PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-8
https://doi.org/10.14719/pst.4421

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RESEARCH ARTICLE

Anti-adipogenic effects of *Parkia speciosa* Hassk. pods extract containing gallic acid and p-coumaric acid on 3T3-L1 adipocytes

Anas Abdullah[1], Norhisham Haron[1*], Emida Mohamed[1], Mohd Izwan Mohamad Yusof[2], Nur Dayana Hassan Cheong[1], Siti Nazrina Camalxaman[1], Ahmad Razali Ishak[3], Sharaniza Ab Rahim[4], Zolkapli Eshak[5] & Suhana Samat[6]

[1]Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Puncak Alam, Selangor, Malaysia

[2]School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia

[3]Centre for Environmental Health and Safety Studies, Faculty of Health Sciences, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Puncak Alam, Selangor, Malaysia

[4]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Sungai Buloh, Selangor, Malaysia

[5]Department of Pharmacology and Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Cawangan Selangor, Puncak Alam, Selangor, Malaysia

[6]Research and Development Unit, Olive Jardin Marketing, Jalan Dato Pati, Kota Bharu, Kelantan

OPEN ACCESS

ARTICLE HISTORY
Received: 22 July 2024
Accepted: 26 February 2025
Available online
Version 1.0 : 07 April 2025
Version 2.0 : 15 April 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Anas A, Norhisham H, Emida M, Mohd IMY, Nur Dayana HC, Siti NC, Ahmad RI, Sharaniza ABR, Zolkapli E, Suhana S. Anti-adipogenic effects of *Parkia speciosa* Hassk. pods extract containing gallic acid and p-coumaric acid on 3T3-L1 adipocytes. Plant Science Today. 2025; 12(2): 1-8. https://doi.org/10.14719/pst.4421

Abstract

Adipogenesis is the process by which preadipocytes develop into mature adipocytes and an increase in adipose mass may lead to obesity. Obesity is categorized as a chronic, low-grade inflammatory condition that can give rise to reactive oxygen species (ROS) production. Antioxidants help mitigate ROS, protecting cells and tissues from oxidative damage. Therefore, this study evaluates the antioxidant activities and inhibitory effects of *Parkia speciosa* Hassk. pod extract (PSPE) on adipogenesis in 3T3-L1 adipocytes. High-performance liquid chromatography (HPLC) detected gallic acid and p-coumaric acid in PSPE, with concentrations of measured at 53.97 ± 0.76 µg/mL and 1.74 ± 0.11 µg/mL, respectively. The IC_{50} values for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric-reducing antioxidant power (FRAP) were determined to be 57.05 ± 0.22 µg/mL and 325.3 ± 4.85 µg $FeSO_4$/mL extract, respectively. MTT assay results indicated that PSPE concentrations ranging from 31.25 to 250 µg/mL maintained over 80% cell viability, while oil red O staining demonstrated reduced lipid accumulation at concentrations of 62.5 and 125 µg/mL after 48 and 72 hours of treatment. These findings suggest that PSPE has potential as a natural antioxidant and anti-adipogenic agent, capable of inhibiting lipid accumulation and mitigating oxidative damage.

Keywords

anti-adipogenic; antioxidant; HPLC; *Parkia speciosa* Hassk. pod

Introduction

Adipogenesis is the process through which preadipocytes mature into adipocytes, specialized cells that store and regulate energy in the form of fat (1). Excessive dietary calorie intake, coupled with inadequate energy expenditure, can lead to an increase in adipose mass and consequently, contribute to obesity. Obesity is a chronic, low-grade inflammatory condition, also known as metaflammation, with an abnormal accumulation of adipose tissue. This inflammatory state can lead to increased production of reactive oxygen species (ROS) (2). ROS are a type of free radical, most of which are highly reactive and

inherently unstable. They can act as either oxidants or reductants, as they are capable of donating or accepting electrons from other molecules (3). These ROS are typically generated during oxygen metabolism and encompass radicals like superoxide, hydroxyl radicals and peroxyl radicals, as well as non-radicals like hydrogen peroxide, hypochlorous acid and ozone. Due to their distinctive chemical properties, ROS can instigate DNA damage in biological systems and trigger the oxidations of lipids and proteins within cells (4).

Antioxidants are substances that play a vital role in preventing oxidation and neutralizing free radicals, thereby protecting cells and tissues from oxidative damage (5). The primary function of antioxidant systems is to maintain oxidants at optimal level rather than eliminate them entirely (6). There are two types of antioxidants: endogenous, which are essential for cellular activities and overall health and exogenous, which may be required during periods of oxidative stress when endogenous antioxidants are insufficient (7).

Parkia speciosa Hassk., also known as stink bean, is a member of the Fabaceae family and belongs to the genus *Parkia* and species *speciosa* (also classified under Leguminosae and Mimosaceae). It is widely distributed throughout tropical regions like Malaysia, Indonesia, Thailand and the Philippines. According to Azizul et al. (8), both the seeds and pods of *Parkia speciosa* Hassk. may help treat chronic health conditions such as inflammation, diabetes, atherosclerosis and sometimes even cancer. Numerous studies have supported the medicinal properties of both the seeds and pods of *Parkia speciosa* Hassk. (9-15). However, the study of phytochemicals contents and antioxidant activities in ethanolic extract of *Parkia speciosa* Hassk. pods and its inhibitory effects on adipogenesis is still deficient due to the limited amount of research conducted. Thus, this study aims to evaluate the phytochemical composition, antioxidant capacity, as well as the adipogenesis inhibitory effect of *Parkia speciosa* Hassk. extract (PSPE) on 3T3-L1 adipocytes.

Materials and Methods

Plant materials and extract preparation

The *Parkia speciosa* Hassk. fruits were collected from Kuala Krai, Kelantan and a voucher specimen (KM 0051/23) was archived at the University Putra Malaysia Herbarium. Approximately 2 Kg of pods were cut into smaller fragments, washed and subjected to a three-day oven-drying process at 40°C. Following drying, the pods were ground into a powder and underwent extraction following the procedure outlined by Fithri et al. (16). Using a 1:2 ratio, the powdered pods were immersed in 70% ethanol at room temperature for four days, after which the supernatant was collected and filtered using No. 1 filter paper (Whatman, England). This process was repeated until the solution became colourless. The ethanol solution was then concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) at 45°C. The resulting concentrated extract was freeze-dried to yield *Parkia speciosa* Hassk. ethanol extract (PSPE), which was stored at -20°C for subsequent use.

HPLC analysis

The Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan) was employed for the phytochemical screening of PSPE. This system comprises an LC-20AT pump connected to a DGU-20A5 degasser, an SPD-20A UV/VIS detector, a SIL-20A autosampler, a CTO-10AS VP column oven and LC solution software (11). Separation was achieved using the Eclipse Plus C18 column (4.6 x 250 mm, 5 µm particle diameter) with the column temperature maintained at 40°C and a pressure limit set at 4000 psi. Mobile phase (A) consisted of 0.1% acetic acid in ultrapure water, while the mobile phase (B) was acetonitrile. A gradient elution method was employed based on the percentage of solvent B over time. Specifically, a linear gradient from 10% to 60% of solvent B was established over 25 minutes. The injection volume was set at 10 µL and the flow rate was adjusted to 1.0 mL/min, with detection carried out at a wavelength of 280 nm. PSPE chromatograms were compared to standards of gallic acid, p-coumaric acid, catechin and kaempferol. Calibration curves were constructed for gallic acid and p-coumaric acid within a concentration range of 20-100 µg/mL.

DPPH free radical scavenging assay

The DPPH free radical scavenging assay was used to determine antioxidant activity, with slight adjustments (17). Briefly, 180 µL of DPPH solution (150 µmol/L) in methanol-water (80:20, v/v) was mixed with 20 µL of the diluted sample in a 96-well microplate. After agitating the mixture for 60 seconds, it was incubated for 40 minutes at room temperature without light. A microplate reader spectrophotometer (Tecan Infinite M200) was used to measure the absorbance at 515 nm against the blank. Each determination was performed in triplicate, with ascorbic acid serving as the positive control. The IC_{50} value, representing the concentration required to scavenge 50% of the free radicals produced by DPPH, was used to assess the scavenging ability of the sample. The IC_{50} values were determined by constructing a calibration curve that related sample concentrations to scavenging activity (%).

Ferric reducing antioxidant power (FRAP) assay

The FRAP value of the PSPE (31.25-1000 µg/mL) was determined with slight adjustments of the methodology (18). A freshly prepared acetate buffer (300 mM, pH 3.6), a 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution were combined to create the FRAP reagent. Prior to use, the FRAP reagent was prepared by mixing 10 mL of TPTZ solution, 10 mL of ferric solution ($FeCl_3.6H_2O$) and 100 mL of acetate buffer in a 10:1:1 ratio. In a 96-well microplate, 25 µL of samples and 125 µL of FRAP reagent were mixed, followed by a 30-minute incubation at 37°C with gentle agitation. The reduction of the colourless ferric complex (Fe^{3+}-tripyridyltriazine) into the blue ferrous complex (Fe^{2+}-tripyridyltriazine) was measured using a UV-Vis microplate reader was used at 593 nm. Ascorbic acid (31.25-1000 µg/mL) was used as the positive control. A calibration curve was established using various concentrations of ferrous sulphate ($FeSO_4$) (3.125-100 µg/mL) and all determinations were done in triplicates.

Cell culture materials

The 3T3-L1 mouse embryonic fibroblast cells and newborn calf serum (NCS) were obtained from the American type culture collection (ATCC), based in Manassas, USA. Fetal bovine serum (FBS) was sourced from Tico Europe (Netherlands). Dulbecco's modified Eagle's medium (DMEM), human recombinant insulin and antibiotic-antimycotic mixed stock were acquired from Nacalai Tesque Inc. situated in Kyoto, Japan. Phosphate-buffered saline (PBS) was obtained from Invitrogen, Inc. headquartered in Carlsbad, CA. 3-isobutyl-1-methylxanthine (IBMX) was purchased from Sigma -Aldrich (St. Louis, USA). Dexamethasone was from MedChem (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) stains obtained from Merck (Germany) and oil red O (ORO) was obtained from R & M chemicals (UK).

Culture, differentiation and treatment of 3T3-L1 cells

The undifferentiated 3T3-L1 fibroblasts (ATCC CL-173) were cultured in complete growth media containing DMEM, supplemented with 10% NCS, 100 units/mL of penicillin and 100 µg/mL of streptomycin with slight modification (19). The cultures were nurtured in T75 flasks in a humidified 5% CO_2 incubator at 37°C until they reached 70-80% confluency. For assay purposes, a 96-well plate was used to seed 3T3-L1 preadipocytes at a density of 1×10^4 cells/well. Two days post-confluence, the preadipocytes were differentiated into mature adipocytes (day 0) by replacing the growth medium with differentiation medium containing DMEM, supplemented with 10% FBS, 0.5 mM IBMX, 10 µg/mL insulin, 0.25 µM dexamethasone, 100 units/mL of penicillin and 100 µg/mL of streptomycin and cultured for three days (day 3). After three days of differentiation, the medium was replaced with maintenance medium containing DMEM, supplemented with 10% FBS, 10 µg/mL insulin, 100 units/mL of penicillin and 100 µg/mL of streptomycin. The media was replaced every three days until mature adipocytes were obtained on day 15. The cells were then subjected to treatment on day 15 with varying concentrations of PSPE (31.25 - 1000 µg/mL).

Cell viability

The effects of PSPE on the viability of mature adipocytes were assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H -tetrazolium bromide (MTT) assay with minor modification (20). Various concentrations of PSPE, ranging from 31.25 to 1000 µg/mL, were administered to the differentiated cells for 72 hours in a humidified 5% CO_2 incubator at 37°C. Cells treated with culture medium only served as control. The MTT assay was performed by adding 20 µL of 5 mg/mL MTT reagent to each well and the plates were incubated at 37°C for 4 hours. To dissolve the MTT-formazan complex formed, the culture media was removed and replaced with 200 µL of dimethyl sulfoxide (DMSO). A microplate reader was used to measure the optical density, which indicates the formazan concentration at 590 nm. Cell viability was measured as a percentage relative to control cells using absorbance, which is correlated with the number of living cells.

Oil red O staining

Lipid accumulation was evaluated following the oil red O staining procedures with minor adjustments (21). Briefly, after treating 3T3-L1 adipocytes with PSPE (31.25-125 µg/mL) for 24, 48 and 72 hours, the medium was removed and the cells were rinsed with PBS. Subsequently, the cells were fixed with 100 µL of 10% formalin for 60 minutes at 24°C, followed by washing with 60% isopropanol and allowing them to dry completely before staining. Lipid droplet staining was performed by adding oil red O working solution to each of the wells for 20 minutes at 24°C, followed by rinsing with distilled water. The cells were then washed again with distilled water. To evaluate the lipid accumulation, 100% isopropanol was added to each well for 10 minutes at 24°C. A microplate reader set to 490 nm was used to quantify the eluted stains after they were transferred to a 96-well plate. The results are presented in reference to the differentiated control cells and comparative analysis was conducted between the treatment doses at each time point.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software, version 9.5.1 (GraphPad Software, Inc. La Jolla, USA). The outcomes of three independent experiments are presented as mean ± standard error of the mean (SEM). Statistical comparisons among the treatment groups were conducted using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for cell viability and oil red O staining assays. To establish statistical significance, a threshold of $p < 0.05$ was used.

Results

Extraction yield

The extraction yield of *Parkia speciosa* Hassk. pods using 70% ethanol was determined to be 37.83%. The methodology used in this study involved soaking 600 g of dried *Parkia speciosa* Hassk. pod powder in 1.2 L of 70% ethanol and shaking it for 3-4 days at room temperature using an orbital shaker. This process was continued by replacing the solvent until it became colourless, which ultimately resulted in a yield of 37.83%.

HPLC analysis

In this study, gallic acid and p-coumaric acid in PSPE were successfully detected and quantitated using reverse-phase HPLC. Gallic acid and p-coumaric acid were identified in PSPE comparing their retention time to those of the standards (Fig. 1). The concentrations of gallic acid and p-coumaric acid in PSPE (10 mg/mL) were 53.97 ± 0.76 µg/mL and 1.74 ± 0.11 µg/mL, respectively (Table 1).

DPPH free radical scavenging assay

The amounts of extract (PSPE) and positive control (ascorbic acid) required to reduce the level of the free radical DPPH by 50% (IC_{50}) were 57.05 ± 0.22 µg/mL and 23.13 ± 0.33 µg/mL, respectively (Fig. 2). These results demonstrate that PSPE had approximately 0.2 times the potency of ascorbic acid.

Ferric reducing antioxidant power (FRAP) assay

The FRAP values of PSPE and ascorbic acid are shown in Table 2. The highest FRAP value demonstrated by PSPE was 325.3 ± 4.85 µg $FeSO_4$/mL, while that of ascorbic acid was 377.5 ± 2.58 µg $FeSO_4$/mL. The results revealed that PSPE exhibited a lower FRAP value at all concentrations compared to ascorbic acid.

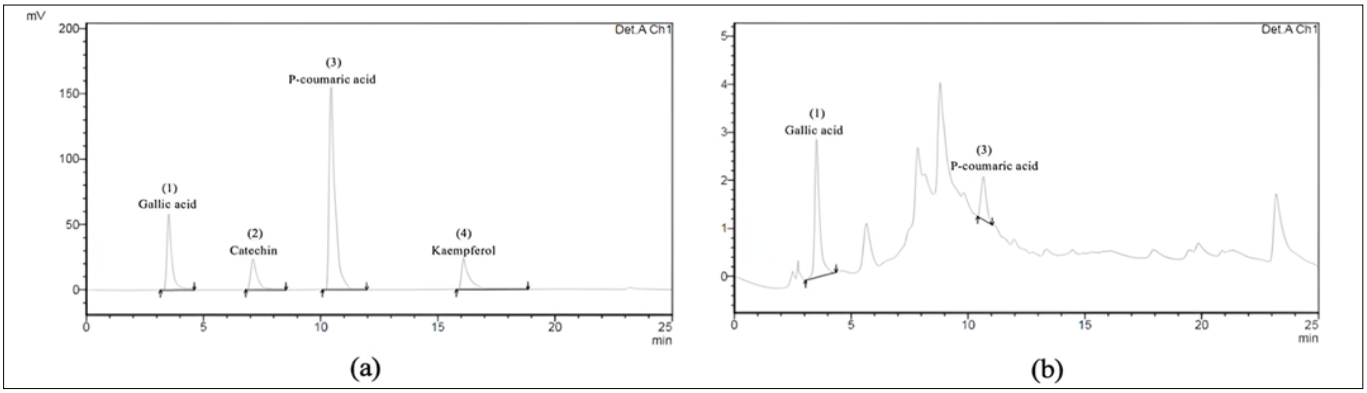

Fig. 1. Chromatograms of (a) standards (100 µg/mL) and (b) PSPE (10 mg/mL).

Table 1. Retention time of gallic acid and p-coumaric acid in 10mg/mL PSPE compared to standard compounds. The concentration of compounds in PSPE reported as mean ± SEM, n=3

	Retention time (min)		Concentration of compounds in PSPE (µg/mL)
	Standard	PSPE	
Gallic acid	3.506	3.495	53.97 ± 0.76
p-coumaric acid	10.442	10.442	1.74 ± 0.11

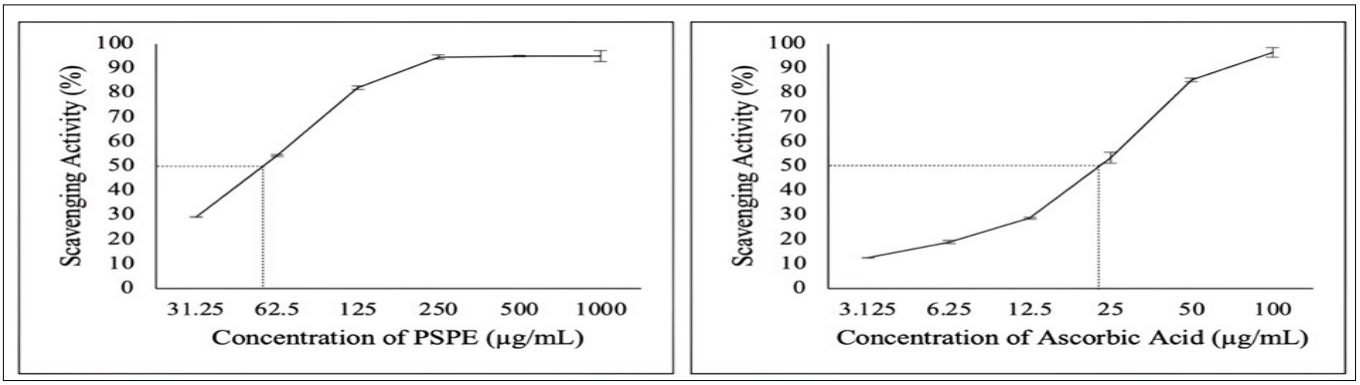

Fig. 2. The DPPH scavenging activity of the ethanolic extract of PSPE and ascorbic acid.

Table 2. The FRAP value of PSPE and ascorbic acid calculated from the standard curve and reported as mean ± SEM, n=3

Concentration (µg/mL)	FRAP value of PSPE (µg $FeSO_4$/mL)	FRAP value of ascorbic acid (µg $FeSO_4$/mL)
31.25	47.64 ± 2.35	96.75 ± 2.47
61.5	92.46 ± 2.87	179.3 ± 0.94
125	150.1 ± 2.94	270.8 ± 5.12
250	230.9 ± 5.71	335.4 ± 1.01
500	294.9 ± 1.34	369.7 ± 0.39
1000	325.3 ± 4.85	377.5 ± 2.58

Cell viability

The MTT assay was performed to assess the effects of PSPE on cell viability and survival before analyzing the functional effects of the extract. Mature 3T3-L1 adipocytes were treated with various concentrations of PSPE (31.25-1000 µg/mL). Table 3 shows the percentages of cell viability relative to the differentiated control cells after 72 hours of cell treatment. The highest concentration of PSPE (1000 µg/mL) resulted in 59.62% cell viability, whereas the lowest concentration of PSPE (31.25 µg/mL) and simvastatin (10 µM) were 99.86% and 99.59% viability, respectively. Cells treated with PSPE at concentrations between 31.25 to 250 µg/mL for 72 hours showed more than 80% cell viability as compared to the control cells and was significantly reduced at concentration 1000 µg/mL (59.62%).

Oil red O staining

The anti-adipogenic potential of the extracts was investigated through intracellular lipid accumulation by oil red O staining in mature adipocytes after treatment for 24, 48 and 72 hours. The cells were stained and the representative images of the droplets were acquired at 10× microscopic field magnification (Fig. 3). At 24 hours, no notable distinctions were observed among the experimental groups. However, at 48 and 72 hours, cells treated with PSPE at concentrations of 62.5 and 125 µg/mL, as well as those treated with simvastatin, displayed a slight reduction in lipid droplets compared to the control group.

The stained cells were then eluted and quantified at 490 nm using a microplate reader. While microscopic examinations did not reveal discernible differences in lipid droplets among the groups, a subsequent quantitative analysis following cell elution revealed significant reductions

Table 3. Percentages of cell viability relative to the differentiated control cells after 72 hours of cell treatment and reported as mean ± SEM, n=3. *Significant reduced from control cells ($p < 0.05$)

Concentration (µg/mL)	Percentage of cell viability (%)
Simvastatin	99.59 ± 5.89
31.25	99.86 ± 5.13
61.5	99.91 ± 10.28
125	93.10 ± 4.35
250	82.15 ± 8.33
500	70.77 ± 9.17
1000	59.62 ± 14.48*

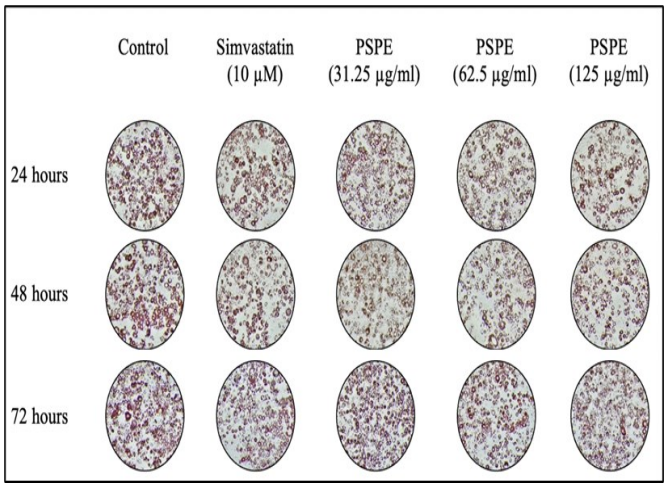

Fig. 3. Images of lipid droplets in control, simvastatin (10 µM) and PSPE (31.25 - 125 µg/mL) treated group acquired at 10X microscopic field magnification for 24 hours, 48 hours and 72 hours.

in some groups. At 24 hours, no significant changes were noted in either microscopic examination or lipid accumulation when comparing the treated cells with the untreated group. However, at 48 hours and 72 hours, the number of lipid droplets in adipocytes was found to be considerably lower in the treated group than in the control group with a significant reduction observed in the quantitative evaluation at concentrations of 62.5 and 125 µg/mL (Fig. 4). These results indicate the anti-adipogenic effects of PSPE (62.5 and 125 µg/mL) and simvastatin began at 48 hours, with no notable differences compared to the effects observed at 72 hours.

Discussion

Extraction yield

The extraction yield of *Parkia speciosa* Hassk. pods in 70% ethanol was 37.83%. Several factors may influence the extraction yield and these include the type of solvent used, the extraction method, the extraction time, as well as the origin of the material or sample (22). This result is slightly different from the previous study, where the extraction yield obtained was 19.66% and 24.7%, respectively (9, 16). Even though the solvent and method of extraction used were the same in our study, the origin of *Parkia speciosa* Hassk. collected was different (16). On the other hand, the solvent and the method of extraction were also different when compared to our study (9). Thus, these may contribute to the difference in the percentage of extraction yield.

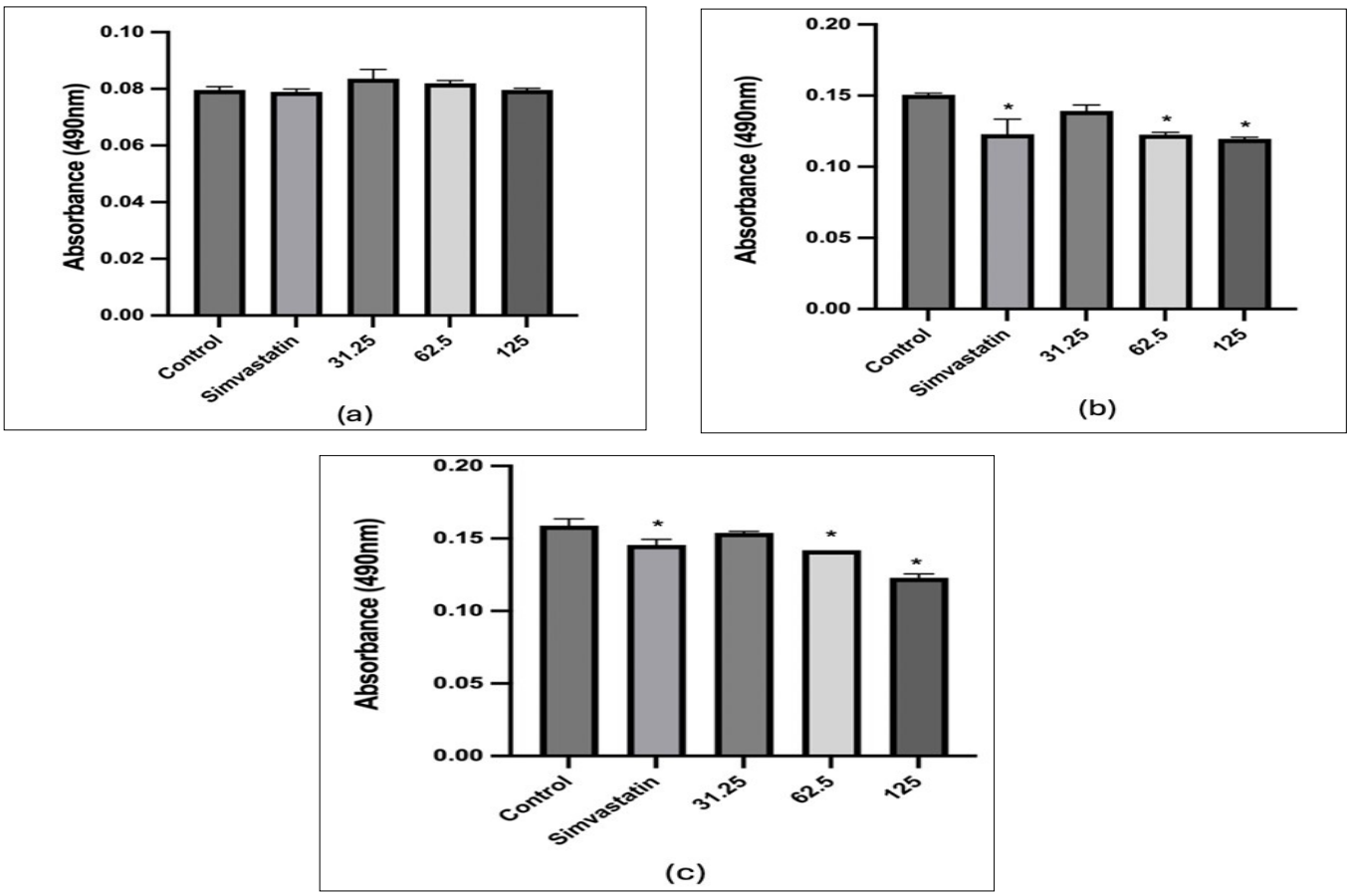

Fig. 4. Lipid accumulation in 3T3-L1 cells after treated with simvastatin (10 µM) and PSPE (31.25 - 125 µg/mL) for (a) 24 hours, (b) 48 hours and (c) 72 hours. Data are expressed as the mean ± SEM of 3 independent experiments (n = 3). *Indicate significant differences with respect to control ($p < 0.05$).

HPLC analysis

Phytochemical compounds which are gallic acid and p-coumaric acid in PSPE were successfully detected and quantified. Even though the phytochemical components of PSPE were discussed in earlier studies, there were no previous reports on the presence of p-coumaric acid found in the extracts of *Parkia speciosa* Hassk. pods (12, 21, 22). Based on the prior studies done, they had identified the compounds in their extract that included gallic acid, chlorogenic acid, ellagic acid, kaempferol, vanillic acid, epicatechin, catechin quercetin and rutin (12, 21-24). The location and season of the collection may have an impact on the phytochemical makeup of the plants (11).

The method of extraction and identification of the compounds used also could affect the compounds detected. Specifically, even though the same detection wavelength was utilized in the study done by Ko et al. (23), the solvent used for the plant extraction in their study was 95% ethanol. Furthermore, the solvent utilized in the mobile phase is also different. These could be the cause of the disparity in our results. To date, no study utilizes 70% ethanol as the extraction solvent in analyzing the phytochemical constituents of *Parkia speciosa* Hassk. pods via HPLC, and according to Gan & Latiff (25), using 70% ethanol as the extraction solvent makes it possible to perfectly attract polar compounds up to the desired semi-polar compounds from a plant. Therefore, in this study, we identified and quantified gallic acid and p-coumaric acid by HPLC analysis. This choice of solvent in our study may have also contributed to the detection of p-coumaric acid in the extract, which has been first reported.

DPPH free radical scavenging assay

This study revealed that the IC_{50} of PSPE and ascorbic acid were 57.05 ± 0.22 µg/mL and 23.13 ± 0.33 µg/mL, respectively. The IC_{50} value of an antioxidant compound is inversely proportional to its antioxidant activity. Thus, the lower antioxidant activity indicates a higher IC_{50} value. The DPPH scavenging activity of *Parkia speciosa* Hassk. extract exhibited a slightly higher IC_{50} value, which was 64.2 µg/mL (23). Even though the same solvent was used in this study, the percentage of the solvent used was 95% ethanol. Thus, this may influence the results since the different extraction solvents may result in different compounds extracted.

On the other hand, a study determined the DPPH scavenging activity by using 70% ethanolic extract of *Parkia speciosa* Hassk. pods, showed that the IC_{50} value was also slightly higher (75.72 µg/mL) when compared to our extract (16). Although the solvent and percentage used were the same as in our study, the location of *Parkia speciosa* Hassk. collected was from Indonesia. Therefore, this could affect the phytochemical composition of the plants since the location and season of the collection may have an impact on the phytochemical makeup of the plants and thus, will affect its antioxidant properties.

Ferric reducing antioxidant power (FRAP) assay

In this study, PSPE had a lower FRAP values compared to ascorbic acid. Although PSPE had lower FRAP values, it demonstrated nearly the same amount of FRAP values with ascorbic acid for all concentrations. This is because ascorbic acid is one of the potent reducing agents that increases the absorption of iron by converting Fe^{3+} to Fe^{2+} (26). In the presence of TPTZ, the ability of an antioxidant to reduce Fe (III) to Fe(II) in a redox reaction is measured by using the FRAP assay, resulting in the formation of an intense blue Fe(II)-TPTZ complex (23, 25). The methanol extract solvent showed that the FRAP value of *Parkia speciosa* Hassk. pod extract was 299.62 µg $FeSO_4$/mL (25). This value was slightly lower than that of PSPE, potentially due to differences in the extraction solvent used.

Furthermore, it was revealed that the IC_{50} value of the extract was 274 ± 16.1 µg/mL (23). Since the data presented and the standard utilized to present the FRAP value for the study were different, the results cannot be compared. However, although the result was incomparable, both studies showed and claimed that there was the presence of antioxidant activities in PSPE when tested using FRAP assay.

Cell viability

The maximum dose of PSPE (1000 µg/mL) resulted in lowest cell viability, while the lowest concentrations of PSPE (31.25 µg/mL) and simvastatin (10 µM) were 99.86% and 99.59%, respectively. These results revealed that as the concentration of the PSPE increased, the viability of 3T3-L1 mature adipocytes decreased. To the best of our knowledge, there is currently no data in the scientific literature that reveals the effects of PSPE treatments on cell viability in 3T3-L1 mature adipocytes. As a result, we are unable to compare the effects of our extracts on cell viability to the findings of other studies. However, experiments that used different plant extracts revealed a similar pattern in which the cell viability of 3T3-L1 adipocytes reduced when higher doses of extract were utilized (27, 28). These reducing patterns may be caused by a variety of factors, including the plant extracts themselves, which may contain cytotoxic substances that might harm cells, the concentration of the extracts utilized and the duration of the treatment.

Oil red O staining

The number of lipid droplets in adipocytes was found to be considerably less in the PSPE-treated group than in the control group at 48 hours and 72 hours, and there was also a significant reduction seen in quantitative evaluation at concentrations of 62.5 and 125 µg/ml. In a study done by Li et al. (28) examining the apoptosis of 3T3-L1 cells by using pomegranate flower extract (PFE), it was found that the relative quantitative oil red O staining resulted in a significant decrease of the 3T3-L1 cells differentiation rate after treating with PFE. Red pepper leaf extracts significantly reduced lipid accumulation when compared to the control group, indicating that the extracts repressed lipid accumulation during differentiation (29). The reduction in lipid accumulation may be associated with the compound found in the plant extracts. The ability of the bioactive compounds in the plant extract to suppress the adipogenesis of mature adipocytes may account for the decrease of lipid droplets in 3T3-L1 adipocytes (30). In our study, gallic acid and p-coumaric acid, which were previously known to have the ability to inhibit adipogenesis, were detected in PSPE. Therefore, this might be one of the factors that contribute to the anti-adipogenic effects of PSPE.

Conclusion

In conclusion, the PSPE exhibited antioxidant activity and anti-adipogenic effects owing to the presence of phenolic compounds. These findings imply that the ethanolic extract of *Parkia speciosa* Hassk. could serve as a promising natural antioxidant and anti-adipogenic agent that is beneficial in suppressing lipid accumulation and thus preventing oxidative damage to the body.

Acknowledgements

This work was supported by the Fundamental Research Grant Scheme (FRGS) funded by Ministry of Higher Education, Malaysia (FRGS/1/2021/WAB11/UITM/02/1). The authors would also like to thank Universiti Teknologi MARA (UiTM) for its support and financial assistance provided for this study.

Authors' contributions

AA and NH participated in the concept and design of the study. AA carried out the experiment, data collection and writing the article. AA and NH participated in the analysis and interpretation of the results. NH, EM, MIMY, NDHC, SNC, ARI, SAR, ZE, SS involved in the critical revision of the article. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Datta P, Sharma A, Pal B, Mohit K. The role of adipokines and adipogenesis in the pathogenesis of osteoarthritis. In: Foti M, Locati M, editors. Cytokine effector functions in tissues: Academic Press: Amsterdam; 2017: p. 99–107. https://doi.org/10.1016/B978-0-12-804214-4.00004-X
2. Naomi R, Teoh SH, Embong H, Balan SS, Othman F, Bahari H, et al. The role of oxidative stress and inflammation in obesity and its impact on cognitive impairments-A narrative review. Antioxidants. 2023;12(5):1071. https://doi.org/10.3390/antiox12051071
3. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Phcog Rev. 2010;4 (8):118–26. https://doi.org/10.4103/0973-7847.70902
4. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, et al. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. Int J Mol Sci. 2017;18(1):96. https://doi.org/10.3390/ijms18010096
5. Babando AA, Ilemona AJ, Adesina AJ, Gangas P, Ibrahim H, Ojo OA. Antioxidants from Nigerian medicinal plants: What are the evidence? In: Mansour MA, editor. Lipid peroxidation research. IntechOpen: 2020. p. 43. https://doi.org/10.5772/intechopen.84454
6. Moussa Z, Judeh ZM, Ahmed SA. Nonenzymatic exogenous and endogenous antioxidants. Free Radic Med Biol. 2019;1:11-22. https://doi.org/10.5772/intechopen.87778
7. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr J. 2015;15:1-22. https://doi.org/10.1186/s12937-016-0186-5
8. Azizul NH, Leong YH, Ahmad NI, Rahman SA. Nutraceutical potential of *Parkia speciosa* (stink bean): A current review. Am J Biomed Sci Res. 2019;4:392-402. https://doi.org/10.34297/ajbsr.2019.04.000842
9. Kamisah Y, Zuhair JSF, Juliana AH, Jaarin K. *Parkia speciosa* empty pod prevents hypertension and cardiac damage in rats given N(G)-nitro-L-arginine methyl ester. Biomed Pharmacother. 2017;96:291–98. https://doi.org/10.1016/j.biopha.2017.09.095
10. Ghasemzadeh A, Jaafar HZE, Bukhori MFM, Rahmat MH, Rahmat A. Assessment and comparison of phytochemical constituents and biological activities of bitter bean (*Parkia speciosa* Hassk.) collected from different locations in Malaysia. Chem Cent J. 2018;12(1):12. https://doi.org/10.1186/s13065-018-0377-6
11. Mustafa NH, Ugusman A, Jalil J, Kamisah Y. Anti-inflammatory property of Parkia speciosa empty pod extract in human umbilical vein endothelial cells. J Appl Pharm Sci. 2018;8(1):152–58. https://doi.org/10.7324/JAPS.2018.8123
12. Sonia N, Dsouza MR, Alisha. Pharmacological evaluation of *Parkia speciosa* Hassk. for antioxidant, anti-inflammatory, anti-diabetic and anti-microbial activities *in vitro*. Int J Life Sci. 2018;A11:49-59.
13. Gui JS, Jalil J, Jubri Z, Kamisah Y. *Parkia speciosa* empty pod extract exerts anti-inflammatory properties by modulating NFκB and MAPK pathways in cardiomyocytes exposed to tumor necrosis factor-α. Cytotechnology. 2019;71(1):79–89. https://doi.org/10.1007/s10616-018-0267-8
14. Fitrya F, Amriani A, Puspa Novita R, Setiorini D. Immunomodulatory effect of *Parkia speciosa* Hassk. pods extract on rat induced by *Salmonella typhimurium*. J Pharm Pharmacogn Res. 2020;8(5):457–65.
15. Singhania N, Chhikara N, Bishnoi S, Garg MK, Panghal A. Bioactive compounds of petai beans (*Parkia speciosa* Hassk.). In: Murthy HN, Paek KY, editors. Bioactive compounds in underutilized vegetables and legumes. Springer: Cham; 2021 p. 1–19. https://doi.org/10.1007/978-3-030-44578-2_30-1
16. Fithri NA, Fitrya F, Shabrina T, Yulanri D. Antioxidant activity analysis and standardization of *Parkia speciosa* (Petai) pods ethanol extract. Sci Technol Indones. 2019;4(1):5-10. https://doi.org/11.26554/sti.2219.4.1.5-11
17. Bobo-García G, Davidov-Pardo G, Arroqui C, Vírseda P, Marín-Arroyo MR, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts and comparison with conventional spectrophotometric methods. J Sci Food Agric. 2015;95(1):204–09. https://doi.org/10.1002/jsfa.6706
18. Abd Manan E, Abd Gani SS, Zaidan UH, Halmi MI. Characterization of antioxidant activities in red dragon fruit (*Hylocereus polyrhizus*) pulp water-based extract. J Adv Res Fluid Mech Therm Sci. 2019;61(2):170-80.
19. Kang JW, Nam D, Kim KH, Huh JE, Lee JD. Effect of gambisan on the inhibition of adipogenesis in 3T3-L1 adipocytes. J Evid Based Complementary Altern Med. 2013;1:789067. https://doi.org/10.1155/2013/789067
20. Oruganti L, Reddy Sankaran K, Dinnupati HG, Kotakadi VS, Meriga B. Anti-adipogenic and lipid-lowering activity of piperine and epigallocatechin gallate in 3T3-L1 adipocytes. Arch Physiol Biochem. 2023;129(5):1152–59. https://doi.org/10.1080/13813455.2021.1908366
21. Hwang JM, Lee MH, Lee JH, Lee JH. *Agastache rugosa* extract and its bioactive compound tilianin suppress adipogenesis and lipogenesis on 3T3-l1 cells. Appl Sci. (Switzerland). 2021;11 (16):7679. https://doi.org/10.3390/app11167679
22. Mohamad M, Ali MW, Ripin A, Ahmad A. Effect of extraction process parameters on the yield of bioactive compounds from the roots of *Eurycoma longifolia*. J Teknol (Sci Engin). 2013;60:51–57. https://doi.org/10.11113/jt.v60.1441

23. Ko HJ, Ang LH, Ng LT. Antioxidant activities and polyphenolic constituents of bitter bean *Parkia speciosa*. Int J Food Prop. 2014;17 (9):1977–86. https://doi.org/10.1080/10942912.2013.775152

24. Siti HN, Jalil J, Asmadi AY, Kamisah Y. *Parkia speciosa* Hassk. Empty pod extract alleviates angiotensin II-induced cardiomyocyte hypertrophy in H9C2 cells by modulating the Ang II/ROS/NO Axis and MAPK pathway. Front Pharma. 2021;12:741623.

25. Gan CY, Latiff AA. Antioxidant *Parkia speciosa* pod powder as potential functional flour in food application: Physicochemical properties' characterization. Food Hydrocoll. 2011;25(5):1174–80. https://doi.org/10.1016/j.foodhyd.2010.11.004

26. Pehlivan FE. Vitamin C: An antioxidant agent. In: Pehlivan FE, editor. Vitamin C. IntechOpen. 2017; p. 23-35. https://doi.org/10.5772/intechopen.69660

27. Etesami B, Ghaseminezhad S, Nowrouzi A, Rashidipour M, Yazdanparast R. Investigation of 3T3-L1 cell differentiation to adipocyte, affected by aqueous seed extract of *Phoenix dactylifera* L. Rep Biochem Mol Biol. 2020;9(1):14.

28. Li T, Zhang L, Jin C, Xiong Y, Cheng YY, Chen K. Pomegranate flower extract bidirectionally regulates the proliferation, differentiation and apoptosis of 3T3-L1 cells through regulation of PPARγ expression mediated by PI3K-AKT signaling pathway. Biomed Pharmacother. 2020;131:110769. https://doi.org/10.1016/j.biopha.2020.110769

29. Oh MJ, Lee H Bin, Yoo G, Park M, Lee CH, Choi I, et al. Anti-obesity effects of red pepper (*Capsicum annuum* L.) leaf extract on 3T3-L1 preadipocytes and high fat diet-fed mice. Food Funct. 2022;14 (1):292–304. https://doi.org/10.1039/d2fo03201e

30. García-Carrasco B, Fernandez-Dacosta R, Dávalos A, Ordovás J, Rodriguez-Casado A. *In vitro* hypolipidemic and antioxidant effects of leaf and root extracts of *Taraxacum officinale*. J Med Sci. 2015;3(2):38–54. https://doi.org/10.3390/medsci3020038