Biosaintifika 16 (2) (2024): 206-212 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Potency of *Elaeocarpus grandiflorus* Leaf Extract as Anti-obesity: *in vivo* and *silico* Study

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Submitted: 2024-02-07. Revised: 2024-05-09. Accepted: 2024-07-09

Abstract. The research aims to determine the effect of ethanol extract of *Elaeocarpus grandiflorus* leaves extract as anti-obesity by *in vivo* and *silico* computational models. The pre-and -post-tests were carried out on 20 female Wistar rats, divided into 4 groups. The control group (K) received no treatment and the experimental groups were treated with *E. grandiflorus* extract of 200 mg/kg BW (P1), 400 mg/kg BW (P2), and 800 mg/kg BW (P3) for 14 days. The body weight, abdominal circumference, and abdominal fat mass were measured on Day 1 and Day 15. The results showed differences in body weight (p=0.02), abdominal circumference (p=0.01), and abdominal fat mass (p=0.00). *In silico* exploration, bioactive compounds of rutin, orientin, luteolin, vitexin, iso orientin, isovitexin, kaempferol, and quercetin were identified, and targeted ELAVL1, IGF1R, CREB1, AKT1, and PIK3R1 of the AMPK signaling pathway that involved in the anti-obesity mechanism. The high binding affinity values was rutin-EVAL1 (-9.3), orientin-ELAVL1 (-8.3), and quercetin- IGFR1 (-8.2). It can be concluded that the ethanol extract of *E. grandiflorus* leaves has the potential to be developed as an anti-obesity agent.

Keywords: Anti-obesity; AMPK pathway; *Elaeocorpus grandiflorus*

How to cite : Nugrahaningsih, W. H., Ramasamy, S., Laraswati, V. A., Yuniastuti, A., & Christijanti, W. (2024). The potency of *Elaeocarpus grandiflorus* Leaf Extract as Anti-obesity: *in vivo* and *silico* Study. *Biosaintifika: Journal of Biology & Biology Education, 16(2),* 206-212.

DOI: http://dx.doi.org/10.15294/biosaintifika.v16i2.3695

INTRODUCTION

Obesity is a metabolic disorder that is caused by the accumulation of fat tissue in the body. Obesity is due to the imbalance between energy intake with energy expenditure (Anyanwu et al., 2020; Wang et al., 2021). Increased body weight is caused by most of the excess energy being stored as fat resulting in excess adiposity (Nisa & Madjid, 2016, Maqsood et al., 2017; Muhammad, 2018;). Predisposition factors for obesity trigger diseases including diabetes, dyslipidemia, cardiovascular disease, hypertension, sleep apnoea, cancer, and osteoarthritis, gastrointestinal disorders, respiratory tract disorders (Koyuncuoğlu Güngör, 2014, Cefalu et al., 2015; Purnell, 2018). Body Mass Index (BMI) is a simple calculation to classify overweight or obesity by measuring weight and height. The results of Basic Health Research (RISKESDAS) stated that in 2018 the prevalence of the Indonesian population aged 18 years and experienced overweight and obesity from 11.5% and 14.8% in 2013 to 13.6% and 21.8% in 2018 (Ministry of Health, 2018)

Some medicinal plants have been reported and used alternatively in preventing obesity and promoting weight loss. Research showed that the secondary metabolite in plants has a pivotal role as an anti-obesity agent. Flavonoids, tannins, saponins, and alkaloids of the leaves of *Phyllanthus emblica* L. (amalaka tree) show good anti-obesity activity (Gundamaraju et al., 2012). A study by Thomas, et. al., (2018) reported that the activity of purple perilla leaves, that is rich in polyphenols such as rosmarinic acid, showed lipid-lowering potential in adipocyte cells and prevented body weight gain in mice.

E. grandiflorus leaves are reported to contain more than 87 types of secondary metabolites such as dicarboxylic acids, tannins, phytosterols, terpenoids, and phenolics, and the most abundant is the flavonoid group. The main composition of flavonoid compounds includes naringin, isoorientin, orientin, vitexin, isovitexin, rutin luteolin, procyanidin, quercetin, epicatechin, and

kaempferol (Habibah et al., 2021). The content of *E. grandiflorus* leaves is almost the same as *P. emblica* leaves which exhibited the potential of an anti-obesity agent by promoting weight reduction in animal models and the activity is correlated with the presence of flavonoids. (Ardiansyah et al., 2018). The research aims to determine the effect of ethanol extract of *Elaeocarpus grandiflorus* leaves extract as anti-obesity by using *in vivo* and *silico* computational models.

METHODS

This research was conducted using *in vivo* and exploratory *in silico*) computational models. The independent variable was treated with various doses of *E. grandiflorus* leaf extract. The dependent variables were body weight (g), abdominal circumference (cm), and abdominal fat mass (g). Body weight was measured using an electric scale and the abdominal circumference of the mice was measured using midline. Abdominal fat mass was measured using an electric scale after mini surgery to carry out the adipose tissue from the abdominal.

Experimental research was carried out with a randomized pre- and post-test with a control group design for body weight and abdominal circumference variables and for abdominal fat mass variable only in the post-test. Ethical approval for animal experimentation was granted by Semarang State University Health Research Ethics Commission with letter number 295/KEPK/EC/2023. *In silico* study explored the target of a bioactive compound of *E. grandiflorus* in the anti-obesity pathway by molecular docking.

In vivo Study

A total of 20 female Wistar rats aged 2-3 months were divided into 4 groups with 5 rats in each group. Rats were placed in cages according to their group at Biology Laboratory, Universitas Negeri Semarang. Each rat was given standard food and water ad libitum. The animal was acclimated for 7 seven days before the study.

The high-fat diet was given every day 2 ml orally on days 1-14. The high-fat diet was given to rats beside standard food. High high-fat diet aims to increase the lipid value of blood and fat tissue. High-fat feed was served by mixing pork oil (50%) and the duck egg yolk (50%). Before giving a high fat diet body (Day 1), the weight and abdominal circumference of the rats were measured.

E. grandiflorus ethanol extract was given on days 7-14. The control group (K) was given high-fat feed without extract. The experimental groups were treated with *E. grandiflorus* extract in doses of 200 mg/kg BW (P1), 400 mg/kg BW (P2), and 800 mg/kg BW (P3). The extract was administered once daily by sonde gavage. On Day 15 the weight and abdominal circumference were measured. After the rats were terminated, the abdominal fat mass was measured.

In silico Study

In this *in silico* study, the following procedures were carried out, (i) unification of bioactive compounds via PubChem via the page (https://pubchem.ncbi.nlm.nih.gov/), (ii) prediction of target proteins on the Similarity Ensemble Approach (SEA) page (https://sea .bkslab.org/), (iii) identifying the interaction of proteins and bioactive compounds on the STRING page (https://string-db.org/), (iv) preparation of target proteins via the PDB page (https://www.rcsb.org/), (v) docking with the PyRx application, and finally (vi) visualization of the results via the Discovery Studio Visualizer software.

Data Analysis

The data obtained were analyzed statistically using SPSS which includes normality, homogeneity, and one-way ANOVA tests to determine differences between groups followed by Least Significant Difference (LSD) tests to determine the significance of differences between groups. Furthermore, the data will be strengthened by *in silico* analysis via molecular docking

RESULTS AND DISCUSSION

This study aims to determine whether there is an anti-obesity effect on female Wistar rats after being treated with the ethanol extract of *E. grandiflorus* leaves and a comparison was done with the group of rats given high-fat feed. Administration of ethanol extract of *E. grandiflorus* leaves was induced in the rats for 7 days to observe for any changes in body weight, abdominal circumference, and fat mass in rats and the observation was presented in Table 1.

Table 1. Mean difference in weight loss, abdominal circumference, and abdominal fat mass

Group	n	Weight (g)			abdominal circumference (cm)			Abdominal fat mass(g)
		Pretest	Postest	Δ	Pretest	Postest	Δ	Postest
K	5	153	157	3	13.6	12.5	1.1	3.7
P1	5	168	168	0	13.1	12	-1.1	2.3
P2	5	155	144	-11	12.8	11.4	-1.4	1.7
P3	5	169	160	-9	13.9	11	-2.9	1.4
Anova (p)				0.02			0.01	0.00

K: a control group of untreated rats, PI: rats treated with *E. grandiflorus* ethanol extract of 200 mg/kg BW, P2: 400 mg/kg BW and P3: 800 mg/kg BW.

This finding shows that administration of *E. grandiflorus* ethanol extract to female Wistar rats with a high-fat diet feeding promotes the decrease in body weight, abdominal circumference, and abdominal fat mass. The highest decrement in body weight was observed in the rats treated with ethanol extract of *E. grandiflorus* leaves at the dosage of 400 mg/kg BW. Abdominal circumference and fat mass tests show a good decreasing effect in rats treated with the dose of 800 mg/kg BW. Abdominal circumference has the highest mean value in P3 when compared with the control P1group, while fat mass has the lowest mean which shows a decrease when compared with the control group.

The decrement in the body weight, abdominal circumference, and fat mass in rats might be due to the presence of flavonoids in *E. grandiflorus* leaves Flavonoids are reposted to have the potential in inhibiting the pancreatic lipase enzyme, and are also capable of reducing food intake, digesting carbohydrates in glucose absorption, releasing insulin, stimulating energy expenditure, modulating adipocyte differentiation, adipogenesis, lipolysis, β- oxidation, and apoptosis (García-Barrado et al., 2020; Rufino et al., 2021).

In silico data shows that flavonoids interact with target proteins between ligands through AMP-activated protein kinase (AMPK) signaling which plays an important role in cell metabolism, mediates phosphorylation of target substrates and energy expenditure, and apart from that, increasing AMPK activity that can stimulate the production of CPT-1 which causes fatty acid oxidation. , Therefore AMPK signaling was identified as one of the targets in the prevention and anti-obesity treatment (Rufino et al., 2021; Wang et al., 2021).

The data obtained from the computational methods were carried out by screening the bioactive compounds from 11 main flavonoid contents to obtain t the prediction target. STRING page aided in obtaining n a network of target protein interactions with bioactive compounds. From the KEGG Pathways results, it is shown that there is an AMPK signaling pathway that has potential as an anti-obesity pathway. Eight (8) flavonoid compounds from 11 flavonoid compounds were identified and their 5 targeted proteins in the AMPK signaling pathway were tabulated in Table 2. Docking is used to obtain binding affinity results which aim to strengthen the working mechanism or influence of the receptor and ligand which are presented in Table 3.

Table 2. Receptor and ligand of bioactive compound on AMPK pathway

Targeted Protein	Bioactive compound
Insulin-Like Growth Factor 1 Receptor (IGF1R)	Quercetin
cAMP responsive element binding protein 1 (CREB1)	Luteolin, Quercetin, Orientin, Vitexin, Isoorientin, Isovitexin, dan Kaempferol
Serine/threonine kinase 1 (AKT1)	Quercetin
Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1)	Quercetin
ELAV-*like Protein 1* (ELAVL1)	Isoorientin, luteolin, Isovitexin, Orientin, Quercetin, Kaempferol, dan Rutin

Table 3. Binding affinity macromolecule-ligand bound of AMPK pathway

Macromolecule	Ligand	Binding Affinity
ELAV-*like Protein 1* (ELAVL1)	Rutin	-9.2
	Orientin	-8.3
	Kaempferol	-7.4
	Isovitexin	-7.4
	Isoorientin	-7.3
	Luteolin	-.,2
	Quercetin	-7,1
Insulin-Like Growth Factor 1 Receptor (IGF1R)	Quercetin	-8.2
cAMP responsive element binding protein 1 (CREB1)	Luteolin	-6.9
	Quercetin	-6.3
	Orientin	-6.6
	Vitexin	-6.5
	Isoorientin	-6.8
	Isovitexin	-6.8
	Kaempferol	-6.8
Serine/threonine kinase 1 (AKT1)	Quercetin	-6.8
Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1)	Quercetin	-6.9

The highest binding affinity value shows that the compound and targeted receptor have a large role in the anti-obesity mechanism. The highest binding affinity values are ELAVL1 with routine of -9.2 ELAVL1 with orientin of -8.3 and the IGF1R receptor with quercetin of -8.2. Apart from that, there are 3 receptors with low binding affinity values, namely AKT1, PIK3R1, and CREB1 but they still have a role in the AMPK signaling pathway as an anti-obesity target pathway which is presented in Figure 1

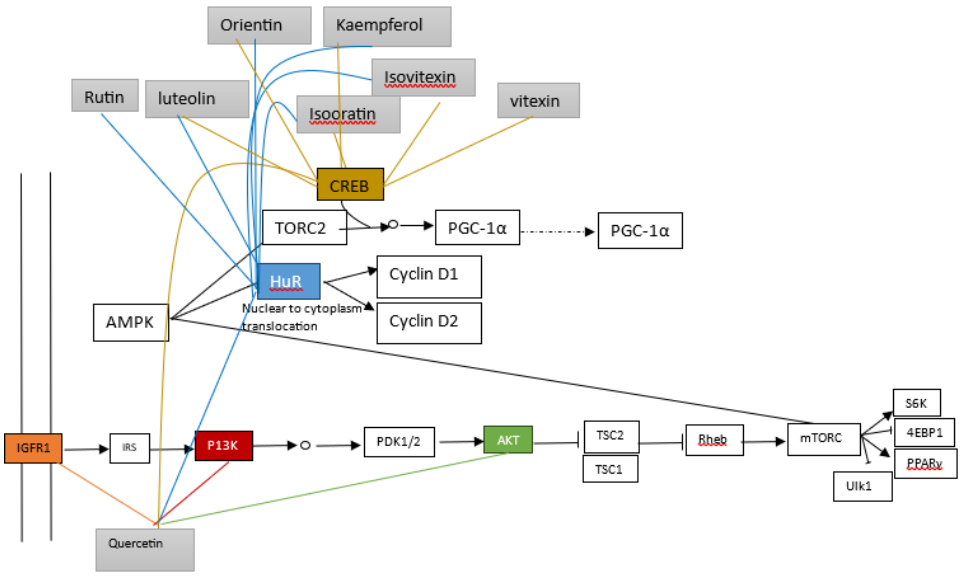

Figure 1. AMPK signaling pathway triggered by bioactive compounds from *E. grandiflorus* ethanol extract

The data obtained from docking with a high binding affinity value, namely -9.2, shows that ELAVL1 interacts very strongly with the routine compound in the AMPK pathway (Figure 2). There might be a possibility of a decrease in activity shown in this study. ELAV 1 – elavl1/Hur is an RNA-binding protein that mediates gene expression through the stabilization of target mRNA and is highly expressed in White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). HuR has also been suggested to regulate the expression of the critical glucose transporter (GLUT-1) in mature adipocytes (Anthony et al., 2020). Meanwhile, the compound rutin can work

in energy expenditure in BAT, and thermogenesis, increasing mitochondrial biogenesis in muscle mediated by the AMPK pathway. Rutin has a relationship with leptin levels produced from WAT and influences food intake and energy expenditure (Yang et al., 2020, Rufino et al., 2021;). From the ability of rutin to interact with ELAVL1 from the AMPK pathway, it can be concluded that the anti-obesity effect is by regulating energy metabolism and increasing energy expenditure in the body through muscle mitochondria.

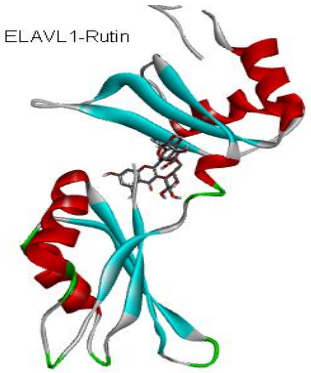

Figure 2. Visualization of ELAVL1-Rutin bound

ELAVL1 also interacts with the compound orientin which has a binding affinity value of -8.3, indicating strong interaction (Figure 3). Orientin is known to have activities such as antioxidant, anti-inflammatory, and anti-adipogenesis and can reduce intracellular triglyceride (TG) accumulation by inhibiting the expression of C/EBPα and PPARγ. Suppressive effect of orientin in the early stages of adipogenesis, orientin decreases intracellular lipid accumulation by reducing the synthesis of fatty acids and TG through reducing C/EBPδ expression and inhibiting PI3K/Akt-FOXO1 signaling in the early stages of adipogenesis (Lam et al., 2016; Nagai et al., 2018)

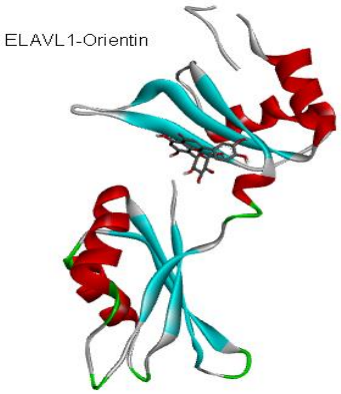

Figure 3. Visualization of ELAVL1-Orientin bound

The binding affinity value of IGF1R-Quercetin is -8.2 (Figure 3). IGF1R is a receptor for IGF1 which is found in the insulin signalling pathway. In this pathway, quercetin can activate the AMPK pathway and is connected to the insulin pathway, where IGFR1 has the same pathway as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt). These two targeted proteins also have the same interaction with other flavonoid compounds which might have an anti-obesity potential. However, the interaction strength is not that strong and this can be seen from the docking data that show low binding affinity values.

Insulin Growth Factor-1 (IGF1) acts through the IGF1 receptor (IGF1R) and Insulin Receptor (IRS), both receptors will stimulate the AKT/P13K pathway which is parallel to the IGF1R. The P13K and AKT pathways play a role in lipid synthesis during adipogenesis through activation of PPARγ. This pathway is a key regulator of cell growth and proliferation, and aberrant activation of this pathway promotes the development of obesity. The PI3K/AKT pathway regulates appetite through the CNS and peripheral tissues. There is a statement that leptin acts in the mediobasal part of the hypothalamus to suppress food intake partly through the PI3K-AKT-FoxO1 pathway and selective inhibition of PI3K eliminates the effects of leptin. mTOR also contributes to appetite regulation in central and peripheral systems. Stimulation of mTOR in the hypothalamus reduces food intake and improves obesity. Apart from that, IGF1 also activates AMPK as an energy-sensing pathway that plays a role in mitochondria (Aghanoori et al., 2019; Wen et al., 2022).

Quercetin can inhibit the P13K/AKT pathway which has an effect in blocking adipocytes (Rufino et al., 2021). In addition, quercetin also downregulated adipogenesis and apoptosis by reducing the action of enzymes related to adipogenesis (Chen et al., 2016). Quercetin reported activating AMPK to inhibit adipogenesis in 3T3-L1 cells so that quercetin can effectively weaken adipogenesis through increasing AMPK regulation and can stimulate the absorption of glucose in muscle cells (Ahn et al., 2008; Kawser Hossain et al., 2016).

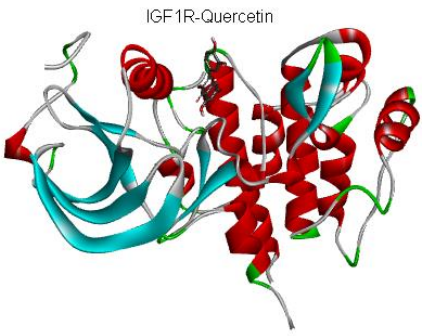

Figure 4. Visualization IGFR1-Quercetin bound

Excess energy is stored as triglycerides through the fatty acid pathway which is oxidized to produce energy when there is a lack of energy in the body. The mechanism of AMPK is to coordinate changes in lipid metabolism from anabolism to catabolism if there is a deficiency (Daval et al., 2006; Wang et al., 2018).

This research has limited testing on experimental animals with a minimum number of samples and the variables used are body weight, abdominal circumference, and fat mass. Therefore, it is necessary to continue this study to ensure that the flavonoid presence in *E. grandiflorus* leaves can be developed as a target for anti-obesity treatment.

CONCLUSION

The present study concluded that *E. grandiflorus* leaf has the potential to be developed as an anti-obesity agent. The *E. grandiflorus* leaf extracts showed a reduction in body weight, abdominal circumference, and abdominal fat mass in the *in vivo* model. Bioactive compounds of rutin, orientin, luteolin, vitexin, iso orientin, isovitexin, kaempferol, and quercetin targeted ELAV-like Protein 1 (ELAVL1), Insulin-Like Growth Factor 1 Receptor (IGF1R), cAMP responsive element binding protein 1 (CREB1), Serine/threonine kinase 1 (AKT1), and Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) in the AMPK signaling pathway. The strongest bound was between rutin- ELAV-like Protein 1 (ELAVL1). Further research can be developed by conducting preclinical tests on experimental animals regarding the pharmacokinetics, pharmacodynamics, and safety of the extract.

ACKNOWLEDGEMENT

This research was funded by the Faculty of Mathematics and Natural Sciences Universitas Negeri Semarang through a research collaboration scheme with contract agreement number 81.17.5/UN37/PPK.04/2023.

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