity by standard Orlistat. **Conclusion:** The mixture of green tea and two other herbs has a lipase inhibitory activity similar to Orlistat, when compared to the single herb. The highest lipase inhibitory activity is found in formula 9.

**Key Words:** green tea; herbs; anti obesity; lipase inhibitor

### **INTRODUCTION**

In our study, we used several types of plants such as breadfruit leaves, (*Artocarpus communis* (Sk)) as antidiabetic<sup>1</sup>, cinnamon leaf (*Cinnamomum verum* (K)) as antidiabetic<sup>2</sup>, *Syzygium polyanthum* (Sl) bay leaf which is high in antioxidants<sup>3</sup>, sapodilla leaf (*Manilkara zapota*) (Sw) as anti-diabetic.<sup>4</sup> Teak extracts can be considered as one of the alternatives to chemical food preservatives and for controlling food.<sup>5</sup>

The Tea leaves (*Camellia sinensis*) is one of the most common drinks consumed globally. Green tea is more than just a drink, its unparalleled healthy nature is due to minimal oxidative processes, as a food supplement against disruptive diseases such as cancer, inflammation, diabetes, obesity, nerve damage, heart disease, autoimmune diseases, microbial pathogens, and osteoarthritis.<sup>6</sup>

Polyphenols, especially epigallocatechin gallate (EGCG) can protect pancreatic beta cell damage from the effects of oxidation. In addition, research by giving green tea orally, found that giving green tea can reduce the increase in blood sugar levels. EGCG in green tea works by inhibiting sodium-glucose transporters in the mucosa. The results showed that diabetic rats treated with green tea showed the lowest pattern of increased blood glucose over time, this showed that green tea had the best level of inhibition of blood glucose absorption compared to

other beverage treatments. In mice treated with a mixture of green tea and mulberry leaf tea still showed inhibition of increased blood glucose levels when compared to those treated in bottled drinking water, but their ability was lower when compared to those receiving green tea or mulberry leaf tea alone.<sup>7</sup>

This study aims to observe anti obesity potential of a single herb or a mixture of green tea and other herbs (*Syzygium polyanthum. Lv*, *Artocarpus communis. Lv*, *Cinnamomum verum. Lv*, *Manilkara zapota. Lv*, *Tectona grandis. Lv*) by the *in vitro* inhibition assay of the lipase enzyme activity. Green tea known to help you lose weight but also has the bitter taste. A randomized design formulation (12 formulas) of herbal tea was conducted by increasing green tea content and mixed with two other herbs that expected to improve flavor and inhibition to lipase enzymes.

### **MATERIAL AND METHODS**

#### **Material**

Green tea leaves obtained from Research Center Of Tea and Quinine (P2TK), Gamboeng, West Java Province, *Syzygium polyanthum Lv*, *Artocarpus communis Lv* comes from Research Center of Biology, Bogor, *Tectona grandis. Lv*, *Cinnamomum verum. Lv* comes from market, *Manilkara zapota. Lv* come from housing area, Aquadest, filter paper, enzim lipase

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inmorfolin propan sulfonat 10 mM (MOPS) dan 1mM EDTA, pH 6.8 , tris buffer (100mMTris-HCl dan 5mM CaCl<sub>2</sub>, pH 7.0) , p-nitrofenil butirat(p-NPB)/DMF 10mM. Enzim lipase were purchased from Tokyo Chemical Industry (TCI), p-nitrofenil butirat(p-NPB) were purchased from Sigma Aldrich. Toluene.

**General Experiment**

LC-ESI-MS were obtained using a Mariner Biospectrometry-Finnigan in ESI negative-mode. Absorbance measurement was conducted in 96 well plate reader using a Thermo Scientific Varioskan™.

**Water Content<sup>8</sup>**

Determination of water content is done using the azeotroph distillation method. Toluene was first saturated by the methods found in Pharmacopoeia Indonesia. A number of ingredients and toluene are put into a distillation flask and then heated. Distillation is carried out at a rate of approximately 2 drops per second, until some of the water is distilled. The volume of water is read on the scale indicated on the distillation apparatus. Moisture content is calculated by the formula:

$$\frac{\text{Volume of water (mL)} \times \text{specific gravity of water (g/mL)} \times 100\%}{\text{Weight sample (g)}}$$

**Ash Content<sup>9</sup>**

Material is incanded slowly at a temperature of 500-600 oC until it turns into white ash. Total ash content is calculated in g per g of air-dried material.

**Sample Preparation**

Green tea and herbaceous leaves *Syzygium polyanthum.Lv*; *Artocarpus communis.Lv*; *Manilkara*

*zapota.Lv*; *Tectona grandis.Lv* ;*Cinnamomum verum.Lv* are then dried at room temperature and made with a size of 20 mesh. Green tea powder and other herbs are homogenized respectively, then weighed with a comparison of the proportion of green tea leaves; herb (*Syzygium polyanthum.Lv*; *Artocarpus communis.Lv*; *Manilkara zapota.Lv*; *Tectona grandis.Lv* ); cinnamon leaves (b / b). A mixture of three leaf powder was put in a tea bag with a total weight of 1.5 g to be brewed in 100 mL of bottled mineral water at 70-90°C for 10 minutes.

**Anti Obesity Activity Test**

The in vitro anti obesity test was measured as lipase inhibitory assay following the method by Kim et al.<sup>10</sup> The 20 µL sampel was added with 20 µL of lipase enzyme (in morpholine propan sulfonic acid 10 mM (MOPS) and 1mM EDTA, pH 6.8) and added with tris buffers (100mMTris-HCl and 5mM CaCl<sub>2</sub>, pH 7.0) to 200 µL incubation for 15 minutes at 37°C then added 5 µL p-nitrophenyl butyrate (p-NPB) / DMF 10mM, then incubated for 30 minutes at 37°C then measured with a wavelength of 405 using Thermo Scientific Varioskan™.

**RESULTS**

Table 1. shows data on water content and ash content of green tea and other herbs. The results show the value of water content between 5.7-9.6% and ash content between.

**Table 1. Ash and Moisture Contents of Gamboeng Green Tea Leaves and Other Herbs on Dry Basis**

Leaves Sampel	Local /English name	Sample Abbreviation	Water Content (%)	Ash Content (%)
<i>Camellia sinensis.Lv</i>	Teh hijau/Green tea	G	4.15	5.96
<i>Syzygium polyanthum.Lv</i>	Salam/Bay	Sl	7.46	6.4
<i>Artocarpus communis. Lv</i>	Sukun/Bread fruit	Sk	6.27	9.6
<i>Manilkara zapota.Lv</i>	Sawo/Sapodila	Sw	6.41	5.7
<i>Cinnamomum verum.Lv</i>	Kayu manis/Cinnamon	K	6.59	4.88
<i>Tectona grandis.Lv</i>	Jati/Teak	Dj	8.97	7.08

**Table 2. Prediction of Chemical Compounds in Gamboeng Green Tea Leaves and Other herbs**

Molecular weight	Orlistat	G	Sk	S <sub>l</sub>	Sw	K	DJ	Compound name
175		v		v			v	L-thianine
194		v						Caffeine
495	v							Orlistat
185	v				v			1,9-Dihydro-6H-purin-6-imine
195								2-Amino-5,8-dihydro-4,6,7(1H)-pteridinetriene,9CI
261		v	v	v		v		Flindersine;1',2'-Dihydro,1'R*,2'S*-dihydroxy
144						v		N,N,N',N'-Tetramethyl-1,4-butanediamine,CI
210					v			Methyluracil
245					v			1-(2,4-Dihydroxyphenyl)-2-(3,5-dihydroxyphenyl)ethylene;(E)-form
225				v				3,7-Dimethyl-1-6-octadien-3-ol;(&plusmn;)-form,2-Methylpropanoyl
458		v						Epigallocatechin gallate

**Table 3. Obesity Activity Test of Green Tea and Other Herbal**

Sample	Composition	% Inhibition Lipase	% Inhibition $\alpha$ -Glu <sup>16</sup>
EGCG	1%	74,95	77.86
Orlistat (anti obesity standard)	1%	46.79	-
Sliming tea	1%	22,16	-
Green tea (G)	1%	47,82	47.31
Cinnamon Lv.( K)	1%	16,23	90.11
Bay leaves (Sl)	1%	30,96	93.00
Bread fruit Lv (Sk)	1%	23,58	57.98
Sapodilla leaves (Sw)	1%	40,83	94.29
Tectona grandis leaves (Dj)	1%	57.18	96.85
G-Sw-K	Formula 1 (4:4:4)	19,39	94.78
	Formula 2 (4:2:1)	27,97	27.96
	Formula 3 (4:1:1)	53,942	99.58
G-Sk-K	Formula 4 (4:4:4)	37,55	55.01
	Formula 5 (4:2:1)	10,34	84.29
	Formula 6 (4:1:1)	36,21	68.52
G-Sl-K	Formula 7 (4:4:4)	28,54	92.04
	Formula 8 (4:2:1)	26,442	93.35
	Formula 9 (4:1:1)	67,29	95.35
G-Dj-K	Formula 10(4:4:4)	3,57	49.78
	Formula 11(4:2:1)	13,60	69.74
	Formula 12(4:1:1)	56,61	63.92

Table 2. shows the LCMS analysis results of the compounds contained in each herb. Flindersine; 1', 2'-Dihydro, 1'R \*, 2'S \* -dihydroxy is found in almost all herbs analyzed. In the orlistat standard, 1,9-Dihydro-6H-purin-6-imine compound is also detected which are also contained in *Manilkara zapota*.Lv. L-Thianine which the compound contained in green tea, is also detected in bay leaf herbs and teak leaves.

Table 3. shows that orlistat which is the standard of commercial known lipase inhibitor compound has 46.79% inhibition, whereas sliming tea as commercial comparison of anti obesity herbs has 22.16% inhibition, There is a difference results of lipase inhibitory activity between the sample of a single and a mixture of herbs. Interestingly, the results shows that formula 3 (mixture of green tea, *Manilkara zapota* and cinnamon, with a ratio of 4: 1: 1). has 53.94 % inhibition, formula 9 (a mixture of green tea, *Syzygium polyanthum* and cinnamon, with a ratio of 4: 1: 1) has 67.32 % inhibition and formula 12 (a mixture of green tea, *Tectona grandis* and cinnamon with a ratio of 4: 1: 1) has 56.61 % inhibition which close to lipase inhibitory activity by standard Orlistat.

## DISCUSSION

Determination of water content aims to provide a maximum limit on the amount of compound lost in the drying process<sup>8</sup>. The results of the analysis of the water content of herbs used in this study can be seen in Table 1. which ranged from 4.15-8.97%. The requirements given by Indonesian National Standard (SNI) for the water content of green tea bag products are a maximum of 10%, therefore results water content results of herbs

used in this study fulfilled the requirement of the SNI standard<sup>11</sup>.

The value of ash content is useful as a parameter for the range of permissible internal and external mineral content, this is related to purity and contamination<sup>8</sup>. The results of ash content analysis of herbs used in this study can be seen in Table 1, which ranged from 4.88-7.08% except for *Artocarpus communis*. The requirements given by Indonesian National Standard (SNI) for the ash content of green tea bag products is a maximum of 7%, therefore results of most herbs used in this study fulfilled the requirement of the SNI standard<sup>11</sup>.

LCMS results from table 2. shows that almost all herbs extracts have compounds with 261 molecular weights, predicted as Flindersine; 1', 2' Dihydro, 1'R \*, 2's \* -dihydroxy, which is a group of Flindersine which has anti-bacterial and anti-fungal activities<sup>12</sup>.

The presence of a hydroxyl group (Flindersine; 1', 2' Dihydro, 1'R \*, 2'S \* -dihydroxy) substituted on the benzene ring contributes to the ability of phenolic compounds to release hydrogen atoms and involved redox reactions with Folin Ciocalteus reagents. The more the number of hydroxyl groups and double bonds are conjugated to phenolic compounds, the greater the potential of the compound to be involved in the redox reaction, as confirmed by Widyawati.<sup>13</sup>

Green tea generally has the highest amount of EGCG, compared to other teas. because green tea is not partially or fully oxidized. Tea made from *Camellia sinensis* has a higher EGCG content than tea made from *assamica* tea plants. Epigallocatechin gallate (EGCG) is considered the most abundant and powerful compound in green tea extracts. EgCg was found to have a high

content in the dried leaves of green tea (7380 mg per 100 g) compared to white tea (4245 mg per 100 g) and black tea (936 mg per 100 g)<sup>14</sup>. Besides many others bioactivities of EGCG<sup>7</sup>, this compound also reported has lipase inhibitory activity<sup>15</sup>.

From table 3, it can be seen that lipase inhibition by a single herbs and by formula of a mixture of green tea with 2 other herbs. For comparison, lipase inhibition by ECGC and Orlistat as the known lipase inhibitors and slimming tea as a commercial herbs for anti obesity also shown in Table 3. Higher lipase inhibitory activities were found in formula containing much higher green tea content and lower content of the other 2 herbs in the formulas with a ratio of 4: 1: 1 (green tea: herb1: herb2) such as formula 3(G:Sw:K=4:1:1), 9(G:Sl:K=4:1:1) and 12 (G:Dj:K=4:1:1), in comparison to formulas with other ratio (4:4:4 and 4:2:1). Formula 9 containing green tea-bay leaf and cinnamon leaf has the highest lipase inhibitory activity (67,292 %). In previous study by Megawati et al.<sup>16</sup> alpha glucosidase inhibitor (Table 3), Formula 3 (99.58% inhibition), Formula 9 (93.35% inhibition) and Formula 12 (63.92% inhibition) also showed the highest  $\alpha$ -glucosidase inhibitory activities compare to the same mixture with different formula ratio. Hence formula with a ratio 4:1:1 showed similar increase effect for both  $\alpha$ -glucosidase and lipase inhibitory activities.

## CONCLUSIONS

The mixture of green tea and two other herbs with ratio of 4:1:1 has a lipase inhibitory activity similar to Orlistat. The highest lipase inhibitory activity is found in formula 9 (a mixture of green tea, *Syzygium polyanthum* and cinnamon) that has 67.322 % inhibition. Further studies are needed to develop potential of this mixture as functional drink for anti obesity.

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