ANTI-OBESEITY ACTIVITY OF EXTRACT UNDER VARIOUS FRACTIONS OF JAMBU AIR SAMARANG (SYZYGIUM SAMARANGENSE) LEAVES ON WISTAR FEMALE RATS

ATUN QOWIYYAH*, SETIADI IHSAN, SIVA HAMDANI, LAELATUS SYIFA

Department of Pharmacy, Faculty of Mathematic and Natural Science, Universitas Garut, Garut, Indonesia
*Corresponding Author: atunqowiyyah@gmail.com

Abstract

Syzygium samarangense was the lipase inhibitor that capable of inhibiting lipid absorption, so it assumes have a potency to be anti-obesity agent. The purpose of this study was to evaluate the anti-obesity effect of extract and various fractions of Syzygium samarangense leaves. The extracts were prepared by maceration method while fractionation process was carried out using liquid-liquid extraction method. This study was also done using Wistar female rats induced by high carbohydrate food for 45 days and Monosodium Glutamate (MSG) subcutaneous injection 2 g/kg body weight of rat for 5 consecutive days followed by 14 days treatment. Induction with high carbohydrate food was continued during the treatment time. Results showed that MSG and high carbohydrate food could induce obesity. The extract (containing n-hexane and ethyl acetate fraction) at a dose of 50 mg/kg body weight of rat had anti-obesity effect by inhibiting body weight. The best activity was shown by the fraction of n-hexane with 85.96% of inhibition percentage. The ethanol extract, n-hexane, and ethyl acetate fractions in Syzygium samarangense leaves did not have laxative and anorexic effect. It also could not decrease fat deposition in liver organ and abdominal fat tissue.

Keywords: Syzygium samarangense, Obesity, Ethanol extract, N-Hexane fraction.
1. Introduction

Obesity has become health issues in the world. In some nations, it is reaching epidemic proportions. Obesity occur when the homeostatic mechanism controlling energy balance become disordered and/or overwhelmed. In 2008, WHO estimated that there were more than 1.4 billion adults having overweight condition, approximately half of whom were obese according to BMI (Body Mass Index) criteria [1]. Obesity often coexist with metabolic and other disorders (particularly hypertension, hypercholesterolemia and type 2 diabetes) together comprising the metabolic syndrome [1].

Orlistat is the only one drug in 2013 licensed in UK for the treatment of obesity. In the intestine, it reacts with serine residues at the active sites of gastric and pancreatic lipases. Then, it is irreversibly inhibiting these enzymes, preventing breakdown of dietary fat to fatty acids and glycerol. It therefore decreases absorption (and correspondingly causes faecal excretion) of some 30% of dietary fat [1]. Although orlistat has side effects include abdominal cramps, flatus with discharge and faecal incontinence, orlistat is well tolerated [1] also relatively safe for long-term obesity therapy, unlike centrally anti-obesity.

Due to problems in safety and tolerability, weight management drugs have presented clinical challenge. As result, in United States, there are no new weight-management drugs. In general, they had received regulatory approval from the date of orlistat approval in 1999 through 2012 [2].

Many well-known drugs have been primary discovered from plants, that they could provide opportunities for developing new products. Myrtaceae family (i.e., 121 genera, 3800–5800 spp.) is one of the most important families in tropical forests. Then, they are aromatic trees or shrubs, which frequently produce edible fruits [3].

The Syzygium genera shows rich medicinal applications, and number of studies has shown it to be a useful medicinal agent. One of them was Syzygium samarangense with common name java apple, java rose apple, samarang rose apple, markopa, water apple, and wax apple [4]. In Indonesia, it is known as jambu air samarang. It is medicinal properties such as antihyperglycemic, spasmolytic, immunomodulator, and antimicrobial activity [4-6].

Other species of Myrtaceae (Myrciaria jaboticaba (Vell.) Berg) can exert a protective role to counter obesity-associated insulin resistance by ameliorated water consumption and energy intake in streptozotocin diabetic rats, reduced plasma total cholesterol levels and triacylglycerol associated to the strong in vitro inhibition of pancreatic lipase [5]. Based on the kemotaxonomic principle where the taxonomic similarity causes the similarity of the chemical content (as well as pharmacological activity) so it is assumed that Syzygium samarangense also has anti-obesity activity.

This research was done to evaluate the anti-obesity effect of extract under various fractions of n-hexane, ethyl acetate, and water in Syzygium samarangense leaves. This study was also tested using female Wistar rats, which induced to obesity using high carbohydrate food and MSG injection as combination between food-induced obesity and monosodium glutamate-induced obesity.
2. Material and Method

2.1. Plant material, chemicals, and equipment

The materials used were *Syzygium samarangense* leaves; 96% of ethanol; ultrapure water; n-hexane, ethyl acetate; tragacanth; orlistat; standard food for rats, rice flour, etc. The chemicals were obtained from local suppliers (PT. Rumah Publikasi Indonesia, Indonesia) with technical and pro analyst quality.

The equipment used were macerator, vacuum rotary evaporator, freeze dryer, analytical balance, digital balance, mortar and stamper, rat cage, oral needle/sonde for rat, syringe 3 cc, and commonly laboratory glassware.

2.2. Collection, determination, and plant material processing

*Syzygium samarangense* leaves were obtained from Garut City, West Java Province, Indonesia; and authenticated in Bandungense Herbarium, School of Life Science and Technology, Institut Teknologi Bandung with 128/I1.CO2.2/PL/2017 voucher specimen number. Material processing include the process of collecting material, wet sortation, washing, drying, dry sortation, and grinding into powder.

2.3. Animal experiments

In animal experiments, we used Wistar female rats. Then, the identification of the position of the centre of pressure of a projectile body was motivated by the need for calculating aerodynamic moments, stability, and structural analyses. Then, the centre-of-pressure position of bodies composed of conical noses and cylindrical after bodies was determined as follows [5].

The approval ethic was obtained from Health Research Ethics Committee, Fakultas Kedokteran, Universitas Padjadjaran, Bandung Indonesia so all animal experiments performed according to the regulation (Approval ethic number: 824/UN.6/KEP/EC/2018). Wistar female rats were purchased from Pusat Ilmu Hayati, Institut Teknologi Bandung, Indonesia. The rats were housed individually and maintained on a standard 12 hours in light/dark cycle at room temperature with free access to water and food, which was similar procedure with previous studies [7].

After acclimatization for a week, they were divided into seven groups, in which each group consisted of 3 rats.

- Group 1: normal untreated (negative control),
- Group 2: obesity rats treated with 1% tragacanth suspension (positive control),
- Group 3: obesity rats treated with orlistat 32.4 mg/kg body weight (comparator drug),
- Group 4: obesity rats treated with 50 mg/kg body weight of extract of *Syzygium samarangense* leaves,
- Group 5: obesity rats treated with n-hexane fraction 0.13 mg/kg body weight of rat,
- Group 6: obesity rats treated with ethyl acetate fraction 1.98 mg/kg body weight of rat,
- Group 7: obesity rats treated with water fraction 4.83 mg/kg body weight of rat.
2.4. Extraction and fractination process

The simplicia powder of *Syzygium samarangense* leaves were extracted by maceration method using 96% ethanol for 3 days (3 x 24 hours) at room temperature with daily solvent replacement. The liquid extracts were evaporated at 40°C under low pressure using a vacuum rotary evaporator [7]. The fractination process was carried out using liquid-liquid extraction with n-hexane, ethyl acetate, and water as solvents. N-hexane and ethyl acetate fraction were evaporated using a vacuum rotary evaporator, while water fraction evaporated using a freeze dryer. The dry extracts and fractions were served as a suspension in 1% tragacanth.

2.5. Obesity induction

The feeding to induce obesity was high carbohydrate food [8] for 45 days (6 weeks) and subcutaneously injection by MSG 2 g/kg body weight of rat in 5 consecutive days in early induction time [9, 10] until obesity condition achieved. Induction with high carbohydrate food continued during treatment time.

2.6. The anti-obesity activity test

This study was conducted on obese Wistar female rats. The dry extract and various fractions were served as suspension in 1% tragacanth. The dosage of ethanol extract was 50 mg/kg body weight of rat, while the fraction dosage calculated based on percentage of fraction weight to extract weight multiply to extract dosage. The treatment was given for 14 days. The dosage of extract was chosen based on orientation process.

Test parameters consisted of body weight which determined every day; food intake, fecal consistency and fecal weight determined on first day, seventh day and fourteenth day during treatment. After treatment phase, the rats were sacrificed, the liver was isolated and weighed while the abdominal fat tissue was isolated [11, 12] followed by extraction the fat tissue using Soxhlet method to obtain netto fat content.

The anti-obesity activity was proven by the inhibition of body weight gain significantly compared to positive control group. Other test parameters were used to predict the mechanism of action of the drug.

2.7. Statistical analysis

All datas expressed as mean (n=3) ± standard deviation (SD) and statistically analysis using ANOVA (Analysis of Variance), LSD (Least Significance Different), Kruskal-Wallis and Mann-Whitney method. p-value of less than 0.05 was considered significant.

3. Results and discussion

3.1. Obesity induction

The in vivo anti-obesity activity test need animal experiments which induced by various methods to achieve obesity condition. Vogel mention these methods such as a food-induced obesity, hypothalamic obesity, gold thioglycose-induced obesity, and monosodium glutamate-induced obesity [9].
Various model of food-induced obesity can have developed in animal experiments. Obesity and metabolic disorders in rats (which resemble the human metabolic disorder syndrome) can have induced by high-fat (HF) diet feeding. However dietary intervention is not standardized, and the HF-induced phenotype varies distinctly among different studies [13]. Other studies showed that high carbohydrate and high fat diet could induce obesity with glucose intolerance, increased plasma lipid concentration, hypertension, left ventricular hypertrophy and fibrosis, liver inflammation, and steatosis [14].

In this study, the induction (which combine of MSG and high carbohydrate food) could increase rat body weight with the range 122.3-188.6% significantly compared to negative control group (p<0.05) which indicated the obese condition was achieved. Mean values of % induction from all group are shown in Fig. 1, while the average body weight of rats is shown in Fig. 2.

![Fig. 1. Percentage induction from all group compared to negative control group.](image1)

![Fig. 2. Average body weight of rats during induction and treatment time.](image2)

**3.2. The anti-obesity activity test**

In this research, high carbohydrate food was given during induction phase also in treatment phase. So, the method was curative and preventive, which mean the rat body weight increases during induction time until obese condition achieved. The extracts which have anti-obesity effect could inhibit the body weight gain of rats in treatment time.
Orlistat as comparator drug show anti-obesity activity by inhibit body weight gain significantly to positive control group (p<0.05) which mean that the method used in this research was valid. The ethanol extract of *Syzygium samarangense*, n-hexane fraction, and ethyl acetate fraction also show anti-obesity activity. The highest percentage inhibition of body weight gain showed by n-hexane fraction of *Syzygium samarangense* leaves with 85.96% inhibition (shown in Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Bodyweight gain (D14-D1) (g)</th>
<th>Inhibition of bodyweight gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>38.00 ± 10.15</td>
<td>-</td>
</tr>
<tr>
<td>Orlistat</td>
<td>9.00 ± 2.65*</td>
<td>76.32</td>
</tr>
<tr>
<td>Ethanol extract 50 mg/kg body weight of rat</td>
<td>10.00 ± 17.06*</td>
<td>73.68</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>5.33 ±11.85*</td>
<td>85.96</td>
</tr>
<tr>
<td>Ethylacetate fraction</td>
<td>10.00 ± 14.18*</td>
<td>73.68</td>
</tr>
<tr>
<td>Water fraction</td>
<td>24.00 ± 21.38</td>
<td>36.84</td>
</tr>
</tbody>
</table>

Note: *) = significantly different to positive control group (p < 0.05), D = day

One of the mechanisms of anti-obesity drug is inhibition activity of pancreatic lipase enzyme. This enzyme inhibition can lower digestion of fats. Thus, it gives effects on their assimilation and absorption. This can allow a reduced calorie intake in obese patients and help in preventing additional weight gain [15]. This also gives that fat absorption inhibited and excreted with feces produce oily and liquid feces. Fecal index and fecal consistency parameter used to predict this mechanism of action of extracts and fraction of *Syzygium samarangense* leaves. While the diet was high carbohydrate (low fat) so this parameter could not give hint on that mechanism. On the other hand, some of natural product claim have anti-obesity effect, unfortunately by laxative effect. It will implicate to decreasing fecal consistency (higher fecal consistency score) and increasing fecal index. It is important to find out whether all extracts give for 14 days treatment affect fecal consistency and fecal index that reflect the laxative effect or pancreatic lipase inhibiting effect.

Note: Score 0 = normal, score 1 = normal faeces, PC = positive control, O = orlistat, EE = ethanol extract, NHF = n-hexane fraction, EAF = ethyl acetate fraction, WF = water fraction

Fig. 3. Rats fecal consistency during treatment.
Table 2. Fecal and food index of rats during treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fecal index (%)</th>
<th>Food index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.91±0.81</td>
<td>1.08±0.75</td>
</tr>
<tr>
<td>Orlistat</td>
<td>1.04±0.40</td>
<td>0.92±0.76</td>
</tr>
<tr>
<td>Ethanol extract 50 mg/kg</td>
<td>1.70±0.44</td>
<td>0.88±0.15</td>
</tr>
<tr>
<td>body weight of rat</td>
<td>1.42±0.49</td>
<td>0.85±0.14</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>1.23±0.45</td>
<td>1.70±1.06</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>1.49±0.68</td>
<td>1.04±0.17</td>
</tr>
<tr>
<td>Water fraction</td>
<td>1.22±0.51</td>
<td>1.04±0.17</td>
</tr>
</tbody>
</table>

Result showed that in Fig. 3 and Table 2, there were no significant difference on rats fecal consistency among all groups during treatment, its ranged between normal (score = 0) and normal flaccid (score = 1), neither did the rats fecal index. There was not laxative effect from orlistat, ethanol extract and all fractions from *Syzygium samarangense*. Regarding the low-fat diet, the effect on inhibitory enzyme activity could not assure.

Food index parameter was used to find another hint about anti-obesity mechanism of extract and fractions. It was known that eating behavior and body weight regulated by serotonin in central nervous system, thus stimulation of this biogenic amine would reduce food intake and weight gain. It also increases energy expenditure in experimental animal or in human. We found no significant difference of food consumption represent as food index among all group compared to positive control group after treatment with orlistat and ethanol extract and all fractions (p>0.05), as shown in Table 2. This indicate that extract and fraction of *Syzygium samarangense* did not have anorexan effect/appetite reducer, so the mechanism of anti-obesity effect did not relate to central nervous system.

After 14-day treatment, the rats were sacrificed then the liver and abdominal fat tissues were isolated followed extraction the fat tissue using Soxhlet method with n-hexane solvent to obtain netto fat content. The significantly increase of liver index may reflect fatty liver condition because in obesity condition, liver is organ that could accumulate fat storage.

Result showed that there was no significant difference on liver index among all groups compared to positive control group after treatment (p>0.05) as showed on Table 3. The ethanol extract and fractions of *Syzygium samarangense* could not decrease fat deposition on liver. Also, there were no significant difference on abdominal fat tissue index among all groups compared to positive control group after treatment (p>0.05). The ethanol extract and fractions of *Syzygium samarangense* could not decrease abdominal fat tissue deposition.

Table 3. Liver organ and abdominal fat tissue index of rats after treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver index (%)</th>
<th>Abdominal fat index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>3.69±0.31</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Orlistat</td>
<td>3.78±0.55</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Ethanol extract 50 mg/kg body weight of rat</td>
<td>4.27±0.76</td>
<td>0.34 ±0.83</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>4.21±0.32</td>
<td>1.56 ±1.62</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>4.45±0.61</td>
<td>0.98 ± 0.58</td>
</tr>
<tr>
<td>Water fraction</td>
<td>3.84±0.29</td>
<td>0.84 ± 0.86</td>
</tr>
</tbody>
</table>
4. Conclusion
The effects of forebody and after body shapes on the aerodynamics at supersonic speeds are analysed in this paper. The ethanol extract, n-hexane fraction, and ethyl acetate fraction of Syzygium samarangense leaves had anti-obesity activity by inhibit body weight gain significantly compared to positive control group (p<0.05) without anorexan or laxative effect, and could not decrease fat deposition in liver and abdominal fat tissue. The highest activity showed by n-hexane fraction with 85.96% inhibition of body weight gain.

Acknowledgements
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References


Syzygium samarangense. (Blume) Merr. & L.M.Perry. Myrtaceae. Various parts of the tree are used in traditional medicine, and some have in fact been shown to possess antibiotic activity. The plant contains tannins and is astringent. Various plant extracts and isolated compounds has already been as rheumatoid arthritis, atherosclerosis and asthma, which all scientifically demonstrated. Turmeric (Curcuma longa), which has show a high prevalence globally. The selective activity of standardized extracts of Jamun fruit was also evident. It was observed that the extracts were more apoptotic against estrogen-dependent mammary breast cancer cells (MCF-7aro) when compared to estrogen-independent cell lines (MDA-MB-231), while no toxicity was observed when tested against nontumorigenic/normal counter parts MCF-10A (Li et al., 2009). Syzygium aromaticum and Syzygium cumini are arguably the most useful, with their fruits and leaves used as components of multiple traditional therapies. The anti-inflammatory activity of S. cumini leaves extracts was evaluated using carrageenan-induced oedema method (acute in vivo model) in the hind paws of rabbits. Rabbit were divided into five experimental groups, each group containing six rabbits (n = 6) and fasted for 18 h prior to the experiment. Evaluation of Analgesic, Anti-Inflammatory and CNS Activities of the Methanolic Extract of Syzygium samarangense Leave. Global J Pharmacol. 2014;8(1):39–s46. Antihyperglycemic activities of leaves of Syzygium samarangense of Bangladesh. KARACTERISASI MORFOLIGIS MAUPUN MOLEKULER DIGUNAKAN UNTUK MENENTUKAN MANA JAMBU SEMARANG DAN MANA JAMBU AIR, DAN MANA BUAH YANG BERPO TENSI SEBAGAI BUAH UNGGUL. Kultivar jambu yang telah memiliki nama dan diperdagangkan umumnya stabil, sementara jambu langka umumnya belum diketahui stabilitasnya. Dengan penelitian ini diharapkan dapat dikembangkan strategi konservasi dan pengembangan jambu unggul untuk meningkatkan perekonomian masyarakat pedesaan. ABSTRACT This study was conducted as the follow up of the discovery of four best jambu cultivars in the first year namely Jambu air Jatiwinang.