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Original Research Article

Caraway (*Carum carvi* L.) Agro-industrial Waste; Anti-obesity Effect and Chemical Characterization

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ABSTRACT

Roots and aerial parts are the agricultural by-products of caraway (*Carum carvi* L.) fruit production. Hereto, the anti-obesity and hypolipidemic effects of caraway waste were assessed using different *in vitro* and *in vivo* tests performed on total ethanol extract (TEE), as well as its fractions. The ethyl acetate fraction (EtOAc fr.) exhibited the highest inhibitory activity on pancreatic lipase (PL), *α*-amylase and DPPH free radical. Accordingly, it showed the highest ameliorative activity against dyslipidaemia among other fractions and the total extract when tested on a high fat diet-fed mice model. It attenuated hepatic steatosis, reduced adipocyte size, ameliorated the lipid profile, and suppressed leptin concentrations as revealed by the histopathological and biochemical examinations. Umbelliferone, *p*-coumaric acid and isoquercitrin were isolated from the bioactive EtOAc fr. and quantified in both, the EtOAc fr. and TEE, using HPLC. Isoquercitrin displayed the highest inhibitory activity towards DPPH and PL (IC_{50}: 10 ± 2.50 and 93.54± 2.62 µg/mL, respectively), whereas umbelliferone was the most active against *α*-amylase (IC_{50}: 50.70 ± 2.83 µg/mL). Molecular docking was carried out to uncover the binding affinities to the enzymes' active sites. Isoquercitrin demonstrated strong interaction in the binding site of lipase (-130.87 kcal·mol^{-1}), via H-bonding with Asp79, Ile78, Arg256, Ser152 and Tyr114, whereas, umbelliferone formed H-bonding with Tyr59 and Leu142 and H-Pi interaction with Gln63 in the amylase enzyme's active site (-65.99 kcal·mol^{-1}). The current research highlights the great potential for caraway waste as a multitarget remedy against obesity, which incites further molecular and clinical studies.

Keywords: Caraway waste, Hypolipidemic, Pancreatic lipase, Isoquercitrin, Umbelliferone, P-coumaric acid

Introduction

Obesity is a multifactorial disorder impacted by environmental and genetic variables. It is distinguished by an excessive fat build-up in adipose tissue and other internal organs.[1] The prevalence of obesity has been on the rise over the past few decades, with around 2.5 billion overweight adults in 2022, 890 million (35%) of whom are obese.[2] It is projected that, by 2035, obese adults will exceed 1.5 billion, leading to significant economic impacts due to increased healthcare costs and reduced productivity.[3,4] In many countries, obesity rates have exceeded 50%. [5] In Egypt, 39.8% of the adult population was found to be obese, according to the '100 million Seha' initiative conducted in 2019, with Egypt ranked as the 18th highest country in terms of global obesity. [6]

Obesity is linked to several health issues such as dyslipidaemia, diabetes, cardiovascular diseases, rheumatoid arthritis, certain cancer types, and liver diseases.[1] It also has a major negative effect on a person's quality of life and can exacerbate depression.[7] In recent years, obesity has been recognised as a threat due to its severity and vulnerability to the pandemic coronavirus disease, leading to increased hospitalisation rates.[8] As a result, obesity contributes to higher morbidity and mortality rates, and it is considered a global epidemic, accounting for 2.8 million deaths annually, resulting in significant healthcare costs.[9]

Various approaches are used to manage obesity such as synthetic drugs and surgical procedures. However, these approaches are unsatisfactory due to their high cost, serious adverse effects, and likelihood of relapse with increased severity.[10,11] Considering the obesity epidemic together with the dissatisfaction with different treatments, there is a pressing need to discover natural phytochemicals with anti-obesity properties that are safe and more effective.[12] Pancreatic lipase inhibition and lipid profile regulation are highlighted as the main mechanistic ways by which most plants exert their anti-obesity potential.[11]

Molecular docking is a widely used computational method that predicts how small-molecule ligands will interact with a target. It has been extensively used in the drug discovery process. Molecular docking has been used to screen a large number of compounds for potential interactions with disease-relevant proteins, leading to the identification of promising therapeutic leads in the pharmaceutical industry.[13] Additionally, molecular docking has been effective in the virtual screening of natural products, leading to the investigation of significant bioactive molecules and aiding researchers in proposing their mode of action.[14,15,16]

Caraway (*Carum carvi* L.), a member of the Apiaceae family, originated in the Mediterranean region and India and is now commonly

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cultivated in temperate regions. It is primarily grown for its fruits, also known as seeds, which are used in the food, cosmetics, beverage and pharmaceutical sectors. The aerial parts and roots represent an agricultural by-product of caraway cultivation, with this discarded material accounting for 66% of the whole plant. Consequently, the valorisation of waste products as potential sources of bioactives is gaining attention.[17]

Caraway fruits have been traditionally used in the management of a variety of illnesses due to their diverse bioactivities. These are attributed to their multiple phytoconstituents, including essential oils, fatty acids, flavonoids and phenolic acids.[18] Research has shown that caraway fruits have a hypolipidemic effect, reducing serum cholesterol, triacylglycerol and VLDL-c, and increasing HDL-c levels in animal models.[19-21] In Iranian-Islamic folk medicine, caraway extract has been prescribed for weight loss and is currently traded as an anti-obesity product in Iran's market.[10] The anti-obesity effect of steam-distilled extract of caraway fruits has been extensively tested in overweight and obese women, combined with exercise.[10,22,23] Carvone, limonene, thymol, *trans*-carveol, γ-terpinene, and carvacrol are the main constituents of the steam-distilled extract, as revealed by GC-MS analysis.[10] Carvacrol prevented obesity in mice fed on a high-fat diet by inducing weight and fat loss, as well as reducing lipid levels through the modulation of gene expressions interrelated to adipogenesis and inflammation.[24] Despite the anti-obesity and hypolipidemic activities of caraway fruits being largely overlooked, its waste products have not yet been explored.

The ongoing work aimed to assess the anti-obesity and hypolipidemic effects of caraway waste employing various *in vitro* and *in vivo* tests on the total ethanolic extract (TEE) and its fractions. The most active fraction has been further analysed to isolate its main constituents and for standardisation using HPLC analysis. This research offers a new perspective on the potential of caraway waste as a multi-target remedy against obesity, emphasising its most active fraction and contributing constituents.

Materials and Methods

Plant material

Caraway (*Carum carvi* L.) waste, which means all the plant parts except the fruits (aerial parts and roots of the plant without the fruits), was obtained from the Botanical Garden of Mansoura University, Mansoura, Egypt (31°02'27.7"N 31°21'11.4"E), in May 2017, at the mature fruiting stage. A voucher specimen no. 13.2.18.2 has been retained at the Pharmacognosy Department's Herbarium, Faculty of Pharmacy, Cairo University.

Chemicals

Acarbose was procured from Sigma Aldrich, Louis, MO, USA, and orlistat from Carbosynth, Berkshire, UK. Gallic acid and quercetin were obtained from the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University. Porcine pancreatic α-amylase (PPA) (EC 3.2.1.1) and porcine pancreatic lipase (PPL) (E.C. 3.1.1.3) were purchased from MP biomedicals, Solon, OH, USA and leptin hormone from Minneapolis, MN, USA. Solvents used in HPLC analysis were of HPLC grade (Sigma Aldrich, Louis, MO, USA), whereas other chemicals and reagents were of analytical grade (El-Nasr for Chemicals, Mansoura, Egypt).

Extraction

Shade-dried, powdered *C. carvi* L. waste (3 kg), sieved to 80 mesh, was extracted by maceration using 95% ethanol (20 L×5) yielding 150 g of the total ethanolic extract (TEE). The TEE (130 g) was suspended in water and partitioned respectively using petroleum ether (40-60°C), dichloromethane and ethyl acetate. Liquid-liquid fractionation yielded 55, 30, 8 and 30 g of petroleum ether fraction (Pet-Eth fr.), dichloromethane fraction (DCM fr.), ethyl acetate fraction (EtOAc fr.) and the remaining aqueous fraction (Aq fr.), respectively. Aliquots from TEE and different fractions were reserved for the *in vitro* assays and the *in vivo* study. The EtOAC fr. was subjected to further isolation procedures.

Isolation and identification of compounds

A 3-gram aliquot of the EtOAC fr. was separated using a vacuum liquid chromatography column (VLC) (7 cm high x 5 cm diameter, 190 g silica gel H). The separation was done using gradient elution, starting with 100% DCM and increasing by 10% increments of EtOAc until 100% EtOAc was reached, followed by 2% increments of MeOH until 20% MeOH was reached. The process was monitored by TLC and the fractions were combined into four main fractions. Fraction 2 (420 mg, 60% EtOAC/DCM - 100% EtOAC) was further separated using Sephadex LH 20 with a DCM: MeOH gradient elution to obtain compounds E_1 and E_2 (10 and 9 mg, respectively).

Fraction 4 (510 mg, with 10-20% MeOH/EtOAC) was further chromatographed on a Sephadex LH-20 column employing MeOH as the eluent to give compound E_3 (7 mg). A BRUKER Ascend TM (400 MHz) spectrometer was employed to run ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of the isolated compounds in DMSO-d_6 or CD_3OD for their identification, with tetramethylsilane (TMS) as the internal standard. Chemical shifts were recorded in parts per million (ppm), relative to TMS, and coupling constants (J) expressed in Hz. The NMR data were processed using ACD/NMR processor (www.acdlabs.com/nmrproc/).

Estimation of total phenolic and flavonoid contents

A UV-visible spectrophotometer (Shimadzu 1601 PC, model TCC240, Japan) was used to assess the total phenolic and flavonoid contents. The total phenolic content of the TEE of caraway waste was assessed using the Folin–Ciocalteu method, expressed as gallic acid equivalent, and the absorbance was recorded at 750 nm.[25] The total flavonoid content of TEE was determined and expressed as quercetin equivalent, with the absorbance recorded at 420 nm.[26]

HPLC standardisation

The TEE (10 mg) and its EtOAC fr. (10 mg) were separately dissolved in methanol at a final concentration of 1mg/mL for each. Stock solutions of compounds E_1-E_3 were prepared separately in methanol at final concentrations of 265 µg/mL, each. Solutions were filtered through 0.45 µm syringe membrane filters before HPLC analysis. A Dionex UltiMate 3000RS HPLC system (Thermo ScientificTM, DionexTM, Sunnyvale, CA, USA) equipped with an LPG-3400RS quaternary pump, a TCC-3000RS column thermostat, a WPS-3000RS autosampler and a DAD-3000RS Diode Array Detector was used for all chromatographic runs.

Chromeleon 7 was the software used for data collection and processing. An Inertsil reversed phase C18 column (5 µm particle size, 150 × 4.6 mm i.d.) was utilized throughout this study. The mobile phase was composed of acetonitrile (solvent A) and 0.3% aqueous *ortho*-phosphoric acid (solvent B). Gradient elution was as follows: 0 – 5 mins (10% A in B), 5 – 24 mins (10-15% A in B), 24 – 40 mins (15-18% A in B), 40-55 mins (18-25% A in B), 55-65 mins (25-30% A in B), at 1.0 mL/min flow rate, 20 µL injection volume and the wavelength of detection was set at 325 nm. Calibration curves, based on seven concentration levels in the range of 4-265 µg/mL, indicated good linearity of compounds E_1-E_3 (correlation coefficients > 0.999). Linear regression equations: $y = 1.4558x - 2.0101$, R^2=0.9995 for E_1, $y = 0.9741x + 1.0644$, R^2=0.9994 for E_2, $y = 0.9783x - 0.8531$, R^2=0.9991 for E_3.

In vitro study

In vitro pancreatic lipase (PL) inhibition assay

The pancreatic lipase (PL) activity was measured using a spectrophotometric technique to detect the release of *p*-nitrophenol, with some modifications to the previously reported procedure.[27] Porcine PL (30-90 units/mg protein) was dissolved in Tris-HCl buffer (5 mg/mL). A 0.1 mL PL solution was pre-incubated with different concentrations of each tested treatment (7.81 – 1000 µg/mL) for 5 minutes at 37°C. Thereafter, 10 mM of *p*-nitrophenyl-butyrate (PNPB) substrate was added and a Tris–HCl buffer was used to complete the volume to 1 mL. The absorbance was recorded at 410 nm employing a UV-visible spectrophotometer. Orlistat (at concentrations of 15.6 to

1000 µg/mL) was employed as the positive standard and the reaction was carried out in the same manner. Each sample was analysed three times separately and the inhibitory activities of the tested treatments were presented as IC_{50} values (µg/mL).

In vitro α-amylase inhibition assay

This assay was conducted employing the chromogenic 3,5-dinitrosalicylic acid (DNSA) method [28] with some modifications. The treatment being tested was solubilised in 0.02 M phosphate buffer at pH 6.9 to achieve concentrations ranging from 1.95 to 1000 µg/mL. A 200 µL *α*-amylase solution (2 units/mL) and 200 µL of each treatment were blended and then incubated for 10 minutes at 37 ℃. Subsequently, 200 µL of 1% starch solution was added and incubated for 3 minutes. To halt the reaction, 200 µL of DNSA reagent was added and the mixture was boiled in a water bath for 10 minutes. After cooling the mixture to room temperature, it was diluted with 5 mL of distilled water, and the absorbance at 540 nm was recorded using a UV-visible spectrophotometer (Shimadzu 1601 PC, model TCC240, Japan). Acarbose at various concentrations (1.95 – 1000 µg/mL) was used as the positive standard, and the reaction was performed as described above. Each sample was analysed three times separately, and the inhibitory activity of the tested treatments was presented as IC_{50} values (µg/mL).

DPPH free radical scavenging capacity assay

The antioxidant activity of tested treatments was estimated via spectrophotometric estimation of their DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity, employing a UV-visible spectrophotometer.[29] Various concentrations of treatments (6.25-200 µg/mL) were added to 100 µM DPPH solution, incubated in the dark for 30 mins at room temperature and the absorbance was determined at 515 nm. As a positive control, ascorbic acid (1.5-12 µg/mL) was used. Each sample was analysed three times. DPPH radical scavenging activity was presented as IC_{50} values (µg/mL).

Statistical analysis

All values were presented as Mean ± SEM. Group comparisons were performed using ANOVA (one-way analysis of variance), followed by post-hoc Tukey's test to analyse the data. Differences were deemed significant at $p < 0.0001$ in the *in vitro* study.

In vivo biological study

Animals

Female Swiss albino mice (20-25 g, 8 weeks old), purchased from the animal house at the Faculty of Pharmacy, Mansoura University, were acclimatised for one week under standard conditions of temperature (22 ± 2°C) and humidity (55 ± 5%) with a 12-hour light/dark cycle, and free access to water and food. All the experiments were conducted following the laboratory animal care and use guide and research ethics committee instructions, Faculty of Pharmacy, Cairo University, serial no MP (2700).

Experimental design

Mice were assigned randomly into thirteen groups (8 mice/group) as follows: (i) Normal diet group (ND): mice were fed a normal diet for 12 weeks, (ii) High-fat diet (HFD) control group: mice were fed a high-fat diet for 12 weeks, (iii) HFD fed mice treated with orlistat (40 mg/kg), (iv-xiii) HFD fed mice treated with TEE, Pet-Eth fr., DCM fr., EtOAc fr. and Aq fr. at two doses (300 and 500 mg/kg). The dose of orlistat was similar to that in a previous report.[30] Doses of caraway were determined according to previous reports.[31,32] All pharmacological treatments started on day one of the fifth week and continued for 8 weeks (once a day) until the end of the 12th week with the HFD.

The normal group and the HFD control group received 0.5% CMC as a vehicle at the beginning of the fifth week and proceeded until the experiment ended. All vehicles and drugs were administered via oral gavage. The composition of the HFD included cheap fats, corn starch, molasses, wheat flour and soybean oil. Mice were fasted overnight at the end of the 12th week. They were then euthanised under anaesthesia using a ketamine/xylazine cocktail (0.1 mL/20 g mouse weight, i.p.) for blood collection via retro-orbital puncture technique. The collected blood was then centrifuged at 4000 rpm, and the serum was refrigerated at -80°C for forthcoming biochemica1 examination. Animals were then given mild anaesthesia and sacrificed by cervical dislocation. Tissues from the liver and white adipose were dissected into pieces and preserved in 10% formalin for histological analysis.

Measurement of body weight

Throughout the experimental period, body weight measurements were taken weekly under non-fasting conditions using an electric balance (Generic, Hy-301, China).

Biochemical analysis

The levels of triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) in the serum were determined using assay kits from Spinreact. LDL-c (low-density lipoprotein cholesterol) and VLDL-c (very low-density lipoprotein cholesterol) were calculated using Friedewald's formula: $LDL = TC - HDL - TG/5$ and $VLDL = TG/5$.[33] The total lipids were determined based on a previous report.[34] Leptin hormone was measured using assay kits from ThermoFisher # KMC2281. The Atherogenic Index of Plasma (AIP) and Coronary Risk Index (CRI) were calculated as follows: $AIP = TC - HDL - c/HDL - c$, $CRI = TC/HDL - c$.[35, 36]

Tissue sample processing and histopathology

Liver and adipose connective tissue sections were preserved in 10% neutral-buffered formalin for 24 hours. Afterwards, the slides were dehydrated in gradient ethanol, cleared in xylene, sectioned at a thickness of 4 µm, inserted in paraffin blocks, and stained with haematoxylin and eosin (H&E).

Statistical analysis

All values were displayed as Mean ± SEM. Statistical analyses were performed using GraphPad Prism V-7. Comparison between groups was performed using ANOVA (one-way analysis of variance), followed by post-hoc Tukey's test to analyse the data. Differences were deemed significant at $p < 0.05$ in the *in vivo* study.

In silico molecular docking

Ligand and protein preparation

The 3D structures of umbelliferone, *p*-coumaric acid, and isoquercetin were downloaded in SDF format from PubChem. Additionally, the 3D structures of alpha-amylase (1ua7) and pancreatic lipase (1LPB) were extracted from the Protein data bank (https://www.rcsb.org/) and were prepared using the quick prep module in MOE, which involved removing water molecules, assigning bond orders, adding hydrogens, optimising hydrogen bonds, correcting charges and minimising the protein complex.

Molecular docking

Molecular docking studies were conducted utilizing iGEMDOCK version 2.1 (2011).[37-39] The target protein's active site was first identified using the software's binding site preparation module, which defined the binding pocket as a sphere with an 8Å radius centred around the co-crystallised ligand. The compounds were then docked using the standard docking mode, which employs a genetic algorithm-based scoring function to estimate binding energies. The docking parameters included a population size of 300, a maximum of 80 generations, and the generation of two poses per compound. The resulting poses were ranked based on their binding scores, and the top poses were manually inspected and visualised to analyse the 2D and 3D interactions between the ligands and the target protein.

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Results and Discussion

Total phenolic and flavonoid contents of caraway waste

Flavonoids and other phenolic compounds are natural plant constituents that play a significant role in managing obesity, along with other important biological activities.[11] The contents of total phenolics and flavonoids of the TEE of caraway waste were 93.9 ± 0.3 µg gallic acid equivalent and 29.87 ± 0.45 µg quercetin equivalent per milligram of extract, respectively (equivalent to 4.7 mg GAE and 1.49 mg QE per gram of dry weight, respectively). Previous studies have reported phenolic contents ranging from 0.22 to 3.9 mg GAE per gram of dry weight in caraway fruits,[40, 41] indicating the potential economic value of the waste studied here.

In vitro pancreatic lipase (PL) inhibitory activities

Dietary fats and carbohydrates are known to be major sources of excess calories.[42] As a result, inhibitors of dietary fat and/or carbohydrate digestion and absorption are being considered as potential treatment approaches for managing obesity by decreasing energy intake through gastrointestinal mechanisms.[43] Pancreatic lipase (PL) is a crucial enzyme implicated in the absorption and digestion of triglycerides, responsible for hydrolysing 50-70% of total dietary fats.[42, 43] Many plants have shown potential in combating obesity by inhibiting PL.[11] Therefore, the PL inhibitory potential of TEE and its fractions (Pet-Eth fr., DCM fr., EtOAc fr. and Aq fr.) were monitored *in vitro*, with the EtOAC fr. showing the most promising activity (IC_{50} 68.46 ± 1.40 µg/mL), demonstrating significantly higher inhibitory activities than the TEE and other tested fractions ($p < 0.0001$) (Table 1). This effect was comparable to the standard drug orlistat (IC_{50} 44.8 ± 0.73 µg/mL).

In vitro α-amylase inhibitory activities

α-Amylase is an enzyme that breaks down carbohydrates and plays a crucial role in digesting dietary carbohydrates such as starch. Inhibiting this enzyme is important for preventing and treating obesity and diabetes.[44] TEE and its fractions (Pet-Eth fr., DCM fr., EtOAc fr. and Aq fr.) were *in vitro* tested for their *α*-amylase inhibitory potential (Table 1). The EtOAc fr. showed significantly potent *α*-amylase inhibitory activity (IC_{50} 37.3 ± 2.61 µg/mL), better than the TEE and other tested fractions ($p < 0.0001$). This effect was comparable to acarbose (IC_{50} 15.28 ± 0.82 µg/mL). In a previous study on caraway fruits' ethanolic extract and its fractions, the ethyl acetate fraction exhibited potent inhibitory activity against *α*-amylase (421.4 ± 7.8 µg/mL), but much less compared to the results found in our investigation.[45]

In vitro antioxidant activities

Oxidative damage provoked by free radicals is one of the aetiologic factors correlated with many chronic illnesses, like obesity, cancer, diabetes, neural disorders and atherogenic processes.[46,47] Among various perspectives to manage obesity, researchers have proven the effectiveness of antioxidant supplementation in reducing obesity and its related disorders.[46,48] So, the antioxidant activity of the TEE of caraway waste and its fractions was screened *in vitro* using the DPPH method (Table 1). The ethyl acetate fraction exhibited the highest radical scavenging activity (IC_{50} 16.00 ± 1.81 µg/mL), compared to ascorbic acid (IC_{50} 6.00 ± 2.19 µg/mL) ($p < 0.0001$). The DPPH radical scavenging capacity of caraway fruits' ethanolic extract and its various fractions was previously investigated (IC_{50} 53.05 to 211.5 µg/mL),[45] revealing a higher antioxidant capacity of the discarded waste under investigation compared to the commonly utilized fruits.

In vivo anti-obesity activity of total ethanolic extract (TEE) of caraway waste and its fractions

The promising inhibitory activities of caraway waste, particularly its ethyl acetate fraction, against PL and *α*-amylase enzymes, along with its antioxidant potential using DPPH assay, prompted further investigation into the anti-obesity effect of this waste and its fractions in a high-fat diet-induced obesity model in mice.

While there are many targets for anti-obesity agents, inducing weight loss is the primary effect that could qualify products as anti-obesity preparations.[49] Therefore, body weight measurement is a key parameter for assessing obesity in murine models. The body weight of all groups was monitored weekly throughout the experimental time frame. By the end of the 12th week, the high-fat diet (HFD) group revealed a significant ($p < 0.05$) increase in body weight (33.2%), compared to the normal diet (ND) group, indicating successful induction of obesity in mice (Table 2).

After 8 weeks of treatment, administration of orlistat and ethyl acetate fraction (300 and 500 mg/kg) significantly decreased gaining weight compared to the HFD group by 52%, 35.8% and 35.3%, respectively, showing no significant difference ($p < 0.05$) from the normal diet group (Table 2). Other tested fractions showed no significant suppression of body weight when compared to the HFD group, indicating that the total ethanolic extract's activity is probably localised in its ethyl acetate fraction.

Table 1: DPPH radical scavenging activities, pancreatic lipase and *α*-amylase inhibitory activities of the total ethanolic extract (TEE) of caraway waste and its fractions

Tested samples	IC_{50} (µg/mL)		
	Pancreatic lipase inhibitory activity	***α*-Amylase inhibitory activity**	**DPPH scavenging activity**
TEE	668.10 ± 4.17^{f}	53.37 ± 2.29^{c}	20.20 ± 1.044^{b}
Pet-Eth fr.	155.80 ± 4.50^{d}	105.70 ± 4.19^{e}	100.00 ± 1.96^{c}
DCM fr.	198.60 ± 3.24^{e}	82.72 ± 1.38^{d}	39.60 ± 3.2^{b}
EtOAC fr.	68.46 ± 1.40^{b}	37.30 ± 2.61^{b}	16.00 ± 1.81^{b}
Aq fr.	102.00 ± 3.55^{c}	79.06 ± 1.82^{d}	23.90 ± 2.33^{b}
Orlistat	44.87 ± 0.73^{a}	-	-
Acarbose	-	15.28 ± 0.82^{a}	-
Ascorbic acid	-	-	6.00 ± 2.19^{a}

Values are presented as Mean± SEM. Groups having different superscript letters (a, b, c, d, e, f) within a column are significantly different from each other (One way analysis of variance (ANOVA) followed by post-hoc Tukey's test, $p < 0.0001$).

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Table 2: Effect of the total ethanolic extract (TEE) of caraway waste and its fractions on final body weight and body weight gain in high-fat diet induced obese mice

Groups	Final body weight (g)	Body weight gain (g/ 8 weeks)[a]
Normal diet (ND)	34.01 ± 0.78	6.4 ± 0.217
High-fat diet (HFD)	45.3 ± 0.42[#]	17.3 ± 0.872[#]
HFD + Orlistat	37.14 ± 0.47[*]	8.3 ± 0.382[*]
HFD + TEE (300 mg/kg)	41.64 ± 0.44[#]	11.5 ± 0.427[#]
HFD + TEE (500 mg/kg)	41.11 ± 0.33[#]	12.1 ± 0.709[#]
HFD + Pet-Eth fr. (300 mg/kg)	43.08 ± 0.90[#]	13.0 ± 1.02[#]
HFD +Pet-Eth fr. (500 mg/kg)	43.75 ± 0.72[#]	15.2 ± 0.836[#]
HFD +DCM fr. (300 mg/kg)	43.45 ± 0.89[#]	14.4 ± 0.637[#]
HFD +DCM fr. (500 mg/kg)	43.75 ± 0.75[#]	15.7 ± 0.172[#]
HFD +EtOAC fr. (300 mg/kg)	39.18 ± 0.36[*]	11.1 ± 0.290[*]
HFD +EtOAC fr. (500 mg/kg)	39.28 ± 0.30[*]	11.2 ± 0.652[*]
HFD + Aq fr. (300 mg/kg)	42.94 ± 1.14[#]	14.3 ± 0.372[#]
HFD + Aq fr. (500 mg/kg)	41.59 ± 0.69[#]	13.9 ± 0.710[#]

Values are presented as Mean± SEM. Comparison between groups was carried out using one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test. [#] Significant difference from normal diet group at $P < 0.05$, * indicates significant difference from HFD group at $p < 0.05$, [a] Body weight gain after 8 weeks of treatment.

A high-fat diet has been demonstrated to increase the size of fat cells through fat accumulation, leading to liver damage.[50] In this study, histological analysis of adipose tissue sections in the HFD group revealed enlarged fat cells with macrophage infiltration, compared to the ND group (Figure 1). However, treatment with ethyl acetate fraction (EtOAC fr.) at both tested doses significantly reduced the size of fat cells without macrophage infiltration, comparable to the effect of orlistat. Additionally, liver sections of the HFD group showed steatosis characterised by numerous lipid droplets (Figure 2). Treatment with the EtOAC fr. and TEE at both tested doses attenuated the extent of steatosis and preserved the normal structure of the liver, particularly the 500 mg/kg dose of the EtOAC fr. (Figure 2). Other treatment groups showed distorted liver architecture with obvious lipid droplets (Figure 2). This suggests that caraway waste, particularly its ethyl acetate fraction, has the potential to prevent weight gain, increase in adipocyte size, and fat deposition in the liver, consequently offering protection against obesity-induced liver injury.

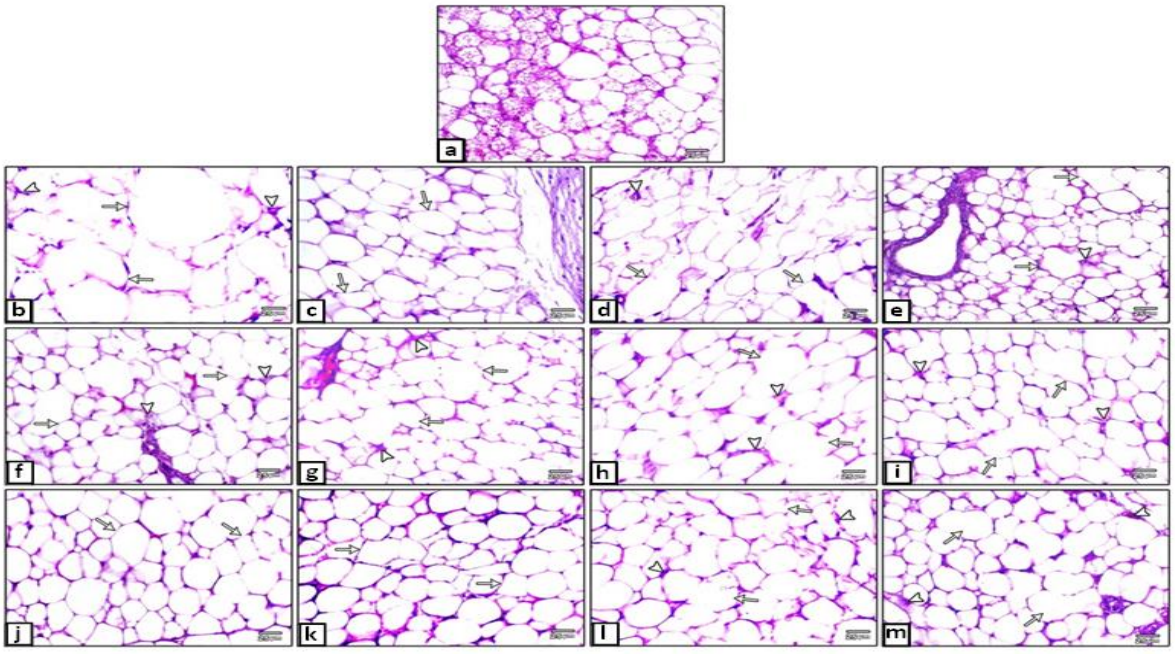

Figure 1: Histological sections of adipose tissues (H&E X 400). (a) ND group, (b) HFD group, (c) Orlistat group, (d) TEE 300 mg/kg group, (e)TEE 500 mg/kg group, (f) Pet-Eth fr. 300 mg/kg group, (g) Pet-Eth fr. 500 mg/kg group, (h) DCM fr. 300 mg/kg group, (i) DCM fr. 500 mg/kg group, (j) EtOAc fr. 300 mg/kg group, (k) EtOAc fr. 500 mg/kg group, (l) Aq fr. 300 mg/kg group, (m) Aq fr. 500 mg/kg group. Arrow indicates adipocytes and arrowhead indicates macrophage infiltration.

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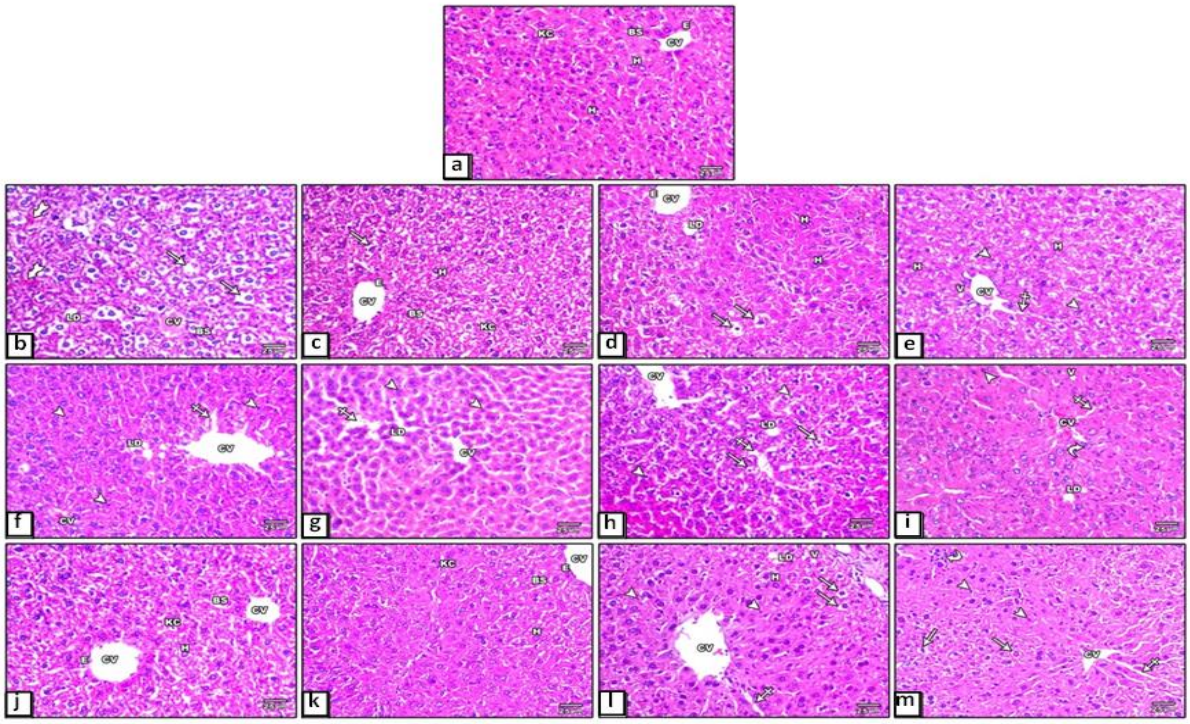

Figure 2: Histological sections of liver tissues (H&E X 400). (a) ND group, (b) HFD group, (c) orlistat group, (d) TEE 300 mg/kg group, (e) TEE 500 mg/kg group, (f) Pet-Eth fr. 300 mg/kg group, (g) Pet-Eth fr. 500 mg/kg group, (h) DCM fr. 300 mg/kg group, (i) DCM fr. 500 mg/kg group, (j) EtOAc fr. 300 mg/kg group, (k) EtOAc fr. 500 mg/kg group, (l) Aq fr. 300 mg/kg group, (m) Aq fr. 500 mg/kg group. (CV) central vein, (E) endothelial cells, (H) Hepatocytes, (BS) blood sinusoids, (KC) Kupffer cells, (tailed arrows) haemorrhage areas, (arrows) vacuolated hepatocytes, (LD) lipid droplets, (arrow heads) pyknotic cells, (crossed arrows) dilated blood sinusoid, (V) vacuoles, (curved arrows) necrotic area.

Leptin is an adipocyte-derived hormone, which is essential for maintaining energy balance and body weight in rodents and humans. Its secretion is positively correlated to fat mass and adipocyte size.[51] Leptin resistance has been observed in obesity, leading to hyperleptinaemia.[51] A rise in circulating leptin has been documented in high-fat diet-fed mice.[52] In this study, a significant increase (2.7-fold, $p < 0.05$) in the serum levels of leptin was noticed in the HFD group as opposed to the normal diet group (Figure 3). However, treatment with the TEE, EtOAc fr., and Aq fr. at both tested doses, as well as orlistat, significantly ($p < 0.05$) suppressed the high-fat diet-induced rise in serum leptin levels. The best results were achieved by orlistat and the ethyl acetate fraction (at 300 and 500 mg/kg), reducing serum leptin levels by 53.3%, 37.1% and 40.7%, respectively. This finding aligns with the drop in leptin levels in response to weight loss.[53]

Obesity or HFD is linked to dyslipidaemia, which is indicated by high levels of triacylglycerol (TG), total cholesterol (TC) and LDL-c, together with low HDL-c levels, thus increasing the risk of cardiovascular diseases.[54] In line with this, HFD-fed mice revealed a significant rise in TG, TC, VLDL-c, LDL-c and total lipids, and a decline in HDL-c (Table 3). However, only orlistat, EtOAC fr., TEE and Aq fr. significantly ameliorated the lipid profile in HFD-fed mice at $p < 0.05$ (Table 3). It is crucial to note that the serum concentrations of TG, TC, VLDL-c, total lipids and HDL-c were significantly improved in HFD-fed mice when administered with orlistat (by 38.8, 39.9, 39, 38.8, and 62.3%, respectively) and EtOAC fr. (at 300 and 500 mg/kg, by 32.2-32.6, 32.2-35.3, 32.2-32.6, 31.7-33.2, and 56.2-58.6%, respectively), showing significant normalisation and remarkable hypolipidemic effects ($p < 0.05$). Additionally, TEE and Aq fr., only at 500 mg/kg, normalised the serum concentrations of TG and VLDL-c at $p < 0.05$.

Coronary risk (CRI) and atherogenic (AI) indices are reliable indicators for forecasting the likelihood of atherosclerosis and heart disease. In particular, AI is a novel marker for obesity prediction and can be used as an optimal indicator of dyslipidaemia and correlated cardiovascular diseases.[55] High AI levels are strongly associated with obesity.[55] In the current study, disruptions in lipid dynamics exposed HFD-fed mice to the risks of cardiovascular progression, as revealed by the significant rise in AI and CRI noticed in the HFD group compared to the normal control group at $p < 0.05$ (Table 3). TEE, EtOAC fr. and Aq fr., at both tested doses, significantly minimised the high-risk values in AI and CRI compared to the HFD group ($p < 0.05$) (Table 3). The EtOAC fr. and TEE demonstrated significantly normal values of CRI (2.22 ± 0.19, 2.09 ± 0.10 at 300 and 500 mg EtOAC fr./kg, respectively, and 2.65 ± 0.15, 2.62 ± 0.06 at 300 and 500 mg TEE/kg, respectively) and AI (1.22 ± 0.19, 1.09 ± 0.10 at 300 and 500 mg EtOAC fr./kg, respectively, and 1.65 ± 0.15, 1.62 ± 0.06 at 300 and 500 mg TEE/kg, respectively), compared to orlistat (1.9 ± 0.09 and 0.93 ± 0.09, respectively) (Table 3). Thus, caraway waste, particularly its ethyl acetate fraction, revealed a significant hypolipidemic effect with cardioprotective potential, which can be considered one of its mechanistic anti-obesity effects.

Phytochemical analysis of the active EtOAC fraction

Following a phytochemical analysis of the bioactive EtOAC fr., three phenolic compounds, designated as E_1-E_3, were isolated. The identities of these compounds were deduced as umbelliferone (E_1), *p*-coumaric acid (E_2) and isoquercitrin (E_3) (Figure 4), based on their spectral data (^{1}H-NMR and ^{13}C-NMR) and their contrast with published data.[56-59]

Spectral data of isolated compounds:

Compound E_1: ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.94 (d, J = 9.4 Hz, 1H, H-4), 7.53 (d, J = 8.4 Hz, 1H, H-5), 6.79 (br d, J = 8.4 Hz, 1H, H-6), 6.72 (br s, 1H, H-8), 6.21 (d, J = 9.4 Hz, 1H, H-3). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 161.83 (C-2), 160.93 (C-7), 155.98 (C-9), 145.01 (C-4), 130.19 (C-5), 113.61 (C-6), 111.86 (C-10), 111.74 (C-3), 102.64 (C-8).

Compound E_2: ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.84 (s, OH), 7.53 (d, J = 15.9 Hz, 1H, H-7), 7.49 (d, J = 8.0 Hz, 2H, H-2, H-6), 6.80 (d, J = 8.0 Hz, 2H, H-3, H-5), 6.30 (d, J = 15.9 Hz, 1H, H-8). ^{13}C NMR (100

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MHz, DMSO-d_6) δ: 160.06 (C-4), 144.62 (C-7), 130.56 (C-2, C-6), 125.74 (C-1), 116.21 (C-3, C-5), 115.83 (C-8), 68.46 (C-9).
Compound E_3: ^{1}H-NMR (400 MHz, CD_3OD) δ 7.70 (br s, 1H, H-2′), 7.58 (br d, *J* = 8.4 Hz, 1H, H-6′), 6.86 (d, *J* = 8.5 Hz, 1H, H-5′), 6.38 (br s, H, H-8), 6.19 (br s, 1H, H-6), 5.26 (d, *J* = 7.4,1H, H-1′′). ^{13}C NMR (100 MHz, CD_3OD) δ: 178.10 (C-4), 164.64 (C-7), 161.67 (C-5), 157.59 (C-2), 157.07 (C-9), 148.45 (C-4′), 144.52 (C-3′), 134.20 (C-3), 121.78 (C-1′), 116.13 (C-5′), 114.59 (C-2′), 104.28 (C-10), 102.85 (C-1′′), 98.47 (C-6), 93.29 (C-8), 77.00 (C-5′′), 76.71 (C-3′′), 74.32 (C-2′′), 69.80 (C-4′′), 61.13(C-6′′).

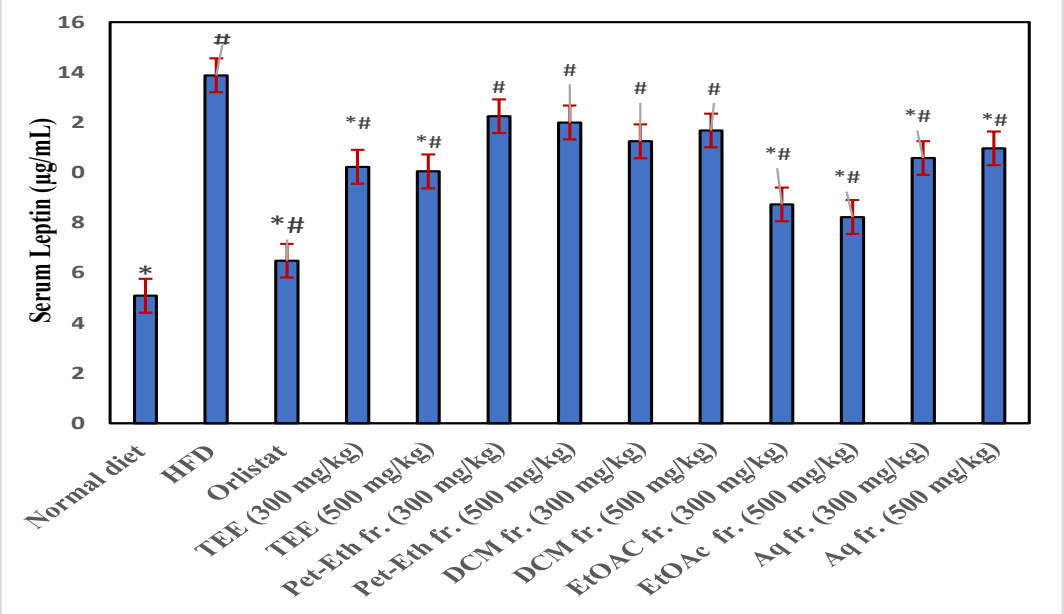
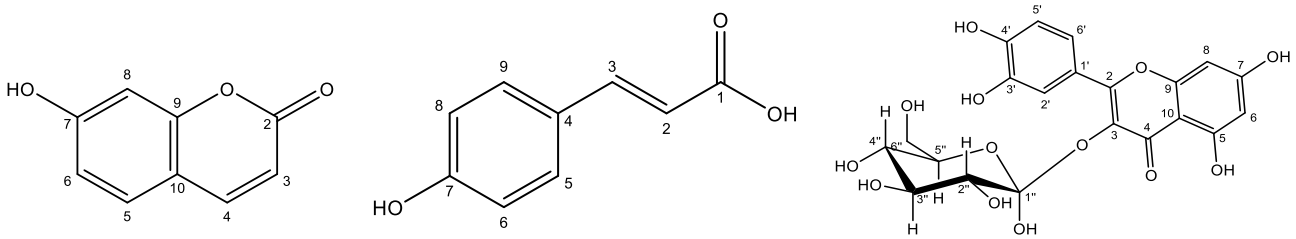


Figure 3: Effect of the total ethanolic extract of caraway waste (TEE) and its fractions on serum leptin hormone in high-fat diet-induced obese mice (HFD). Results expressed as Mean ± SEM. Comparison between groups was carried out using one-way analysis of variance (ANOVA), followed by the post-hoc Tuckey test. *Significant difference from HFD group at $p < 0.05$, #Significant difference from normal diet group at $p < 0.05$.



E_1 (Umbelliferonee) E_2 (*p*-coumaric acid) E_3 (*isoquercetrin*)

Figure 4: Chemical structures of isolated compounds.

Table 3: Effect of the total ethanolic extract (TEE) of caraway waste and its fractions on lipid profile, coronary risk index (CRI) and atherogenic index (AI) in high-fat diet induced obese mice.

Groups	Serum TG (mg/dl)	Serum TC (mg/dl)	Serum HDL-c (mg/dl)	Serum LDL-c (mg/dl)	Serum VLDL-c (mg/dl)	Total lipids (mg/dl)	CRI	AI
Normal diet (ND)	53.00 ± 3.25*	93.58 ± 3.36*	67.00 ± 2.31*	20.85 ± 1.86*	10.60 ± 0.65*	193.3 ± 8.37*	1.42 ± 0.05*	0.42 ± 0.05*
High-fat diet (HFD)	98.33 ± 4.46$^{\#}$	181.6 ± 6.74$^{\#}$	35.72 ± 1.98$^{\#}$	125.7 ± 6.01$^{\#}$	19.67 ± 0.89$^{\#}$	360.8 ± 10.54$^{\#}$	4.84 ± 0.22$^{\#}$	3.83 ± 0.23$^{\#}$
HFD + Orlistat	59.93 ± 2.87*	109.1 ± 2.82*	57.96 ± 2.81*	40.51 ± 2.70*$^{\#}$	11.99 ± 0.57*	220.7 ± 6.48*	1.90 ±0.09*	0.93 ± 0.09*
HFD + TEE (300 mg/kg)	75.83 ± 3.52*$^{\#}$	126.6 ± 8.19*$^{\#}$	46.91 ± 2.11$^{\#*}$	80.44 ± 4.34*$^{\#}$	15.17 ± 0.70*$^{\#}$	261.1 ± 14.30*$^{\#}$	2.65 ± 0.15*	1.65 ± 0.15*
HFD + TEE (500 mg/kg)	72.47 ± 2.36*	123.7 ± 3.51*	48.22 ± 1.31$^{\#*}$	64.45 ± 2.44*$^{\#}$	14.49 ± 0.47*	258.9 ± 6.67*$^{\#}$	2.62 ± 0.06*	1.62 ± 0.06*
HFD + Pet-Eth fr.	82.33 ± 5.02$^{\#}$	158.5 ± 8.59$^{\#}$	40.07 ± 2.75$^{\#}$	100.0 ± 7.86$^{\#}$	16.47 ± 1.01$^{\#}$	265.15 ± 7.25$^{\#}$	3.93 ± 0.26$^{\#}$	2.93 ± 0.26$^{\#}$

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(300 mg/kg)								
HFD + Pet-Eth fr. (500 mg/kg)	80.67 ± 4.81$^{\#}$	162.1 ± 6.71$^{\#}$	41.43 ± 2.46$^{\#}$	102.7 ± 5.93$^{\#}$	15.70 ± 1.23$^{\#}$	258.34 ± 8.55$^{\#}$	3.91 ± 0.28$^{\#}$	2.91 ± 0.28$^{\#}$
HFD + DCM fr. (300 mg/kg)	86.75 ± 4.43$^{\#}$	157.4 ± 5.59$^{\#}$	43.17 ± 2.27$^{\#}$	100.7 ± 5.61$^{\#}$	17.35 ± 0.89$^{\#}$	152.34 ± 6.52$^{\#}$	3.7 ± 0.16$^{\#}$	2.70 ± 0.16$^{\#}$
HFD +DCM fr. (500 mg/kg)	83.67 ± 6.15$^{\#}$	156.1 ± 6.26$^{\#}$	43.24 ± 2.31$^{\#}$	102.4 ± 3.44$^{\#}$	16.73 ± 1.23$^{\#}$	148.62 ± 9.26$^{\#}$	3.61 ± 0.25$^{\#}$	2.61 ± 0.25$^{\#}$
HFD + EtOAC fr. (300 mg/kg)	66.67 ± 3.20*	123.2 ± 5.98*	55.79 ± 1.65*	63.54 ± 5.30*$^{\#}$	13.33 ± 0.64*	246.3 ± 11.68*	2.22 ± 0.19*	1.22 ± 0.19*
HFD + EtOAC fr. (500 mg/kg)	66.30 ± 2.17*	117.5 ± 5.17*	56.64 ± 1.89*	54.06 ± 3.61*$^{\#}$	13.26 ± 0.43*	240.9 ± 7.33*	2.09 ±0.10*	1.09 ± 0.10*
HFD + Aq fr. (300 mg/kg)	74.53 ± 3.50*$^{\#}$	139.3 ± 11.08*$^{\#}$	47.14 ± 1.43$^{\#*}$	83.71 ± 6.83*$^{\#}$	14.91 ± 0.70*$^{\#}$	284.1 ± 16.72*$^{\#}$	3.05 ± 0.27*$^{\#}$	2.05 ± 0.27*$^{\#}$
HFD + Aq fr. (500 mg/kg)	71.00 ± 3.78*	134.6 ± 3.66*$^{\#}$	49.70 ± 2.65*$^{\#}$	73.48 ± 5.08*$^{\#}$	14.2 ± 0.76*	270.4 ± 5.70*$^{\#}$	2.79 ± 0.19*$^{\#}$	1.79 ± 0.19*$^{\#}$

Values are presented as Mean± SEM. Comparison between groups was carried out using one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test. *Significant difference from HFD group at $P< 0.05$, #Significant difference from normal diet group at $p < 0.05$. Tc, total cholesterol; TG, triacylglycerol.

Table 4: Radical scavenging activities, pancreatic lipase and *α*-amylase inhibitory activities and docking scores of isolated compounds of caraway waste.

		Pancreatic lipase		***α*-Amylase**	
Tested samples	**DPPH Scavenging Activity (IC_{50}, µg/mL)**	**Inhibitory Activity (IC_{50}, µg/mL)**	**Docking Score (Kcal/mol)**	**Inhibitory Activity (IC_{50}, µg/mL)**	**Docking Score (Kcal/mol)**
Umbelliferone	46.20 ± 0.95^{c}	516.2 ± 5.68^{d}	-25.98	50.70 ± 2.83^{b}	-65.99
p-Coumaric acid	27.47 ± 1.96^{b}	416.2 ± 3.69^{c}	-69.06	71.59 ± 1.46^{c}	-61.83
Isoquercitrin	10.00 ± 2.50^{a}	93.54 ± 2.62^{b}	-130.87	95.98 ± 1.50^{d}	-40.9
Orlistat	-	44.87 ± 0.74^{a}	-128	-	-
Acarbose	-	-	-	15.28 ± 0.82^{a}	-140. 61
Ascorbic acid	6.00 ± 2.19^{a}	-	-	-	-

Values are presented as Mean± SEM. Groups having different superscript letters (a, b, c, d) within a column are significantly different from each other (One way analysis of variance (ANOVA) followed by post-hoc Tukey's test, $p < 0.0001$).

HPLC standardisation

Three phenolic compounds viz., umbelliferone (E_1), *p*-coumaric acid (E_2) and isoquercitrin (E_3) were identified and quantified in the TEE of caraway waste and its EtOAC fr. for their standardisation (Figure 5). The main compound, umbelliferone (E_1), was found to be 0.261±0.027 and 0.78±0.014 g%, followed by *p*-coumaric acid (E_2) at 0.239±0.009 and 0.715±0.017 g%, and isoquercitrin (E_3) at 0.103±0.023 and 0.28±0.014 g% in the TEE and EtOAC fr., respectively. These compounds were found to be richer in the EtOAc fr. relative to TEE, which could explain the higher efficacy of this fraction.

The isolated compounds, namely umbelliferone, *p*-coumaric acid, and isoquercitrin, obtained through bioactivity-guided fractionation, were then tested *in vitro* for their lipase and *α*-amylase inhibitory activities, together with their DPPH radical scavenging potential. Additionally, molecular docking of the isolated compounds to digestive enzymes was performed.

In vitro pancreatic lipase (PL) activities of isolated compounds

Among the compounds tested, isoquercitrin exhibited the highest pancreatic lipase inhibitory activity (IC_{50} 93.54 ± 2.62 µg/mL), with a significance at $p < 0.0001$, while *p*-coumaric acid and umbelliferone showed weaker activities (IC_{50} 416.2 ± 3.69 and 516.2 ± 5.69 µg/mL, respectively) in contrast to orlistat (IC_{50} 44.87 ± 0.74 µg/mL) (Table 4). Our results align with previous data, showing strong pancreatic lipase inhibitory activity for isoquercitrin and its active metabolite quercetin, while phenolic acids like ferulic and *p*-coumaric acids demonstrated weaker activity among the polyphenolic compounds tested.[60,61] However, another study reported negligible inhibition of isoquercitrin on pancreatic lipase.[62] The weak inhibitory effects of umbelliferone and *p*-coumaric acid against pancreatic lipase in the current study are in line with earlier findings.[60,61,63,64]

In vitro α-amylase inhibitory activities of isolated compounds

Umbelliferone significantly ($p < 0.0001$) displayed the highest inhibition against *α*-amylase, then came *p*-coumaric acid and isoquercitrin. The IC_{50} values for umbelliferone, *p*-coumaric acid and isoquercitrin were 50.7 ± 2.83, 71.59 ± 1.46, and 95.98 ± 1.5 µg/mL, respectively, compared to the positive standard acarbose (IC_{50} of 15.28 ± 0.82 µg/mL). Previous studies have suggested significant *α*-amylase inhibitory activity for umbelliferone,[65,66] albeit one study reported negligible activity.[67] In the current study, *p*-coumaric acid showed moderate inhibition, contrasting with a previous publication reporting lower inhibition potency.[68]

In vitro antioxidant properties of the isolated compounds

In terms of antioxidant properties, isoquercitrin and ascorbic acid significantly demonstrated the greatest capacity to scavenge DPPH radicals, followed by *p*-coumaric acid and umbelliferone, which exhibited moderate activities at $p < 0.0001$. These findings are consistent with previous reports.[69-71]

Molecular docking of isolated compounds

Molecular docking revealed that isoquercitrin strongly interacted with several amino acids in the lipase binding site, such as Asp79, Ile78, Arg256, Ser152 and Tyr114, through hydrogen bonding (Figure 6). These interactions with amino acid residues have been reported previously.[63,72] Isoquercitrin also demonstrated the highest docking score (-130.87 kcal·mol^{-1}), which was higher than that of orlistat (-128 kcal·mol^{-1}) (Table 4). The weak *in vitro* inhibitory activity of umbelliferone noted in this study could be explained in the light of its molecular docking, as it failed to interact with the key amino acids in the lipase binding site due to its small molecular size. However, it did

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form one hydrogen bond with Ala259 (Table 4, Figure 6). Similarly, *p*-coumaric acid formed one hydrogen bond in the lipase binding site with only one amino acid, Phe77 (Figure 6). The weak inhibitory activity of *p*-coumaric acid might be explained by the lack of strong interaction.[64] The docking scores of umbelliferone and *p*-coumaric acid (-25.98 and -69.06 kcal·mol^{-1}, respectively) are consistent with their relatively weak *in vitro* activities.

In the context of *α*-amylase, umbelliferone formed hydrogen bonds with Tyr59 and Leu142 and had an H-Pi interaction with Gln63 in the enzyme's active site (Figure 6). Previous research indicated that Tyr59 and Gln63 are crucial for achieving effective inhibitory action.[73,74] Additionally, a previous computational study demonstrated that the amino acids Arg346, Lys352 and Asp353 are involved in the active interaction of umbelliferone with *α*-amylase.[75] Umbelliferone had the highest docking score (-65.99 kcal·mol^{-1}), although much lower than acarbose (-140.61 kcal·mol^{-1}), which aligns with its *in vitro* activity.

The phenolic acid, *p*-coumaric acid, showed hydrogen bonds with Trp56 and Arg315, and H-Pi interaction with Tyr59, in line with previous findings.[68,76] On the other hand, isoquercitrin formed a hydrogen bond with Ser56 and exhibited weak interactions with Asp274 and Tyr58 through Van der Waals' forces within the enzyme's active site (Figure 6). The lack of interaction with Tyr59 and Gln63 may explain its relatively lower inhibition power. It should be noted that this is the first docking simulation study of isoquercitrin in the active site of lipase. Umbelliferone, *p*-coumaric acid and isoquercitrin collectively contributed to the inhibitory activity of the EtOAC fr. towards *α*-amylase, while isoquercitrin was mainly responsible for its PL inhibitory activity.

The isolated compounds have been previously reported to possess additional mechanisms for combating obesity. Umbelliferone was shown to have an anti-obesity effect via reducing the lipid content of adipocytes and lowering levels of hyperlipidaemia, glucose and body weight in obese rats fed a high-fat diet. Additionally, it demonstrated amylase inhibitory activity. It also improved hypertriglyceridaemia in different animal models (diabetic rats and HFD mice) by regulating the antioxidant defence system, inhibiting hepatic lipid accumulation and increasing adiponectin levels.[77-79]

Furthermore, *p*-coumaric acid has been found to prevent obesity in different murine models via activating brown adipose tissue (BAT)-mediated thermogenesis, alleviating hyperlipidaemia, ameliorating cardiovascular and antiatherogenic indices, reducing adiposity and hepatic steatosis, and lowering leptin levels along with its antioxidant potential.[80-82] In addition, peanut sprout (PS), which is rich in *p*-coumaric acid, has been found to improve obesity in induced obese mice. *p*-Coumaric acid exerts its lipid-lowering effect via promoting oxidation of fatty acids and mitochondrial activities in 3T3-L1 cells.[83]

Previous reports have shown the anti-obesity effects of isoquercitrin in both *in vitro* and *in vivo* systems. Our data, along with previous data, suggest that isoquercitrin attenuates obesity by inhibiting lipogenesis, adipocyte differentiation and pancreatic lipase activity. Additionally, it decreases total body weight and adipocyte size, improves serum lipid levels, mitigates liver damage and attenuates abnormal adipokine release by decreasing serum leptin levels and increasing adiponectin. Additionally, isoquercitrin has antioxidant and anti-inflammatory properties.[84-86]

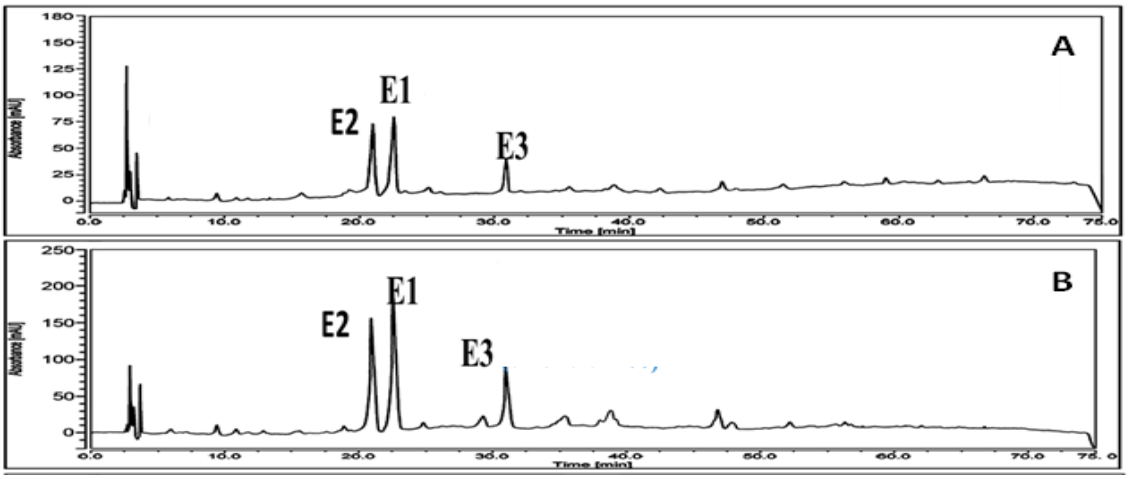

Figure 5: HPLC chromatograms of the total ethanolic extract of caraway waste (A) and its ethyl acetate fraction (B) at λ 325 nm.

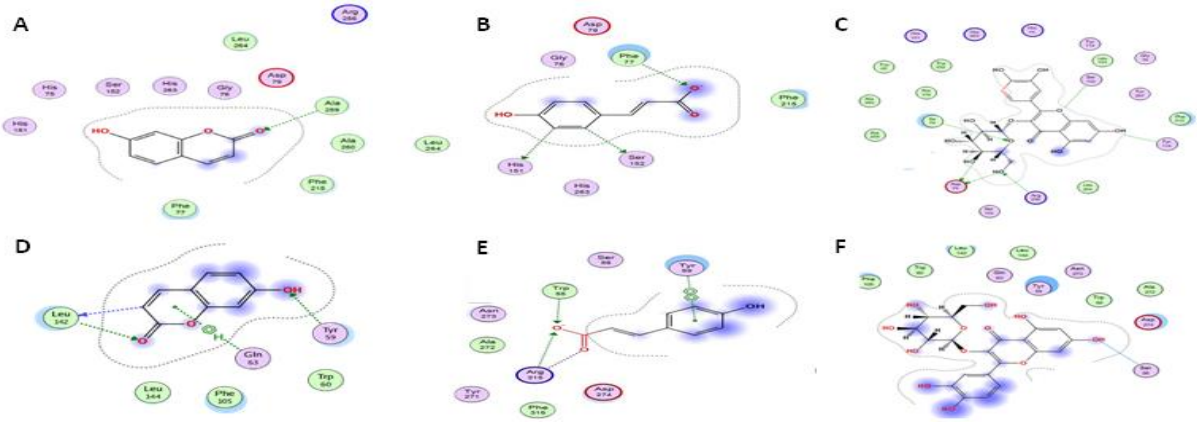

Figure 6: Molecular interactions between isolated compounds and pancreatic lipase and *α*-amylase active sites. Molecular interactions between umbelliferone (A), *p*-coumaric acid (B), isoquercitrin (C) and pancreatic lipase active site. Molecular interaction between umbelliferone (D), *p*-coumaric acid (E), isoquercitrin (F) and *α*-amylase active site. Green dotted arrows indicate hydrogen bonding, green dotted lines indicate arene hydrogen interactions, blue dotted arrows indicate hydrogen-carbon interactions.

Conclusion

In conclusion, caraway waste (TEE) and its EtOAC fr. have shown potential anti-obesity effects, via reducing body weight gain, improving lipid profile, attenuating hepatic steatosis, protecting against liver damage, and inhibiting pancreatic lipase and amylase enzymes, along with their antioxidative potential. The major constituents in the active EtOAC fr. (umbelliferone, *p*-coumaric acid and isoquercitrin) contributed synergistically to its anti-obesity effect through different mechanisms. This is the first study on the anti-obesity efficacy of caraway waste, suggesting its use as a potential multi-target complementary medicine for hyperlipidaemia, oxidative stress and obesity, and it is worth further experimental and clinical investigations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

Identification of the plant was carried out by Dr. Mohamed Elgebaly, taxonomy specialist at El-Orman Garden, Giza.

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