

**Anti-Obesity and Antihyperlipidemic Effects of *Musa cavendishii***Mohammed S. Abdel-Raziq<sup>1</sup>, Fatma M. Abdel Bar<sup>2,3\*</sup>,Sylvia A. Ashamallah<sup>4</sup>, Nashwa Barakat<sup>5</sup> and Ahmed A. Gohar<sup>1</sup><sup>1</sup> Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt<sup>2</sup> Department of Pharmacognosy, Faculty of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia<sup>3</sup> Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt<sup>4</sup> Pathology Department, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt<sup>5</sup> Animal Research Facility, Urology and Nephrology Center, Mansoura University, Mansoura 35516, Egypt

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**Abstract:** Obesity is a major risk factor in many health problems. This study explored the anti-obesity and antihyperlipidemic effects of *Musa cavendishii* Lamb. leaves. Doses of 1200 mg/kg of *M. cavendishii* methanol extract, or 300 mg/kg of fractions, were given to C57BL/6J mice fed with a high-fat diet (HFD) for 7 weeks. The reduction in fat volume was determined using CT scan and histopathological examination of mice livers. The results showed that the *n*-hexane and the EtOAc fractions reduced the body fat volume to 2.0% and 2.2% respectively, compared to 20.1% in the HFD group. Also, treated groups showed almost normal liver architecture with no fat vacuoles compared to the HFD group. Moreover, the treated groups showed a reduction in the levels of some obesity-related biochemical parameters, including plasma glucose, cholesterol, and triglycerides levels. Chromatographic fractionation of the bioactive *n*-hexane extract afforded four known compounds *viz.*, palmitic acid (**1**), 5-methyl-5-(4,8,12-trimethyl-tridecyl)-dihydro-furan-2-one (**2**), cycloeucaleanol (**3**), and stigmaterol (**4**). These compounds were *in vitro* and *in silico* studied for their pancreatic lipase (PL) inhibition. Compound **2** showed remarkable PL inhibition (89.8%) compared to orlistat (85.0%) at 200  $\mu$ M, which was in full agreement with the docking scores (-7.19 and -4.05, respectively).

**Keywords:** *Musa cavendishii*; anti-obesity; antihyperlipidemic; banana leaf; anti-adipogenesis; pancreatic lipase. © 2021 ACG Publications. All rights reserved.

**1. Introduction**

Obesity can be defined as an extreme body fat buildup that may have negative effects on health. Internationally, around 1.5 billion persons are overweight. They comprise 200 million obese men and 300 million obese women [1]. In Egypt, the health issues survey (2015), has recorded a simple key finding that; around 3 in 4 women and 6 in 10 men age 15-59 are overweight or obese [2]. Obesity is a potent risk factor for cardiovascular diseases, type 2 diabetes, cancer, female reproductive

\* Corresponding author: E-Mail: [f.abdelbar@psau.edu.sa](mailto:f.abdelbar@psau.edu.sa); [fatma\\_maar@yahoo.com](mailto:fatma_maar@yahoo.com); Phone: 0966-54540-3617

health problems, psychosocial problems, dyslipidemia, and morphological problems. The various combinations of these metabolic and morphologic risk factors and diseases are generally known as the "Metabolic Syndrome" [3, 4]. The prevalence of obesity is continuously raising at a shocking rate whatever the level of country development and is becoming a key health factor linked to many diseases and myriad social costs [5].

FDA-approved drugs such as sibutramine, orlistat, fluoxetine, mazindol, amfepramone, metformin, ephedrine, caffeine, and phentermine for the treatment of obesity. These drugs have several side effects in addition to their expensive cost. Including but limited to, they cause an elevation in blood pressure and heart rate, insomnia, dry mouth, rhinitis, constipation, anxiety, drowsiness, malabsorption of fat-soluble vitamins, and nervousness [6].

The history of the population cannot be imagined without studying plants as medicines, as the use of herbs in folklore for treatment is as ancient as man. Pharmacological studies have acknowledged the value of medicinal plants as a potential source of bioactive phytochemicals that serve as drugs or lead compounds in drug discovery and design. Many of these phytochemicals have been promoted to become current drug candidates [7].

The genus *Musa* L. Musaceae (banana) comprises about 30 to 40 species of perennial rhizomatous herbs that are native to Southeast Asia, the Pacific islands, and Australia. Genus *Musa* was reported to contain triterpenoids mainly of cycloartane-type [8-10], hemiterpenoids [11, 12], diterpenoids [13], flavonoids [14-17], catecholamines [18] and acyl steryl glycosides [19].

Several *Musa* sp. have reputed for many pharmacological activities such as anti-gastric ulcer [20], analgesic [21], hypoglycemic [22, 23], hypocholesterolaemic [24], antihypertensive [25], antioxidant [26], wound healing [27] and antimalarial [28].

Few researchers have investigated the hypolipidemic effect of *Musa* sp. and no studies addressed its anti-obesity effect. In this research, the leaves of *Musa cavendishii* Lamb. were investigated for murine obesity using high fat-fed C57BL/6J mice for the assessment of hypolipidemic and hypoglycemic effects and for hypoglycemic effects. Furthermore, the most active fractions were subjected to phytochemical investigation to identify the phytochemicals responsible for these effects.

## 2. Materials and Methods

### 2.1. Plant Material

The leaves of *Musa cavendishii* Lamb., family Musaceae were collected in July 2012, from Mansoura University gardens, Mansoura, Egypt. The authenticity of the plants was confirmed by Dr. Nabil Fahmy, Professor, Department of Vegetable and Ornamental Plants, Faculty of Agriculture, Mansoura University, Egypt. The leaves were cut and air-dried at room temperature. A specimen was kept at the herbarium of the Department of Pharmacognosy, Mansoura University, Egypt (Mansoura-7-012).

### 2.2. Biological Study

#### 2.2.1. Animals

C57BL/6J Laboratory mice were obtained from Charles River Laboratory, Germany. The mice were housed in an animal care facility at the Urology and Nephrology Center, Mansoura University, Egypt. The study protocol was received and approved by Research Ethics Committee, Faculty of Pharmacy, Mansoura University, for the use and care of the experimental animals (Code: 2012-17, April 3<sup>rd</sup>, 2012).

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### 2.2.2. Diets

Low-fat (D12450J) and high-fat diet (D12492) were purchased from Research Diets Inc. (New Brunswick, NJ, USA).

### 2.2.3. Biochemical Analyses

Glucose, total cholesterol, and triglycerides kits were purchased from Spintreact Inc., Girona, Spain.

### 2.2.4. Preparation of the Crude Extracts

The air-dried powdered leaves (1 kg) were extracted by maceration with distilled methanol (6 X 5L). The combined methanol extracts were concentrated to a syrupy consistency and then allowed to dry off in a desiccator over anhydrous CaCl<sub>2</sub> to a constant weight (120 g).

### 2.2.5. Liquid-Liquid Fractionation

The total leaf extract (TLE) was suspended in a small volume of methanol, diluted with an equal volume of dist.H<sub>2</sub>O, transferred to a separating funnel, and successively partitioned with *n*-hexane (Hex fraction), methylene chloride (MC fraction), and ethyl acetate (EA fraction). The extracts were evaporated to dryness under reduced pressure and kept frozen for further studies.

### 2.2.6. Preparation of the Oral Liquid Dosage Forms

About 15 g of each extract were dissolved in the least amount of a suitable solvent then adsorbed on Avicel PH 101 (Ak Scientific, Union City, CA, USA) by ratio 1:2. Micronization was performed and then suspended in sterile distilled water before oral dosing to the mice.

### 2.2.7. Protocols for Animal Study

Male C57BL/6J laboratory mice (40-36 g, 6-8 weeks-old) were bred in full barrier units in Experimental Animal House of Urology and Nephrology Center, Mansoura University, and housed in shoebox-cages on corn-cob bedding (one per cage). Mice were acclimatized for one week, maintained on a twelve-hour light-dark cycle with *ad libitum* access to diet and water. After the adaptation period, animals were randomly divided into groups of six; low-fat diet (LFD, 10% kcal fat), high-fat diet (HFD, 60% kcal fat), high-fat diet plus 1200 mg/kg/day of *M. cavendishii* total extract (HFD+TLE), high-fat diet plus 300 mg/kg/day of hexane, CH<sub>2</sub>CH<sub>2</sub> or EtOAc fractions (HFD+HEX, HFD+MC and HFD+EA, respectively) and high-fat diet plus 500 mg/kg/day green tea total extract (HFD+GTE) as a positive control. The energy densities of LFD and HFD were 3.85 and 5.24 kcal/g, respectively. Mice were kept on the specified diets for 7 weeks, with a weekly recording of body weight. After 7 weeks of treatment, animals were starved for 7 hr and blood samples were collected by inserting capillary into the medial canthus of the eye. Blood plasma was obtained by centrifugation at 2000 rpm for 20 min and kept for further analysis at -80 °C. Mice were anesthetized, the abdomens were opened, livers were removed and harvested. Specimens of liver tissue were frozen at -80 °C [29].

### 2.2.8. Pathological Examination

For histological investigation, liver tissues were soaked in 10% buffered formalin overnight, paraffin-embedded, and sections were cut at a 5 µm thickness. Slides were then stained with hematoxylin and eosin (H&E). Frozen liver tissue samples were sectioned into 8 µm-thick sections. Lipid depositions were identified by staining with Oil red O. Digital images were picked up with a Nikon Eclipse 80i microscope, connected to Nikon Digital DXM 1200C camera [29].

### 2.2.9. Non-invasive Determination of Fat Volume

#### 2.2.9.1. Image Acquisition

Injectable anesthesia of the mouse was performed using Ketamine/xylazine cocktail (75 mg/kg ketamine + 5 mg/kg xylazine) I.p., producing 20-30 min. anesthesia. The mouse was suspended in a supine position on the PHILIPS BRILLIANCE 64 SLICE multidetector scanners, and then acquiring the image using Philips Brilliance™ Extended Workspace, V3.01.5000. Acquisitions were performed at slice thickness 0.9 mm length, using 600 projections. The X-ray source was set to a current of 200  $\mu$ A and voltage of 45 kV. After imaging completion, mice were returned to a recovery cage until ambulatory [30].

#### 2.2.9.2. Image Analysis and Visualization

It was carried out using Philips Brilliance™ CT V2.6.2.21004. Images are segmented in Philips Brilliance™ CT according to tissue density—first for total volume and then for fat volume. Total volumes and fat volumes derived from the segmented images were calculated in  $\text{cm}^3$  and recorded.

#### 2.2.9.3. Statistical Analysis

Data analysis and presentation were expressed as the mean  $\pm$  standard error of the mean (SEM). The statistical significance of differences between means for different treated groups was analyzed by Student's t-tests and ANOVA (one-way analysis of variance) using GraphPad Prism version 7.00 for Windows, GraphPad Software (La Jolla California USA).

### 2.3. Phytochemical Study

#### 2.3.1. Chemicals Used for the Phytochemical Study

The solvents used for extraction and chromatographic separation were purchased from EL-Nasr Company for Pharmaceutical Chemicals, Egypt. TLC was performed on precoated silica gel 60 GF<sub>254</sub> 20 x 20 cm, 0.2 mm thick (Merck or Macherey-Nagel, Germany) and RP-18 silica gel plates 5x 20 cm, 200  $\mu$ m layer thickness, Partisil® KC18F silica gel 60A with a fluorescent indicator (Whatman®, USA). For column chromatography, silica gel (230-400) mesh (Natland, USA) or Diol-functionalized silica gel (40-75  $\mu$ m), (Sigma-aldrich, USA), Sephadex LH20, (GE Healthcare, USA), phase-bonded octadecyl-silica gel (RP-18, Merck, Germany) were used.

#### 2.3.2. Phytochemical Investigation of the *n*-hexane Fraction

The *n*-hexane extract (Hex) of *M. cavendishii* (20 g) was fractionated over a silica gel column (50 x 4.5 cm i.d., 300 g silica), using petroleum ether and eluted with *n*-hexane-EtOAc mixtures with increased polarity. Hundred mL fractions were collected and monitored by silica gel TLC [EtOAc-*n*-hexane, 1:9 v/v] or RP-18 TLC plates [MeOH-H<sub>2</sub>O, 8.5: 1.5 v/v] and vanillin/sulfuric acid spray reagent. Similar fractions were pooled together, concentrated, and subjected to further chromatographic fractionation. Four compounds were obtained in a pure form designated from **1-4**. The detailed isolation procedure is supplemented in supplementary materials.

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## 2.4. Pancreatic Lipase Activity Assessment

## 2.4.1. In Silico Study

Molecular Operating Environment (MOE), Version 2009.10, Chemical Computing Group, Inc., Montreal, Quebec, Canada, 2009, <http://www.chemcomp.com>. X-ray crystal structure of pancreatic lipase (PL) was downloaded from protein data bank (PDB ID: 1LPB)[31].

## 2.4.2. In Vitro Pancreatic Lipase Inhibition Assay

For pancreatic lipase (PL) inhibitory assay, porcine PL (E.C. 3.1.1.3, MP biomedical, Solon, OH, USA), *p*-nitrophenylbutyrate, PNPB (Carbosynth, Berkshire, UK), orlistat (Carbosynth, Berkshire, UK), acetonitrile (Sigma Chemicals, USA) and phosphate buffer pH 8 were used.

## 2.4.3. In Vitro Pancreatic Lipase Inhibition Assay Procedure

Pancreatic lipase inhibition assay was carried out by the previously described chromogenic method with modifications [32] using *p*-nitrophenylbutyrate, PNPB as a substrate and in a 96-well plate. The assay was started by incubating 100  $\mu$ l PL (50 USP unit/ ml, using olive oil as a substrate, in buffer, centrifuged at 2000 RPM for 5 minutes to remove the insoluble matter) with 25  $\mu$ l of 200  $\mu$ M of the compound solution (dissolved in 3% DMSO, diluted with acetonitrile) at 37 for 2 h. Then, 75  $\mu$ l of PNPB (20mM, in acetonitrile) was added and further incubated for 30 min. After incubation, the absorbance was measured at 405 nm and the % inhibition of PL was calculated from:

$$\%Inhibition = 1 - \frac{A.test - A.test\ blank}{A.enzyme - A.enzyme\ blank} \times 100$$

Where, *A.test* and *A.blank* represented the absorbance at 405 nm of the reaction mixture containing the testing samples, with and without PL, respectively. While, *A.enzyme* and *A.enzyme blank* represent the absorbance in absence of the test sample, with and without PL, respectively.

## 3. Results and Discussion

3.1. Effect of Orally Administered Extract and Fractions of *M. Cavendishii* on Body Weight

To investigate the effect of *M. cavendishii* leaf extracts on body weight, male C57BL/6J mice were used. After 7 weeks of treatment, all experimental groups showed significant body weight loss as compared to the high-fat diet group (HFD, obese group) where ( $p < 0.001$ ).

**Table 1.** Effect of total leaf extract (TLE) and different fractions (Hex, MC and EA) of *M. cavendishii* on body weight (g) of C57BL/6J mice fed with high-fat diet (HFD), compared to green tea extract (GTE) after seven weeks of oral administration

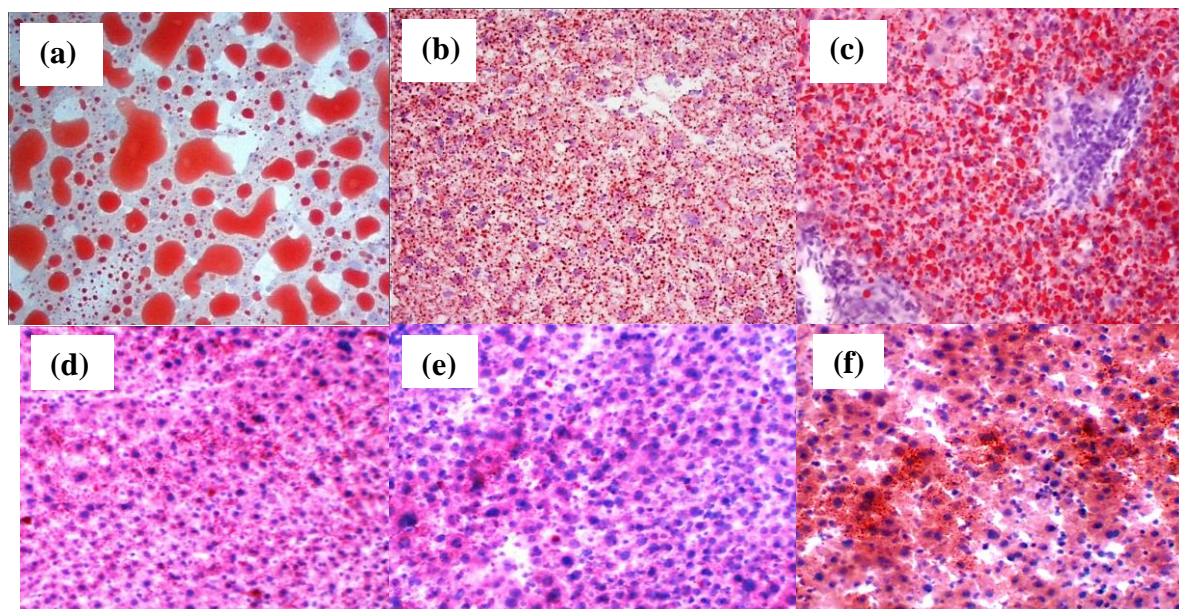
Groups/ Weeks	Average body weight (g)						
	LFD	HFD	LFD+TLE	HFD+Hex	HFD+MC	HFD+EA	HFD+GTE
Week 0	24.25±	25.08±0.33	24.17±0.40	24.7±0.25	25.58±0.29	24.08±0.27	24.25±0.35
Week 1	24.42±	27.5±0.26	25.17±0.28	26.42±0.64	27.33±0.31	25.5±0.37	25.08±0.47
Week 2	24.42±	30.17±0.42	25.25±0.5	28.08±0.30	28.58±0.42	26.67±0.36	26.58±0.44
Week 3	24.92±	31.25±0.53	23.67±0.51	28.58±0.37	29.67±0.31	27.5±0.41	27.42±0.35
Week 4	25.08±	33±0.53	23.92±0.42	29.08±0.55	30.08±0.37	28.83±0.48	28.33±0.48
Week 5	25.58±	34.92±0.47	25±0.39	25.58±0.20	29.17±0.53	27.25±0.36	29.42±0.47
Week 6	26±	36.75±0.28	25.25±0.53	24.92±0.35	29.17±0.53	27.25±0.48	30.17±0.46
Week 7	26.75±	40.25±0.38	26±0.58	25.75±0.31	29.67±0.67	27.75±0.48	30.67±0.25

Table 1 demonstrates that TLE, Hex, MC, and EA extracts of *M. cavendishii* showed a significant decrease in mean body weight (26.0, 25.75, 29.67, and 27.75 g, respectively) compared to green tea extract as a positive control (GTE, 30.67g), high fat diet-fed group (HFD, 40.25 g) and the low fat-fed group as a negative control (LFD, 26.75 g). Therefore, the present study provides evidence that total leaf extract, the *n*-hexane and EtOAc fractions of *M. cavendishii* showed a promising effect

on diet-induced obesity. After seven weeks of oral administration of TLE, Hex, MC, or EA extracts, all treated groups showed a significant decrease in mean body weight with Hex showed the highest effect compared to the high fat diet-fed group (Table 1).

### 3.2. Histopathological Examination of Mice Liver

A high-fat diet is known to increase adipocyte size through fat deposition causing liver injury [33]. The effect of TLE and the more active fractions (Hex and EA), on hepatic steatosis of mice groups fed with HFD, was investigated. Frozen sections of liver stained with oil red O were performed and they showed large fat lobules in the group fed with HFD, Figure 1(a). Obviously, all treated groups showed normal liver architecture with no or minor fat vacuoles, figures 1(d-f). The treated groups with *M. cavendishii* extracts (TLE, Hex, and EA extracts), Figures 1(d, e, and f), respectively, showed normal liver architecture with no or minor fat vacuoles as compared to the HFD group, Figure 1(a). Their architecture is almost comparable to the LFD-fed group, Figure 1(b). Green tea extract (GTE) was used as a positive control that also showed near-normal liver architecture with small clear unstained fat vacuoles as compared to the HFD group, Figure 1(c). Thus, histopathological examination of the liver of the high-fat-fed mice indicated that oral treatment with *M. cavendishii* extracts maintained normal liver architecture, prevented fat deposition, and subsequently protected against liver damage caused by fat lobules accumulation in hepatocytes.



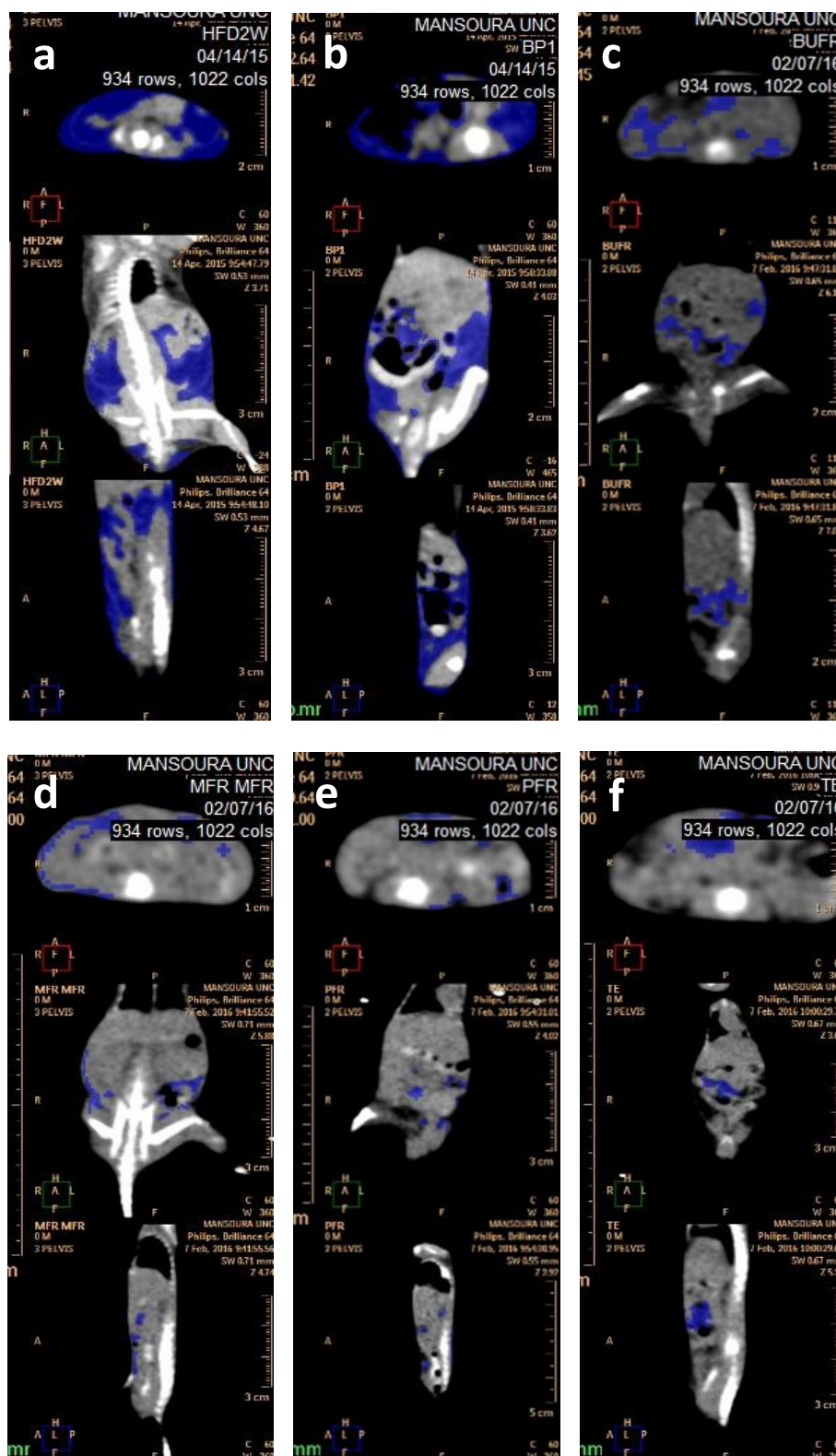
**Figure 1.** Histological analysis of C57BL/6J mice groups' liver stained with Oil Red O stain (X100), performed on frozen liver sections. (a) High fat diet, HFD; (b) Low fat diet, LFD; (c) Green tea total extract, HFD+GTE; (d) *M. cavendishii* total leaf extract, HFD+TLE; (e) *n*-Hexane fraction, HFD+Hex; (f) EA fraction, HFD+EA.

### 3.3. Effect of *M. Cavendishii* Extracts and Fractions on The Volume of Subcutaneous, Intra-abdominal, and Pelvic Fat of Mice Deduced from CT Scan

Moreover, non-invasive determination of fat volume by image acquisition using CT scan showed a remarkable decrease in the fat volume for groups treated with TLE, Hex, and EA extracts, Figure 2(d, e and f), respectively. These data were compared to that of the obese group (HFD), Figure 2(a) and normal group (LFD), Figure 2(b). Total volumes and fat volumes derived from the CT scans were calculated and recorded in table 2. A decrease in fat volume was observed in TLE, Hex and EA extracts treated groups as deduced from CT scan, Figure 2(d, e, and f), respectively. These data of the tested extract and fractions were compared to that of the obese group (HFD), Figure 2(a) and normal group (LFD), Figure 2(b). The TLE of *M. cavendishii* showed a fat ratio is 5.0% as compared to the

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HFD fed group (20.1%). The Hex and EA fractions showed a reduction in the fat volume (2.0% and 2.2%, respectively). The standard green tea extract (GTE) showed a fat volume of 10.8% that is close to the normal LFD group (11.8%), Table 2 and Figure 2(c).



**Figure 2.** Representative CT images segmented for fat (axial, coronal, and sagittal slices), fat volume in blue. Treated groups are (a) High fat diet, HFD; (b) Low fat diet, LFD; (c) Green tea total extract, HFD+GTE; (d) *M. cavendishii* total leaf extract, HFD+TLE; (e) *n*-Hexane fraction, HFD+Hex; (f) EA fraction, HFD+EA

**Table 2.** Total volume, fat volume, and percent fat/total volume ratios for treated (TLE, Hex, MC, EA), normal (LFD) and obese (HFD) C57Bl/6J mice groups, compared to green tea extract (GTE)

Group	Total volume (cm <sup>3</sup> )*	Fat volume (cm <sup>3</sup> )*	Ratio Percent
Normal (LFD)	22.9	2.7	11.8 %
Obese (HFD)	36.4	7.3	20.1 %
HFD+TLE	16.1	0.39	5.0 %
HFD+Hex	18.7	0.38	2.0 %
HFD+MC	20.9	1.1	5.3 %
HFD+EA	19.3	0.42	2.2 %
Green tea (HFD+GTE)	27.8	3.0	10.8 %

\*Total and fat volumes were derived from segmented images using PHILIPS BRILLIANCE software.

### 3.4. Effect of *M. Cavendishii* Extracts and Fractions on Some Obesity-Related Biochemical Parameters

#### 3.4.1. Effect of TLE, Hex, MC, and EA Extracts of *M. Cavendishii* on Fasting Plasma Glucose Level

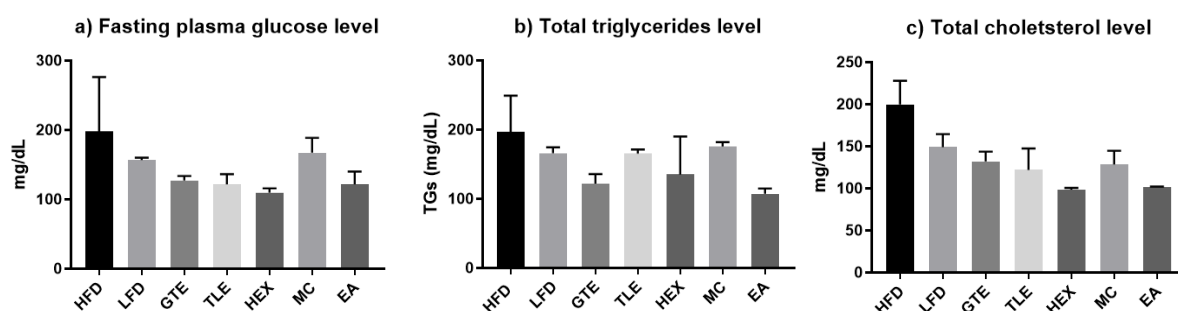
The effect of different *M. cavendishii* leaf extract on some obesity-related biochemical parameters such as fasting plasma glucose, cholesterol, and triglycerides levels were investigated. After 7 weeks of treatment, vehicle-treated obese mice showed an insignificant difference decrease in plasma glucose where ( $p>0.05$ ) in multiple comparisons using a one-way ANOVA test even from the normal group. It is worth saying that a little number of mice in the obese group (fed with HFD) became diabetic. On the other hand, the LFD group and groups treated with *M. cavendishii* extracts were not diabetic. It was revealed that groups treated with TLE, Hex, MC, and EA showed a marked drop in fasting plasma glucose level, Figure 3(a). This may refer to the anti-diabetic activity of the tested extracts and fractions upon running a mouse model of diet-induced diabetes.

#### 3.4.2. Effect of Different Extracts of *M. Cavendishii* on Total Plasma Triglycerides Level

After 7 weeks of treatment, vehicle-treated obese mice showed significant differences among means decrease in triglycerides level as compared to the normal control group ( $p<0.05$ ). In comparison with the treated groups, Hex and EA fractions showed triglycerides lowering effects comparable to that of the positive control (GTE), Figure 3(b).

#### 3.4.3. Effect of Different Extracts of *M. Cavendishii* on Total Plasma Cholesterol Level

After 7 weeks of treatment, vehicle-treated obese mice showed significant differences among means ( $p<0.05$ ) decrease in total cholesterol level as compared to the normal control group and all the treated groups. Figure 3(c) demonstrates that Hex and EA fractions showed a reduction in total cholesterol levels.



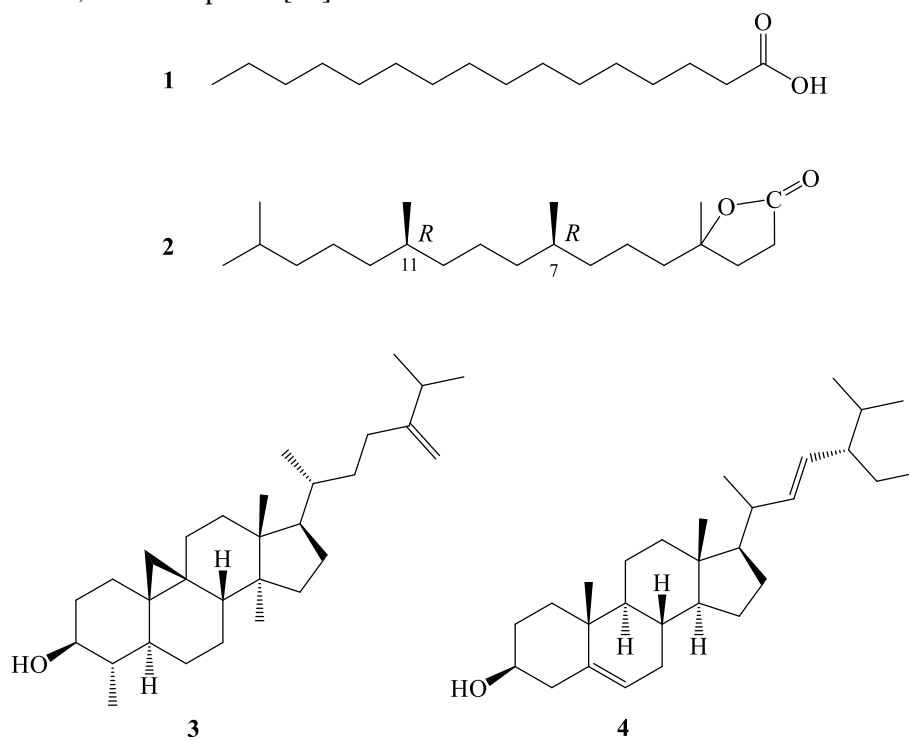
**Figure 3.** Effect of *M. cavendishii* extracts and fractions on some obesity-related biochemical parameters: (a) Fasting plasma glucose level; (b) Total triglycerides level; (c) Total cholesterol level, of C57Bl/6J mice. Treated groups are high-fat diet (HFD), low-fat diet (LFD), green tea extract (GTE), *M. cavendishii* total leaf extract (TLE), methylene chloride fraction (MC), *n*-hexane fraction (Hex), and ethyl acetate fraction (EA). Results are expressed in mg/dL as Mean  $\pm$  SEM (n=6). The multiple comparisons were made using ANOVA followed by Dunnett's at  $p<0.05$ .



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## 3.5. Phytochemical Investigation of The Active Fractions

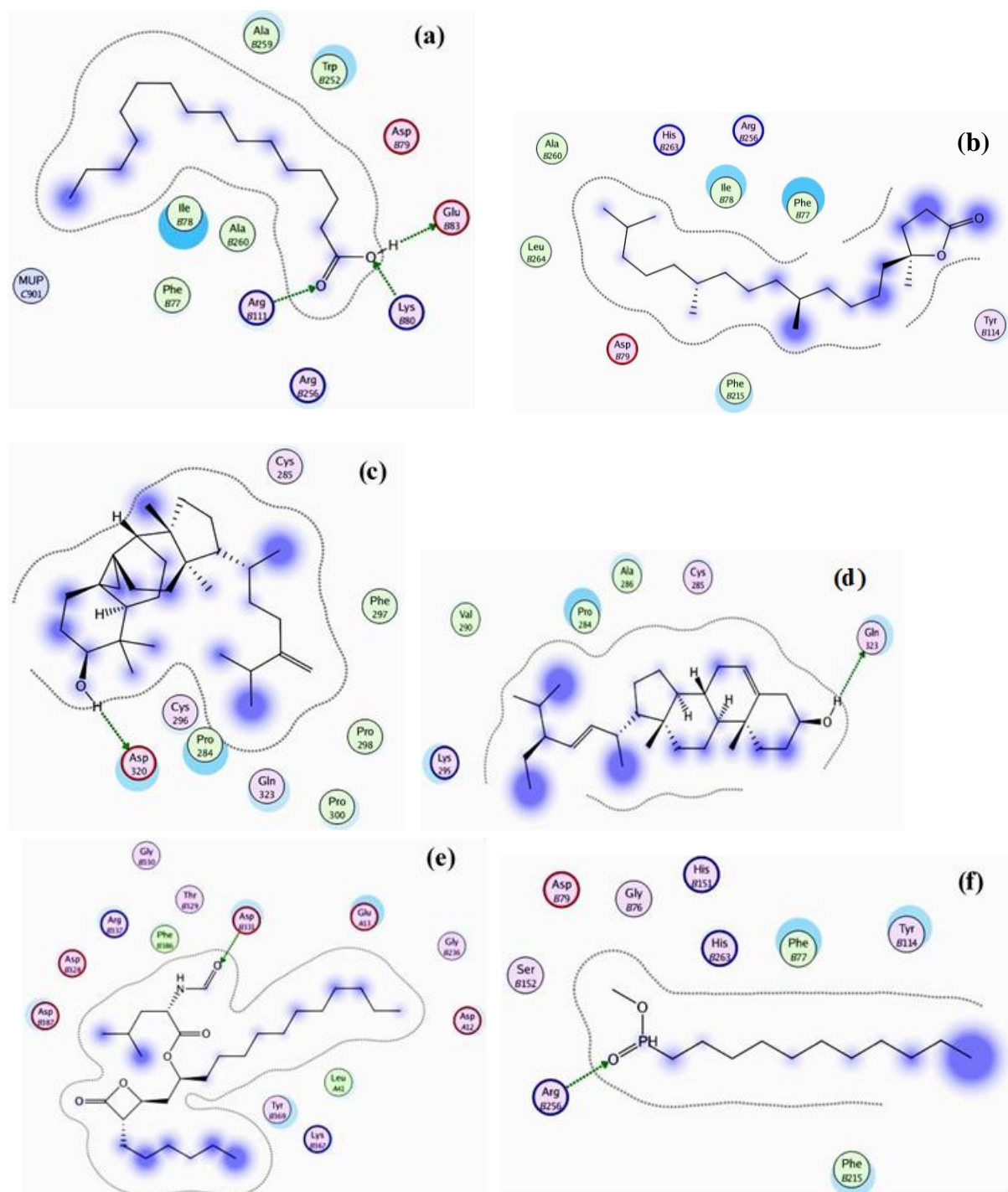
Investigation of the *n*-hexane fraction led to the isolation of four known compounds from *M. cavendishii*, viz. palmitic acid (**1**), 5-methyl-5-(4,8,12-trimethyl-tridecyl)-dihydro-furan-2-one (**2**) [34], cycloeucalenol (**3**) [35] and stigmasterol (**4**) [9]. It is worth noted that this is the first report of cycloeucalenol and compound **2** from *Musa* spp. Intensive investigation of EtOAc extract of *M. cavendishii* afforded eight compounds which were previously reported by our workgroup viz., (6*S*,9*S*)-roseoside, (6*S*,9*R*)-roseoside, kaempferol-3-*O*-rutinoside, *p*-hydroxybenzoic acid, rutoside, catechuic acid, quercetin, and kaempferol [17].



**Figure 4.** Structures of compounds **1-4**

3.6. *In Silico* Pancreatic Lipase Assessment

Virtual screening of the isolated compounds (**1-4**) was performed into the active site of PL (PDB ID: 1LPB) for the determination of the binding mode to the PL active site, Figure 5 (a-d). Careful investigation of the attachment pattern of palmitic acid, the phytol derivative **2**, and orlistat (Figure 5 (a), (b) and (e), respectively), displayed that they showed strong binding to the active site of lipase enzyme through the hydrophobic aliphatic hydrocarbon chain. On the other hand, compound **2** and orlistat showed strong inhibition activity (89.9 % and 85 %, respectively) contrary to palmitic acid (22.2%). Consequently, it could be concluded that the lactone ring either in **2** or in orlistat is essential for activity, although it is exposed out of ligand in case of **2**, Figure 5(b). Compound **2** mainly interacts with the greasy amino acids; Leu264, Ile78, Phe77, Phe215 and Ala260. As a conclusion, firstly it is worth saying that inhibition of lipase could become better through the interaction of the ligand with the hydrophobic amino acids rather than the polar ones. Secondly, the lactone ring appears to be essential for the inhibition activity. Additionally, the docking score of **2** (-7.19 kcal/mol) is in full agreement with the obtained *in vitro* PL inhibitory activity (89.9%), Table 3. However, compound **3** showed a low docking score (-3.81 kcal/mol) which is contradictory with the obtained PL inhibitory activity (66.97%) suggesting a different binding pocket for **3** than that for the co-crystallized ligand (methoxyundecylphosphinic acid, MPA).



**Figure 5.** Docking interactions of compounds isolated from the *n*-hexane fraction of *M. cavendishii* Lamb. with pancreatic lipase (PDB: 1LPB); **(a)** Palmitic acid **1**, **(b)** 5-Methyl-5-(4,8,12-trimethyl-tridecyl)-dihydro-furan-2-one **2**, **(c)** Cyclooeucalenol **3**, **(d)** Stigmasterol **4**, **(e)** Orlistat (A pancreatic lipase inhibitor standard); **(f)** Methoxyundecylphosphinic acid, MPA (Co-crystallized ligand). The green arrow represents side chain acceptor/donor; the blue arrow represents the backbone acceptor/donor; the blue shadow represents ligand exposure.

Biological activities of *Musa cavendishii*3.1. *In Vitro* Pancreatic Lipase Activity Assessment

Lipids are the main source of undesirable calories during weight loss. Therefore, the inhibition of lipid absorption and assimilation is a promising protocol for the prevention and treatment of obesity. Pancreatic lipase (PL) is a crucial enzyme for the breakdown of dietary triglycerides. The approved anti-obesity drug, orlistat, acts through PL inhibition with a modest action on body weight. Recently, plant extracts and other natural products, including vegetables, fruits, marine, and microbial metabolites received great attention from many researchers as potential lipase inhibitors. Natural products in general are considered complements to exercise and lifestyle modifications aimed at maintaining weight loss [36].

The isolated compounds from the *n*-hexane fraction *M. cavendishii* were screened for their PL inhibition. The results recorded in table 3, showed that that the most active lipase inhibitors were compounds **2** and **3** with percentages of inhibition at 200  $\mu$ M equals 89.8% and 66.97%, respectively. This activity of **2** was higher than that of orlistat (85.0% inhibition). Therefore, these results suggested that 5-methyl-5-(4,8,12-trimethyl-tridecyl)-dihydro-furan-2-one (**2**) and cycloeucalenol (**3**) may contribute to the *in vivo* anti-obesity and antihyperlipidemic activities of the *n*-hexane extract. However, palmitic acid **1** and stigmasterol **4** showed weak inhibitory activity.

**Table 3.** Pancreatic lipase (PL) inhibitory activity and docking scores of compounds isolated from the *n*-hexane extract of *M. cavendishii* using the PNPB chromogenic method

Compounds	% Inhibition at 200 $\mu$ M*	Free energy of binding $dG$ kcal/mol**
1	22.2	- 5.45
2	89.9	-7.19
3	66.97	-3.81
4	13.82	-4.20
Orlistat	85.0	-4.50
MPA	-	-6.90

\*The final concentration of all compound was 200  $\mu$ M.

\*\*Calculated by MOE for the tested compounds.

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### Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

### ORCID

Mohammed S. Abdel-Raziq: [0000-0002-2847-545X](https://orcid.org/0000-0002-2847-545X)

Fatma M. Abdel Bar: [0000-0003-0823-4015](https://orcid.org/0000-0003-0823-4015)

Sylvia A. Ashamallah: [0000-0002-6395-769X](https://orcid.org/0000-0002-6395-769X)

Nashwa Barakat: [0000-0002-4350-7325](https://orcid.org/0000-0002-4350-7325)

Ahmed A. Gohar: [0000-0001-5906-1543](https://orcid.org/0000-0001-5906-1543)

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