INHIBITORY EFFECT OF NON-POLAR AND SEMI-POLAR FRACTIONS OF ETHANOLIC EXTRACT OF *Guazuma ulmifolia* Lamk. LEAVES ON RAT PREADIPOCYTES PROLIFERATION AND DIFFERENTIATION

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INTRODUCTION

Jati Belanda Leaves (*G. ulmifolia* Lamk.) has been used traditionally to reduce obesity [1)]. *G. ulmifolia* Lamk. leaves extract contained alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, and steroids [2)]. Phytochemical screening results conducted by Iswantini *et al*. showed that the flavonoids and tannins were detected high levels in this extract [3)]. Flavonoids contained in the leaves of *G. ulmifolia* Lamk. were catechin, kaempferol glycosides, procyanidin B-2, procyanidin B-5, procyanidin C-1[4)].

Obesity can occur when the body of excess adipose tissue (fat), particularly white adipose. Excess white adipose tissue (WAT) is considered as the main cause of obesity [5)]. The growth of adipose tissue occurs due to the formation of new adipocytes and increase in size of adipocytes, called adipogenesis. Adipogenesis involves the multiplication of cells (proliferation) and cell maturation (differentiation). The treatment which can affect the size and number of adipocytes and signals expression are involved in the inhibition or stimulation of specific adipokines have been used to prove the bioactivity associated with antiobesity [6)].

Natural products that have a specific target in this pathway have the potential to prevent and treat obesity. Some polyphenols, including flavonoids have anti-obesity effects and also specific effects on adipocytes [7)]. The presence of the flavonoids i.e. catechin, kaempferol, tilirosida, and prosianidin in *G. ulmifolia* Lamk leaves. may affect adipogenesis.

This research will be conducted extraction and fractionation leaves of *G. ulmifolia* Lamk. Extracts, non-polar fractions and semi-polar fractions tested their effect on the proliferation and differentiation of Wistar rat preadiposit.

MATERIALS AND METHODS

Material

G. ulmifolia Lamk leaves obtained from Meru Betiri National Park and determined by the Indonesian Institute of Sciences, Purwodadi. Herbarium stored in Pharmacognosy Laboratory, Faculty of Pharmacy, Jember University. Cell cultures used primary preadipocyt cells culture, breded at the Laboratory of Physiology, School of Medicine, Brawijaya University.

The organic solvent used for the extraction and fractionation were ethanol redistilation, n-hexane p.a (Merck), ethyl acetate p.a (Merck) and chloroform p.a (Merck).

The materials used in the primary preadipocyt cells culture were collagenase type I (Sigma), the culture medium DMEM, HEPES, NaHCO3, biotin, D-pantothenate, FBS, Penicillin and Streptomycin, inducers of differentiation consisting of insulin, dexamethasone, IBMX and transferrin.

Collecting and Drying G. ulmifolia Leaves

The Leaves were collected from Betiri Meru National Park at an altitude of 900-1223 m above sea level and an average rainfall of 2,300 mm/year. Before to collection, the plant determined in LIPI Purwodadi, East Java. The leaves were taken from fifth leaves from the top end of the stems to the base of the stems. The leaves were sorted, i.e, removed the damaged leaves and other impurities, then washed with running water. After that, leaves drained and then dried until dry. Finally the leaves crushed for obtain leaf powder.

Extraction and Fractionation

Extraction and fractionation methods, as were done by Saifuddin et.al. with a few modification [8)]. A total of 100 g of powdered leaves of *G. ulmifolia* Lamk. macerated in 70% ethanol for 24 hours, then filtered to produce maserat. In the same way, the residue was macerated and filtered again. This process was repeated once more, then maserat collected together and then concentrated at low temperature and pressure to obtain viscous extract. Subsequently, extract added as much water and partitioned with n-hexane. Then, the water-ethanol fraction partitioned with chloroform to obtain chloroform fraction. Fractions n-hexane and

chloroform concentrated at low pressure and temperature to obtain a viscous fractions. Subsequently these fractions were tested their effects on proliferation and differentiation of preadipocytes Wistar rats.

Isolation and Culturing Cells

Preadipocytes isolated from adipose tissue of Wistar rat aged 4-8 weeks. The visceral fat tissue was cut in a sterile condition and as much as possible cleared from the blood vessels. Tissue washed twice with PBS, then washed again with FBS culture medium, and then chopped into small pieces. The tissue taken using tweezers and placed in tubes containing a solution of 0.2% collagenase type I.

Tissue incubated in a waterbath shaker for 1-2 hours at a temperature of 37 °C, and then centrifuged at 1000 rpm for 7 minutes. The supernatant was be disposed and the pellet was taken then added serum free medium, homogenized and centrifuged at 1000 rpm for 7 minutes. Furthermore, the supernatant was be disposed and the pellet was taken then added to the culture medium containing 10% FBS and homogenized, then planting in the culture plate. Furthermore incubated at 37 °C, 5% CO_2 for 24 hours. The cells were washed once every two days [9].

Stimulation of adipocyte differentiation

After the second day preadipocytes were grown in adipogenic medium (DMEM/F12 added 100 U/mL penicillin and 100 U/mL streptomycin, 66 nM insulin, 100 nM dexamethasone, 0.5 mM IBMX and 10 µg/ml transferrin) to induce cells differentiation. Cells suspension grown on a culture plate with the conditions of incubation temperature of 37 °C, 5% CO_2 for 24 hours [10].

Treatment of Cell Culture

After incubation with adipogenic medium, cells were washed and treated with the non-polar and semi-polar fractions. The concentration of these fractions was 500 ppm. After they were added fractions, cell cultures were incubated at 37 °C, 5% CO2 and 95% humidity for 24 hours [10].

Quantification proliferation and differentiation of adipocytes

Before and after incubated in adipogenic medium and treated with nonpolar and semipolar fractions, cells undergoing proliferation and differentiation counted. Cells were counted in the 25 of the visual field. Cells proliferation were calculated based on the number of cells, both cells undergoing differentiation or not. Cells differentiation were calculated based on the number of cells undergoing morphological changes into mature adipocytes [10].

RESULT

Extraction and Fractionation

The extraction of 100 g of powdered leaves of *G. ulmifolia* Lamk. yielded 7.5 g of dry extract. Furthermore, the extract was fractionated with n-hexane (non-polar) and chloroform (polar). Fractionation results as shown in Table 1 below.

Tabel 1. The result of fractionation

Fraction	Weight (g)	Rendemen (%)
n-Hexane	2.7	36.0
Chloroform	0.2	2.7

The result of observation of chromatogram profile on silica TLC plates using an eluent of chloroform : methanol : water (40: 10: 1) and n-hexane : ethylacetate (2 : 1) and visualization with ammonia showed yellow stains that were probably flavonoid compounds. Chromatogram profile both factions as Figure 1 below.

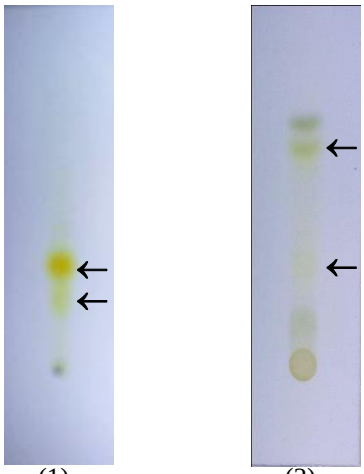

Figure 1. Chromatogram profile of n-hexane fraction (1) and chloroform fraction (2). The arrows indicated the possibility of flavonoid compounds.

Effect of extract (E.etOH), non-polar fraction (F.n-Hex) and semi-polar fraction (F.$CHCL_3$) treatments on the proliferation of preadipocytes, as shown in Figure 2 below.

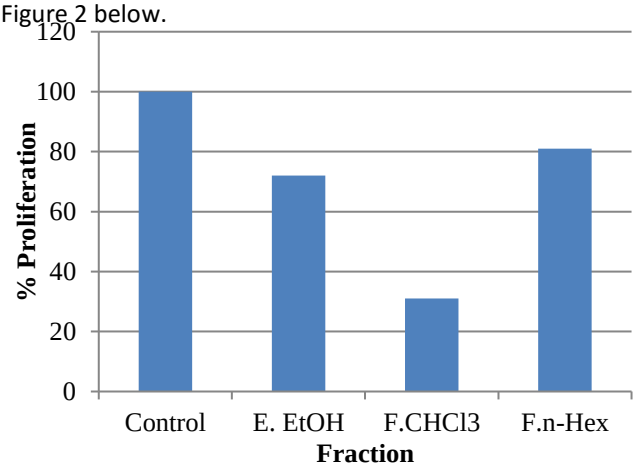

Figure 2. Effect of extracts and fractions *G. ulmifolia* Lamk. on the proliferation of preadipocytes Wistar rats

Effect of extract (E.etOH), non-polar fraction (F.n-Hex) and semi-polar fraction (F.$CHCL_3$) treatments on the differentiation of preadipocytes, as shown in Figure 3 below.

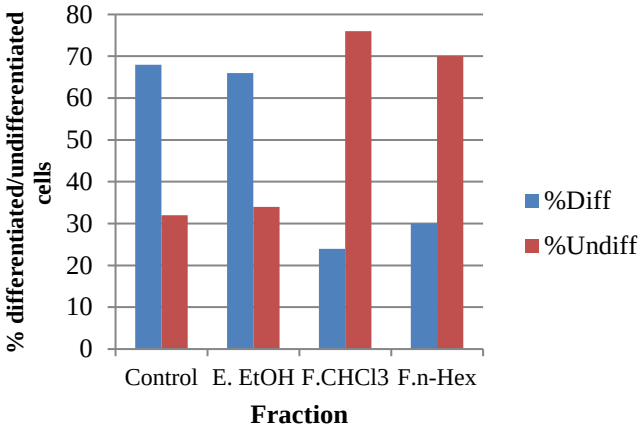

Figure 2. Effect of extracts and fractions *G. ulmifolia* Lamk. on the differentiation of preadipocytes Wistar rats

DISCUSSION

Obesity is not just caused by the hypertrophy but also hyperplasia of adipose tissue. Hyperplasia and hypertrophy of adipose tissue associated with adipogenesis mechanism that includes proliferation and differentiation of preadipocytes into adipocytes. The efforts inhibition of proliferation and differentiation of adipocytes as the basic mechanism of obesity has been conducted in various ways, such as by the use of leaves of *G. ulmifolia* Lamk.

The results of this study showed that the ethanol extract of leaves of *G. ulmifolia*, the non-polar and semi-polar fractions able to inhibit the proliferation of Wistar rats preadipocytes. Semi-polar fraction ($CHCl_3$ fraction) inhibits the proliferation better than extracts and non-polar fractions (n-hexane fraction), which is indicated by the proliferation percentage is only 30% (Figure 2). *G. ulmifolia* leaves extract does not affect to the differentiation of preadipocytes, but its fractions can inhibit differentiation. Semi-polar fraction showed better inhibition than nonpolar fractions. This is indicated by the percentage of undifferentiated cells i.e. 75% (Figure 3).

G. ulmifolia Lamk. leaves effect in inhibiting the proliferation and differentiation of preadipocytes Wistar rats possibility associated with the flavonoid. This possibility is based on several studies about the effects of some flavonoids in inhibiting adipogenesis. Epigallocatechin gallate (EGCG) from green tea with a concentration of 100 µM is able to inhibit the proliferation and differentiation in the primary cultures of human visceral preadiposit [11]. Quercetin, a flavonol, concentration of 200 µM is able inhibit the proliferation and differentiation of preadipocyte cultures of Rattus norvegicus Wistar strain. This inhibition mediated by decreased expression of C/EBP-α [12]. Prosianidin mixture of grape seed consisting of monomer (16.55%), dimer (18.77%), trimer (16%), tetramer (9.3%), oligomer prosianidin (5-13 units; 35.7%) , and phenolic acids (4.22%) in vitro may decrease the mRNA levels of PPAR-γ in cultures of 3T3-L1 preadipocytes. In vivo, administration of prosianidin can reduce mRNA levels of PPAR-γ and C/EBP-α [13]. Harmon and Harp found that ganistein and naringenin can inhibit proliferation preadipocytes [14]. Other flavonoids, luteolin inhibits the intracellular triglyceride accumulation in cultured 3T3-L1 preadipocytes were accompanied by a decrease in the expression of the transcription factor of adipogenic, namely PPAR-γ and C/EBP-α [15]. Flavonoids derived from Citrus aurantium may prevent adipogenesis through the inhibition of gene expression of PPAR-γ and C/EBP-α [16]. Mirisetin, a flavonol, with a concentration of 30 µM can inhibit adipocyte differentiation through decreased expression of PPAR-γ and C/EBP-α [17].

CONCLUSION

G. ulmifolia Lamk leaves extract and its fractions can inhibit the proliferation and differentiation of rat preadipocytes. Semi-polar fraction showed inhibition better than others

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