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Research Article

Phytochemical Analysis and Anti-obesity Effects of *Kalanchoe pinnata* Root-Stem Methanol Extract

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Abstract

Background: For a long time, *Kalanchoe pinnata* has been recognized for its therapeutic effects, primarily on metabolic diseases such as obesity. The current study aims to explore the weight reduction potential of the *K. pinnata's* root-stem mixture and characterize its phytochemical profile.

Methods: The bioactive compounds of the root-stem powder were identified using qualitative and quantitative analyses. The Fourier transform infrared (FTIR) and ultra-violet/visible (UV/Vis) spectroscopies were used to determine the functional groups. Volatile compounds were profiled using gas chromatography–mass spectrometry (GC–MS) analysis. *In vitro*, a pancreatic lipase inhibition assay was used to evaluate the anti-obesity potential of the methanol extract. An *in vivo* study was also conducted on high-fat diet-fed mice to investigate the lipid profiles and histopathological changes in the pancreas, liver, and kidney following treatment with the extract.

Results: Phytochemical screening revealed the presence of compounds such as flavonoids, polyphenols, saponins, and glycosaponins, known for improving metabolic disorders. The GC-MS showed the presence of volatile compounds. *K. pinnata* root-stem methanol extract exhibited significant inhibition of pancreatic lipase activity. The improved lipid profiles of the rats, weight reduction, and histopathological examinations revealed reduced inflammation and the restoration of normal pancreatic, liver, and kidney architecture, similar to the effects of Orlistat.

Conclusion: This study confirms *K. pinnata's* anti-obesity effects. Thus, further clinical studies and investigation into the extract's mechanism of action are needed to verify its safety and efficacy.

Keywords: *Kalanchoe pinnata*, obesity, Orlistat, medicinal plant, pancreatic lipase

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1. Introduction

Carrying excess weight or being classified as obese, which occurs due to an abnormal buildup of fat in body tissues, presents health risks. The body mass index (BMI) is a simple matrix to evaluate overall physical fitness. A higher BMI is associated with health problems that reduce one's quality of life (QoL) [1, 2]. Experiencing weight bias can lead to stigma, resulting in social prejudice against individuals with larger bodies. This bias exposes them to misconceptions and stereotypes that undermine their worth and dignity. This focus on appearance-based ideals of body weight is essential to understanding how weight stigma can harm the effectiveness of health promotion campaigns worldwide. In response to this concern, the World Obesity Federation (WOF) brought together a global group of practitioners, researchers, policymakers, youth advocates, and people with first-hand experience of obesity to explore how narratives of global obesity may be reinforcing weight stigma [3].

Many etiologic causes, like genetics, diet, environment, and epigenetics, are associated with obesity. Individuals of all ages, genders, and ethnicities, residing in different regions of the world, can face weight-related issues. This is accompanied by a global rise in morbidity and mortality, especially in cases associated with co-morbidities like diabetes, hypertension, cancer, atherosclerosis, and heart disease [4]. If a person's BMI is between 25 and 29.9, they are considered overweight, and if it is greater than 30, they are considered obese. The leading cause of obesity is a chronic energy imbalance between calories consumed and calories burned. New research indicates that the quantity of nutrients is less critical for weight control and disease prevention than the quality of dietary sources. Genetic factors are also crucial in a person's tendency to weigh more [5, 6]. Obesity can stem from poor nutrition, lack of exercise, a sedentary lifestyle, or as a side effect of certain medications. Due to the risk of obesity-related illnesses and expensive medical treatments, obesity becomes the primary focus for cost-containment efforts. Careful assessment of patient health factors that affect metabolism, energy intake, and expenditure is necessary for physicians to treat obesity effectively. [7].

Obesity develops gradually with lipid accumulation in adipose tissue and volume expansions in organs and tissues, including the liver and skeletal muscles. The excess lipids build up in different body parts over time, with most of this fat being stored in subcutaneous adipose tissue [8]. Obese patients may have increased immune cells and macrophages in their adipose tissue, which can remodel tissue and release proinflammatory cytokines that may cause insulin resistance. Since adipose tissue surrounds the kidney, renal compression-induced elevation in blood pressure could potentially contribute to the hypertension commonly seen in obese patients. Obesity increases the risk of osteoarthritis because excess weight mechanically strains joints. For this reason, effective therapy is critical to managing and curing obesity and improving the patient's QoL [9].

Lifestyle modification remains the core of managing obesity in the absence of targeted pharmaceutical therapies. GABAA receptor activators, sympathomimetics, pancreatic lipase inhibitors, opioid antagonists, serotonin, 2C receptor agonists, dopamine–norepinephrine reuptake inhibitors, and GLP-1 receptor agonists are some of the drugs used in the treatment of obesity today.

Although they vary in efficacy and adverse effect profiles, they significantly reduce weight compared to lifestyle changes alone [10]. Although obesity is common and has adverse health effects, very few people use anti-obesity medications [11].

Over the past few years, there has been a significant change in the public awareness of health risks related to obesity and the social push to be lean, fit, and healthy. Future research is needed to develop safer and more effective drugs that can help achieve long-term weight loss in obese patients. Medicinal plants have been used for thousands of years for various purposes. There could be a safe and affordable treatment for obesity using medicinal plants [12, 13]. Plants are essential to the treatment of obesity since they are known to produce primary metabolites that are needed for a living cell. They also make an enormous range of secondary metabolites, some of which may have medicinal applications. Most people, especially in developing and underdeveloped nations, do not have access to modern medical treatment and instead rely on tried-and-true traditional medical practices [14]. Considering the importance of medicinal plants in treating and managing various disorders, the present study shall focus on *Kalanchoe pinnata*.

K. pinnata, also known as the miracle plant or life plant, is widely used to treat ailments such as diabetes, arthritis, inflammation, wounds, miscellaneous pains, and heart-related disorders. It is one of the xerophytic succulents that belong to the Crassulaceae family. It is an enriched source of active bioactive compounds such as polyphenols, glycosides, triterpenoids, alkaloids, tannins, bufadienolides, and flavonoids. These compounds are attributed to *K. pinnata's* biological activities. So far, studies have been conducted to substantiate the therapeutic role of *K. pinnata*, including its anti-inflammatory, antidiabetic, and wound-healing properties. However, its anti-obesity effect on metabolic diseases is yet unknown [15]. Therefore, the present study is an initial research investigating the plant's phytochemical constituents and anti-obesity effects. It focuses on the plant's root-stem mixture, as these are usually secondary repositories of many metabolites, like alkaloids, flavonoids, glycosaponins, and phenolic compounds, mainly engaged in lipid metabolism alterations and inhibiting adipogenesis.

2. Methods

2.1. Chemicals

Folin-Ciocalteu (FC) reagent, carboxy methyl cellulose (CMC), aluminum chloride ($AlCl_3$), quercetin, sodium hydroxide (NaOH), ethanol, acetic anhydride, n-hexane, chloroform, sulfuric acid (H_2SO_4), Biuret ($C_2H_5N_3O_2$), Molisch reagent, methanol (CH_3OH), Gallic acid ($C_7H_6O_5$), bovine serum albumin (BSA), glucose ($C_6H_{12}O_6$), and pancreatic lipase were among the reagents utilized in the analysis and obtained from Sigma and Merck.

2.2. Instruments

This study utilized an ultraviolet–visible (UV–Vis) spectrophotometer, Fourier transform infrared spectroscopy (FTIR), a centrifuge, an incubator, a water bath, a rotary evaporator, an analytical balance, a pH meter, pipettes, micropipettes, a fume hood, and a laminar flow cabinet.

2.3. Collection, authentication, and drying plant material

The stems and roots of *K. pinnata* were collected in January 2024 from the Kasur region of Pakistan.

The authenticity of the plant was confirmed by Prof. Dr. Zaheer from Government College University (GCU) Lahore, Pakistan, with the voucher number 4055. The required plant parts were separated. The stems and roots of the plant were left to dry for 25 days. Then, the dried samples were powdered.

2.4. Extraction, drying, and storing the extracts

The mixture of *K. pinnata* was extracted and macerated in methanol for 7 days. This mixture powder was frequently shaken during the maceration period, and after 7 days, it was filtered. The extract was then dried in a rotary evaporator. Once dry, it was left in a labelled vial kept at 4°C.

2.5. Qualitative and quantitative analysis of the phytochemicals

The qualitative analysis was conducted based on the previous study. In the quantitative analysis, the estimation of primary metabolites (protein, carbohydrates, and lipids) [16–18] and secondary metabolites (flavonoids, glycosaponins, and polyphenols) [19–21] was carried out using established methods.

2.6. Analytical studies

2.6.1. FTIR analysis

FTIR scans of *K. pinnata* roots and stems of the methanol extract were made using an Agilent company instrument.

2.6.2. UV/Vis profiling

A 1 mg/mL solution of the extract in methanol was prepared for UV/Vis spectroscopy, and the sample was scanned from 200 nm to 800 nm to capture the spectrum.

2.6.3. GC-MS analysis

The GCMS-QP2010 system was used. The injection temperature was set to 200°C to facilitate the injectable-type introduction of a sample with a split ratio. The sample was introduced into the column in split mode at 0°C. Flow control was maintained by keeping the system pressure within 100 kPa. An overall flow rate of 165.04 mL/min, based on a column flow of 1.69 mL/min, ensured precise control of the analytes' passage through the column. A linear velocity was established for the best separation efficiency. The speed was 47.2 cm/s. The ion source temperature was set at 200°C to ensure the analytes were transferred successfully to the mass spectrometer. The GC-MS interface temperature was set at 250°C, ensuring a 3-minute solvent cut time to minimize interference from the solvent peak, thereby securing accurate detection and quantification of the target compound.

2.7. *In vitro* studies

The pancreatic lipase inhibition assay was conducted following the already published protocol. The extract was mixed with Dimethyl sulfoxide (DMSO) to obtain a 1 mg/mL solution. The standard drug Orlistat was used to compare the values. Pancreatic lipase (50 μL), reaction buffer (30 μL), the extract, and the standard drug (1 mg/mL) were mixed. The mixture was then shaken continuously and incubated at 37°C for 10 minutes. To initiate the reaction, 100 μL of 4-NPP (1 mM) was added to the solution, and the color change resulting from the synthesis of 2, 4-dinitrophenol was observed. The experiment was repeated thrice, and readings

were taken at 405 nm [22, 23]. The following formula was used to determine the % inhibition:

$$\%\ inhibition = (Abs\ of\ enzyme - Abs\ of\ the\ extract/Abs\ of\ enzyme) \times 100.$$

2.8. *In vivo* studies

This study used Sprague Dawley male rats aged 4–5 weeks, weighing 150–200 g. The environment where the rats were kept had a temperature of 22 ± 2°C, humidity of 40–60%, and a light–dark cycle of 12 hours ± 1 hour. They had free access to water and food. The high-fat diet (HFD) consisted of 29.5 g of beef shallow, 22.0 g of casein, 23.0 g of starch, 17.9 g of cellulose, 4.0 g of L-cysteine, 0.3 g of choline chloride, 11.8 g of vitamin mixture, and 10.5 g of mineral mixture.

2.9. Experiment

After a week of housing, the rats were randomly assigned numbers. They were fed an HFD for 4 weeks, except for the normal control group. The rats on the HFD showed a noticeable increase in body fat after 4 weeks compared to those fed the regular diet, which led to the start of the treatment protocol. The extract or the positive control, Orlistat, was given orally five times a week by gavage in a solution containing 0.5% CMC for 8 weeks. Normal control group rats were administered an equivalent volume of 0.5% CMC. Their food intake and body weight were recorded three times a week. Before sacrifice, all animals involved in the experiment fasted overnight. Their organs, including the liver, kidney, and pancreas, were examined histologically after blood samples were collected [24, 25].

The summary of each animal group (n = 6) is as follows:

(i) Group I: Normal control

(ii) Group II: Animals were treated with HFD for disease control

(iii) Group III: Extract-treated rats were administered methanol extract (500 mg/kg) orally following HFD-induced obesity

(iv) Group IV: Standard treatment was given, which included the oral administration of 10 mg/kg of Orlistat after a high-fat diet

2.10. Statistical analysis

The statistical analyses were performed using GraphPad Prism and Microsoft Excel. Phytochemicals were quantified using a regression analysis, with the results from an *in vitro* study being presented as mean standard deviation (±SD). One-way ANOVA was applied in combination with a post-hoc Tukey test to calculate the standard error mean (SEM) of data from an *in vivo* study. P values < 0.05 were considered significant.

3. Results

3.1. Phytochemical analysis

Qualitative and quantitative analysis of the phytochemicals of the root-stem mixture of *K. pinnata* was carried out. The phytochemicals were qualitatively analyzed using several standard methods. Any color change that occurred during the reaction was noted. The results of the qualitative analysis are presented in Table **1**. The methanol extract contained proteins, carbohydrates, and lipids, which indicated that it had nutritional value. Additionally, polyphenols and flavonoids suggested that the plant could be medicinally important.

Table 1: Qualitative analysis of the phytochemicals in the methanol extract of the root-stem mixture.

Test	Results
Proteins	+++
Lipids	+
Carbohydrates	+++
Flavonoids	++
Polyphenols	++
Saponins	+++

In the quantitative evaluation of phytochemicals, primary and secondary metabolites were measured. Table **2** shows the summary of the estimation for the primary metabolite. Protein content was calculated using the standard bovine serum albumin (BSA) curve equation, which is $y = 0.0005\times + 0.0058$, where $R^2 = 0.9803$ (Figure **1**). Similarly, carbohydrate concentration was determined based on the glucose calibration curve described by the equation $y = 0.0004\times + 0.0058$, where $R^2 = 0.9908$ (Figure **2**). The nutritional relevance of the plant extract was demonstrated by the presence of proteins (25.55 ± 0.043), lipids (13.19 ± 0.061), and carbohydrates (41.34 ± 0.051) in the plant methanol extract.

Secondary metabolite estimation results are presented in Table **3**. The total polyphenol content was measured using the gallic acid standard curve (Figure **3**): $y = 0.0024\times + 0.0166$, where $R^2 = 0.9874$. The total flavonoid contents were determined using the quercetin calibration curve (Figure **4**): $y = 0.0032\times + 0.0058$ and $R^2 = 0.9922$. The results of this study confirmed the presence of key secondary metabolites, including glycosaponins (91.05 ± 0.044), flavonoids (134.21 ± 0.039), and polyphenols (121.76 ± 0.047).

Table 2: Quantitative estimation of the primary metabolites in the methanol extract of the root-stem mixture (mean mg/g ± SD).

Sample	Total proteins	Total carbohydrates	Total lipids
Methanol extract of *Kalanchoe pinnata* root-stem mixture	25.55 ± 0.043	41.34 ± 0.051	13.19 ± 0.061

Table 3: Quantitative estimation of secondary metabolites in the methanol extract of the root-stem mixture (mean µg/g ± SD).

Sample	Total polyphenols	Total flavonoids	Glycosaponins
Methanol extract of *Kalanchoe pinnata* root-stem mixture	121.76 ± 0.047	134.21 ± 0.039	134.21 ± 0.039

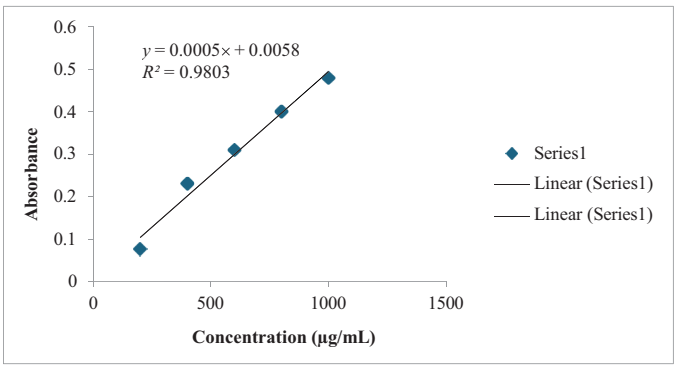

Figure 1: BSA standard curve.

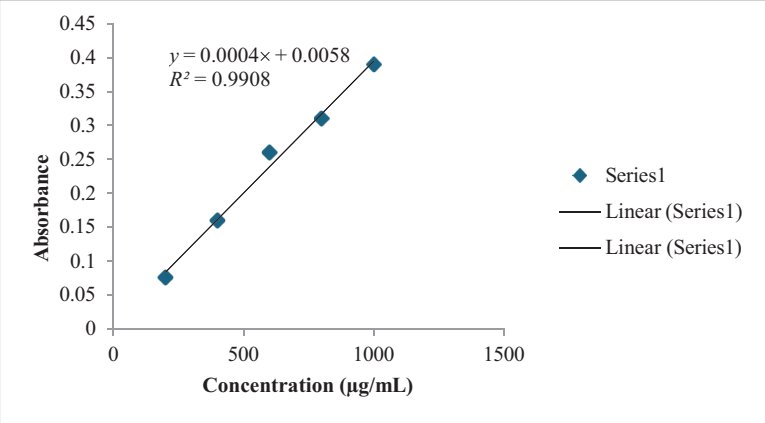

Figure 2: Glucose standard curve.

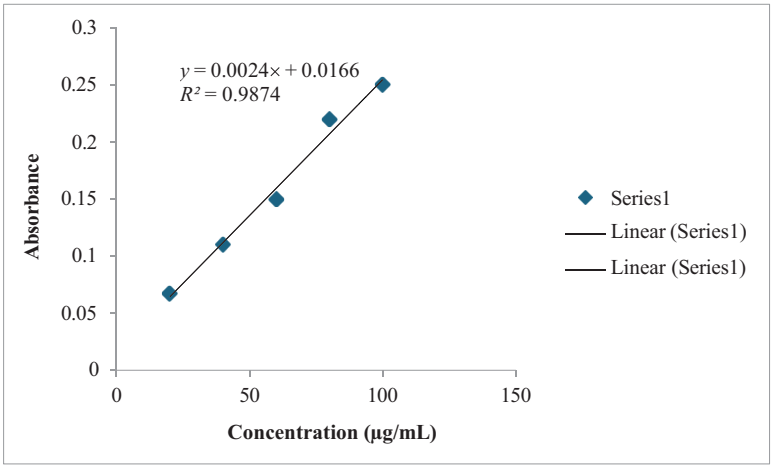

Figure 3: Gallic acid standard curve.

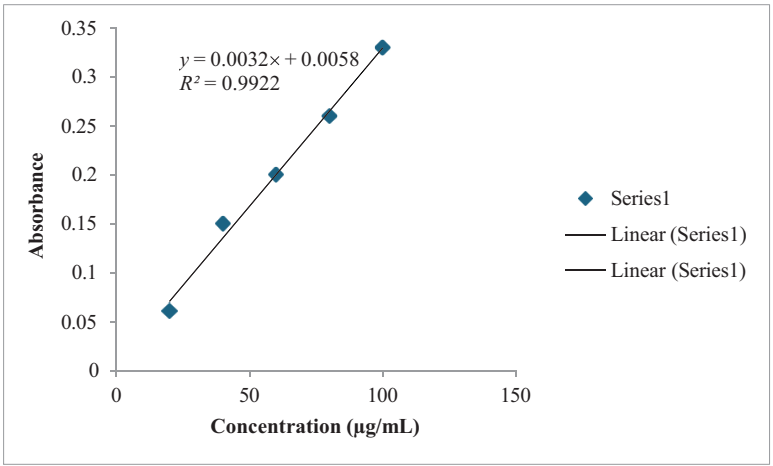

Figure 4: Quercetin standard curve.

3.2. Analytical studies

3.2.1. FTIR spectroscopy

The FTIR spectrum of the powder mixture of *K. pinnata* root-stem is shown in Figure **5**. It indicates the existence of many functional groups, which include -C-O (stretching, 1200 cm^{-1}), -C=C (stretching, 1636 cm^{-1}), -C=O (stretching, 1784 cm^{-1}), -C=O (1921 cm^{-1}), -C=O (conjugation, 2015 cm^{-1}), -C≡C (stretching, 2331 cm^{-1}), -C-H (stretching, 2850 cm^{-1}), and O-H (stretching, 3300 cm^{-1}). Functional groups are atomic configurations within molecules that give specific chemical properties and reactivity. A host of organic compounds depend on carbonyl groups (C=O) to define how they will behave in reactions. Double and triple bonds characterize alkenes (C=C) and alkynes (C≡C) as essential building blocks for many chemical reactions and material syntheses. Alcohol and phenols play a significant role in synthesis and biological mechanisms due to hydroxyl groups (O-H) that enhance solubility and reactivity. Such functional groups have combined input to enhance crucial roles in the structure and operation of organic molecules.

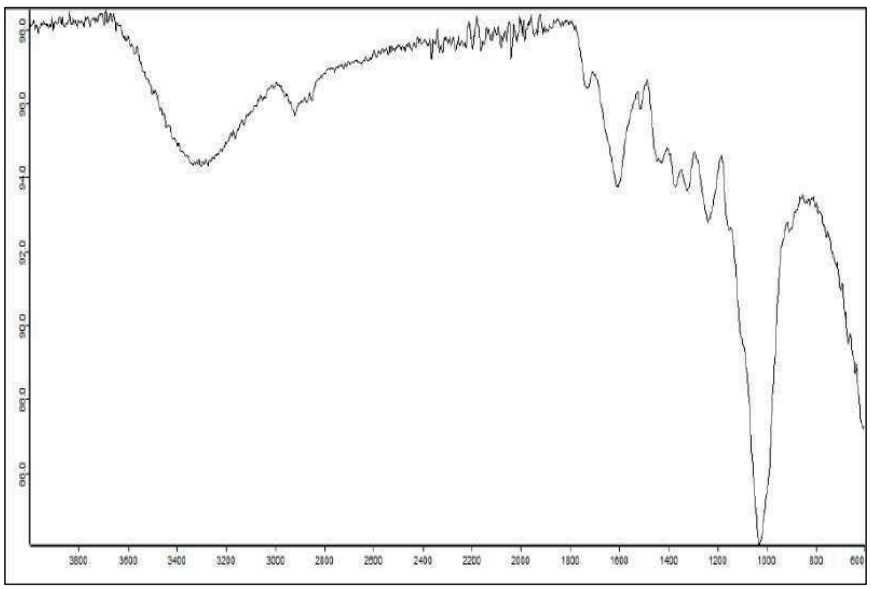

Figure 5: FTIR spectra of *Kalanchoe pinnata* root-stem powder mixture.

3.2.2. UV/Vis spectroscopy

Figure **6** displays the UV/Vis spectrum of *K. pinnata* root-stem powder. The four highest peaks at 220 (peak 1), 254 (peak 2), 280 (peak 3), and 305 (peak 4) were identified in the scan. The appearance of the 220 nm peak in a UV/Vis scan can indicate π to π^* transitions in carbonyls or electron-rich aromatic compounds. The peak at 254 nm is associated with aromatic compounds and underscores the transitions from π to π^* in conjugated systems. In parallel with this, 280 and 305 nm peaks are often related to aromatic compounds. The 280 nm peak is related to π to π^* transitions, while the 300 nm peak is associated with n to π^* transitions in carbonyl or aromatic systems. These peaks contain valuable information concerning the structural and electronic properties of the compound under study.

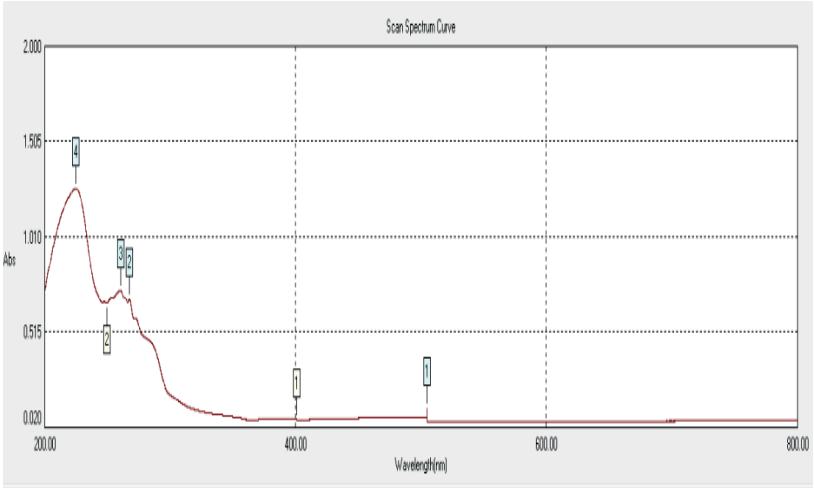

Figure 6: UV/Vis profiling of the methanol extract of *Kalanchoe pinnata* root-stem powder.

3.2.3. GC-MS analysis

The results of GC-MS are shown in Figure **7**. The GC-MS spectrum was interpreted using the National Institute of Standards and Technology (NIST) library and revealed the presence of various compounds (Table **4**).

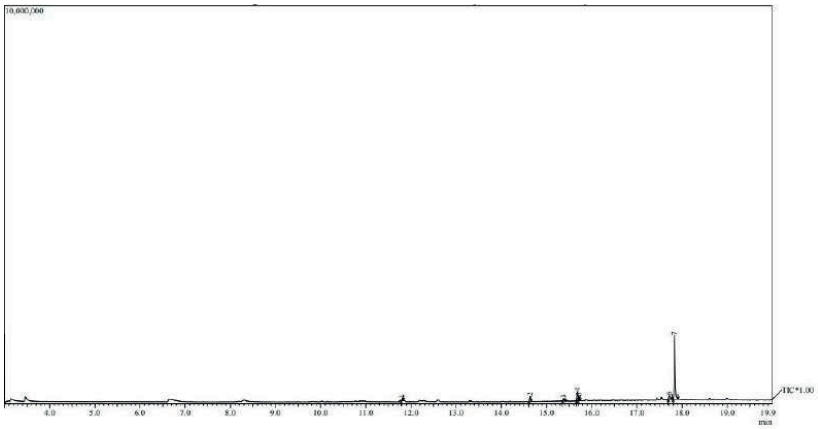

Figure 7: GC-MS spectra of the methanol extract of *Kalanchoe pinnata* root-stem powder.

Table 4: Compounds identified in the methanol extract of *Kalanchoe pinnata* root-stem powder using GC-MS analysis.

Peak No	Compound	Molecular formula	Retention time	Area%
1	1,2-Benzenedicarboxylic acid	C8H6O4	11.801	2.30
2	Octadecanoic acid, ethyl ester	C20H40O2	14.633	4.00
3	Phytol	C20H40O	15.375	2.25
4	9,12-Octadecadienoic acid	C19H34O2	15.681	7.80
5	9,12,15-Octadecatrienoic acid	C21H36O4	15.718	4.51
6	Hexadecanoic acid	C19H38O4	17.716	5.98
7	Di-n-octyl phthalate	C24H38O4C	17.830	73.16

3.2.4. In vitro studies

Through the pancreatic lipase inhibition test, the effect of the extract and Orlistat on the activity of pancreatic enzymes was determined at various concentrations (Figure **8**). The study results showed that Orlistat and the extract have a concentration-dependent effect. In addition, the half maximal inhibitory concentration (IC_{50}) values of Orlistat and the extract were determined. The IC50 of Orlistat was found to be 403.81 μg/mL (y = 0.0723× + 20.811), whereas the IC50 of the methanol extract of the stem-root was found to be 475.93 μg/mL (y = 0.074× + 14.781).

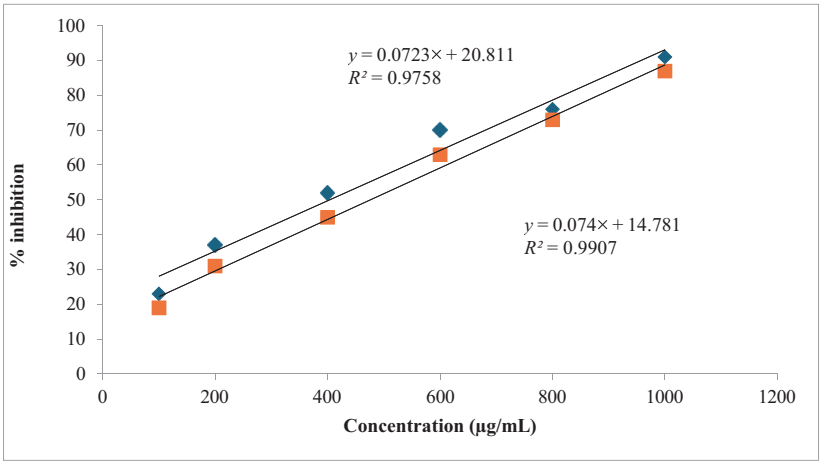

Figure 8: Concentration-dependent inhibition of pancreatic lipase by methanol extract of *Kalanchoe pinnata* root-stem powder.

3.2.5. In vivo studies

Regarding the impact of a high-fat diet on weight change, the results showed that from day 3 to day 14, there was a significant difference (P < 0.05) in weight change between the disease group and the normal group (Figure **9**). While there was a significant weight loss compared to the disease group from days 10–14 during extract and/or Orlistat treatment (standard), this outcome was not statistically different from the controls (P > 0.05).

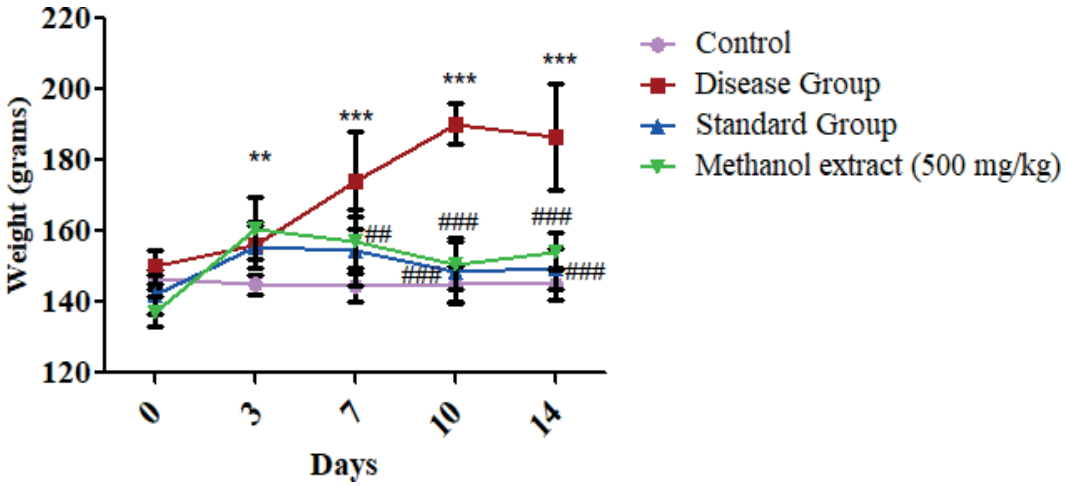

Figure 9: Effect of the methanol extract of *Kalanchoe pinnata* root-stem powder and Orlistat (standard) on body weight (grams) of the rats. *Disease group compared to the normal group; #extract and Orlistat (standard) groups compared to the disease group. Differences were considered significant at P < 0.05. *,#P < 0.05; **,##P < 0.01; ***,###P < 0.001.

3.2.6. Effects on biochemical parameters

Tables **5**–**7** summarize the findings from the lipid profile, liver function tests (LFTs), and renal function tests (RFTs). The results showed that the methanol extract of *K. pinnata* root-stem significantly improved the lipid profile, LFTs, and RFTs.

Table 5: Effects of methanol extract of *Kalanchoe pinnata* root-stem powder on lipid profile.

Treatment	Total lipids (mg/dL)	Cholesterol (mg/dL)	TGs (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	VLDL cholesterol (mg/dL)
Control	233 ± 0.41	63 ± 0.55	83 ± 0.87	29 ± 0.71	61 ± 0.37	18 ± 0.81
Disease	271 ± 0.53***	88 ± 0.29**	92 ± 0.49*	24 ± 0.39**	86 ± 0.79***	23 ± 0.36**
Standard	234 ± 0.07###	68 ± 0.54###	85 ± 0.34##	29 ± 0.26##	66 ± 0.046###	20 ± 0.63#
Methanol extract (500 mg/kg)	235 ± 0.16###	71 ± 0.56##	83 ± 0.78##	28 ± 0.63#	65 ± 0.53###	19 ± 0.44###

*Disease group compared to the normal group; #extract and Orlistat (standard) groups compared to the disease group. TG, triglycerides; HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins. Differences were considered significant at $P < 0.05$. *,#$P < 0.05$; **,##$P < 0.01$; ***,###$P < 0.001$.

Table 6: Effects of methanol extract of *Kalanchoe pinnata* root-stem powder on LFTs.

Treatment	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Indirect bilirubin (mg/dL)	ALT U/L	AST U/L	ALP U/L	Total proteins g/dL	Albumin g/dL	Globulin g/dL
Control	0.61 ± 0.53	0.30 ± 0.45	0.31 ± 0.23	52 ± 0.43	65 ± 0.54	88 ± 0.09	6.61 ± 0.26	4.60 ± 0.38	2.01 ± 0.41
Disease	0.74 ± 0.56*	0.34 ± 0.83	0.34 ± 0.28	67 ± 0.56**	117 ± 0.28***	151 ± 0.35***	7.17 ± 0.41*	4.17 ± 0.65	3.00 ± 0.62*
Standard	0.62 ± 0.56#	0.31 ± 0.49	0.31 ± 0.39	50 ± 0.27###	62 ± 0.23###	85 ± 0.19###	6.34 ± 0.25##	4.32 ± 0.51	2.02 ± 0.32#
Methanol extract (500 mg/kg)	0.64 ± 0.08#	0.32 ± 0.15	0.32 ± 0.38	54 ± 0.81##	72 ± 0.63###	110 ± 0.36###	6.64 ± 0.62##	4.41 ± 0.73	2.23 ± 0.54#

*Disease group compared to the normal group; #extract and Orlistat (standard) groups compared to the disease group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Differences were considered significant at $P < 0.05$. *,#$P < 0.05$; **,##$P < 0.01$; ***,###$P < 0.001$.

Table 7: Effects of methanol extract of *Kalanchoe pinnata* root-stem powder on RFTs.

Treatment	Urea (mg/dl)	BUN (mg/dl)	Creatinine µmol/L
Control	23.00 ± 0.38	12.33 ± 0.31	55.00 ± 0.58
Disease	35.00 ± 0.39***	17.73 ± 0.89**	78.00 ± 0.29***
Standard	21.00 ± 0.47###	11.41 ± 0.43###	56.00 ± 0.08###
Methanol extract (500 mg/kg)	26.00 ± 0.81##	13.95 ± 0.33##	56.00 ± 0.76###

*Disease group compared to the normal group; #extract and Orlistat (standard) groups compared to the disease group. BUN, blood urea nitrogen. Differences were considered significant at $P < 0.05$. *,#$P < 0.05$; **,##$P < 0.01$; ***,###$P < 0.001$.

3.3. Histopathology

The histological findings from the pancreas, liver, and kidney are presented in Figures **10**, **11**, and **12**, respectively. The histopathological examination of the pancreas, liver, and kidney revealed marked variations between the untreated, disease, standard, and methanol extract of *K. pinnata* root-stem powder groups. The pancreas of the disease group presented with oxidative stress, increased inflammation, and islet hyperplasia, all of which hinder insulin secretion. Acinar cells were healthy; the islets were well-defined in orlistat-treated groups, and the results were comparable to the control group's pancreas architecture. Regarding islet integrity and inflammation, the standard treatment group presented with notable protective effects.

On the other hand, the extract treatment group partially restored the normal structure of the pancreas and improved β-cell function. There were signs of possible progression toward nonalcoholic fatty liver disease in the livers of the disease group, including extensive steatosis ballooning degeneration and inflammatory infiltrates. Compared to the standard group, the extract group showed minimal steatosis and preserved liver architecture, suggesting a protective effect against obesity-induced liver damage. Further, the study indicated that the extract treatment group retained tubular integrity and structure, whereas in the standard treatment groups, little protection against obesity-related renal damage was seen.

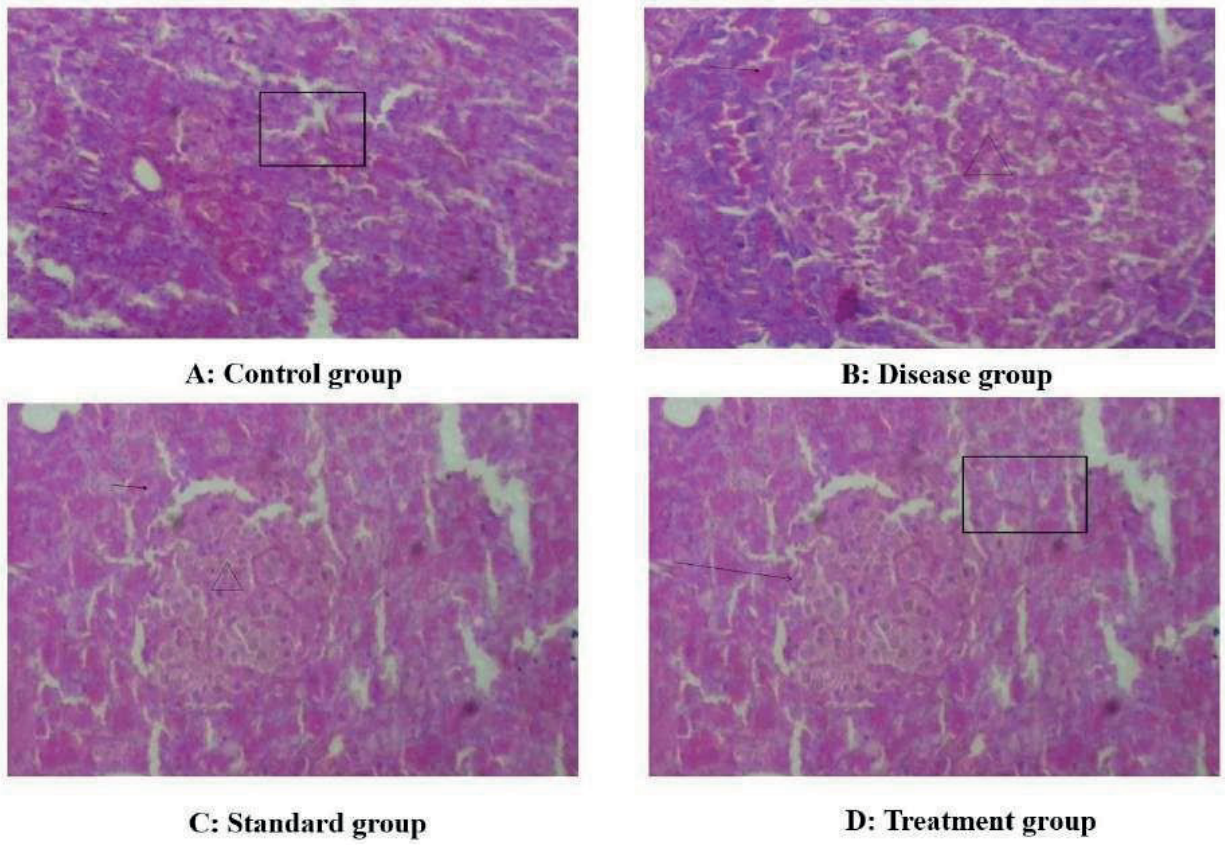

Figure 10: Effect of treatment (extract) and Orlistat on the pancreas (H&E staining, 10×).

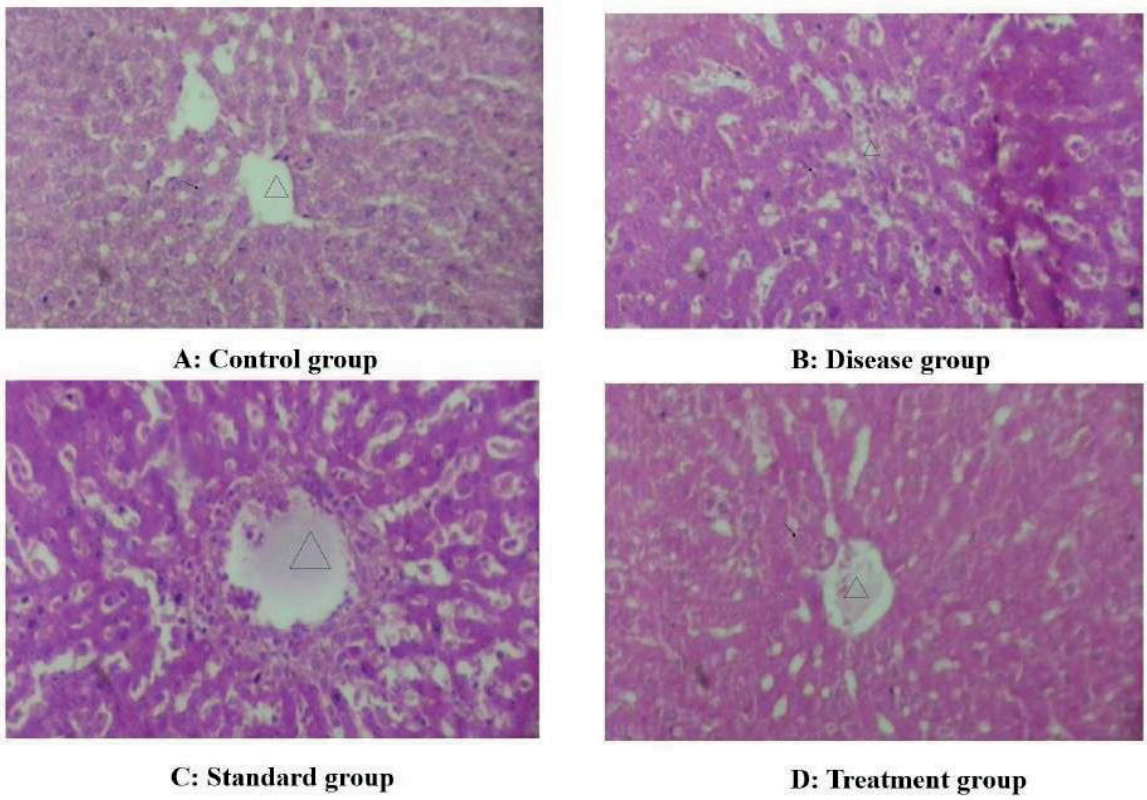

Figure 11: Effect of treatment (extract) and Orlistat on the liver (H&E staining, 10×).

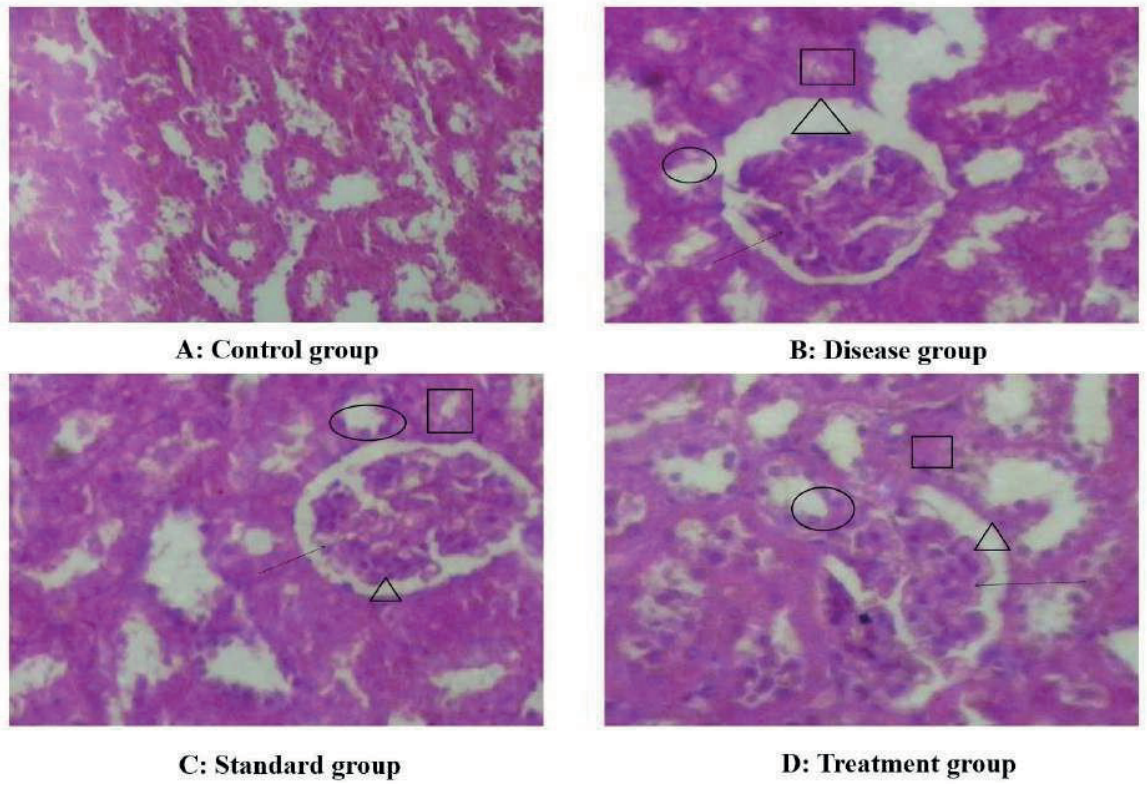

Figure 12: Effect of treatment (extract) and Orlistat on the kidney (H&E staining, 10×).

4. Discussion

Abnormal build-up of body fat, which is characteristic of obesity, is a major global health problem. Obese people are more likely to suffer from chronic illnesses, including type 2 diabetes, cardiovascular disease, and some cancers. Among the numerous underlying causes of obesity are behavioral, genetic, physiological, and environmental factors. One of the contributing factors is mismatched energy expenditure and calorie intake, which is made worse by high-calorie diets and sedentary lifestyles. For obesity treatment, a comprehensive strategy is needed that involves behavioral therapy, food changes, exercise, medication, or surgery. Of late, there has been more interest in natural alternatives, especially medicinal ones [26, 27].

Medicinal plants have been used in traditional medicine for their therapeutic properties. These plants are promising adjunctive approaches in treating obesity since they contain bioactive compounds that can alter metabolic pathways, reduce fat absorption, boost fat metabolism, and suppress appetite. Many different therapeutic plants contain alkaloids, saponins, flavonoids, and polyphenols, all of which can have anti-obesity effects [28–30]. Naturally, medicinal plants are less likely to cause side effects compared to synthetic drugs in managing obesity. The plant's lipid-lowering, anti-inflammatory, and antioxidant properties improve general health and prevent obesity-related complications. Despite the mounting evidence that shows medicinal plants have anti-obesity effects, challenges remain in standardizing dosages and ensuring safety and efficacy in plant therapies. Therefore, more extensive clinical studies are required to provide evidence of these plants' long-term benefits and mechanisms of action for managing obesity [31].

K. pinnata has historically been valued for its medicinal properties. Its leaves are commonly used in traditional medicine to treat wounds, ulcers, respiratory issues, and digestive problems. It is believed to possess anti-inflammatory, analgesic, and antibacterial qualities. *K. pinnata* remains an essential plant in both traditional and modern herbal medicine because of its versatility and multiple uses [32]. This plant has gained significant attention due to its abundant presence of polyphenols and other bioactive compounds, components that are believed to enhance lipid metabolism, reduce inflammation, and increase metabolic activities, contributing to the plant's anti-obesity effects. *K. pinnata* extracts may help reduce body weight and body fat percentage by altering lipid profiles and promoting fat breakdown, making it a possible natural method for weight management. *K. pinnata* may improve insulin sensitivity and minimize the risk of developing type 2 diabetes and obesity-related cardiovascular diseases by suppressing inflammatory responses [33].

K. pinnata polyphenols, saponins, and glycosaponins are essential for obesity management in numerous ways. Owing to their long-known anti-inflammatory and antioxidant properties, polyphenols have been associated with improved sensitivity to insulin and promoted lipid metabolism, leading to reduced body weight and fat accumulation. Besides their hypoglycemic effects, saponins enhance glucose metabolism, promote cholesterol elimination, and decrease the intestinal absorption of dietary fats. *K. pinnata* is a promising natural agent for managing obesity because it contains these bioactive compounds [34]. Significant functional groups were identified

in *K. pinnata* extracts through FTIR and UV/Vis spectroscopy, indicating the presence of bioactive compounds with potential therapeutic applications [35]. GC/mass identified several key volatile compounds, such as phytol, octadecatrienoic acid, and hexadecanoic acid. Octadecanoic acid, another term for stearic acid, is a saturated fatty acid that may have a role in lipid metabolism, aid in weight management, and improve metabolic health. Also notable are the antioxidant and anti-inflammatory properties of phytol, a diterpene alcohol, helping reduce the consequences associated with obesity. Some fatty acids have been shown to reduce inflammation and increase insulin sensitivity. A saturated fatty acid, hexadecanoic acid (palmitic acid), is necessary for cellular processes and can influence metabolic pathways [36].

The reported anti-obesity effects of *K. pinnata* are linked to its high levels of flavonoids, saponins, and polyphenols. Flavonoids have been shown to influence lipid metabolism by inhibiting key enzymes like pancreatic lipase, reducing fat absorption in the intestines, and increasing fatty acid oxidation. Saponins have been found to form insoluble complexes with cholesterol, thus lowering its bioavailability and improving lipid profiles. Polyphenols induce their anti-obesity effects through the modulation of adipogenesis, increased lipolysis, and heightened energy expenditure via modification of signaling pathways like AMPK and PPARγ. The bioactive molecules might act synergistically to yield the noted pharmacological response, as seen in the pancreatic lipase assay. Further research on this aspect is needed to identify its specific molecular mechanisms [14, 15, 34, 37].

The *in vivo* study on rats demonstrated that *K. pinnata* extracts exhibited considerable effects on body weight and lipid profiles, which is consistent with our findings. The treatment resulted in appreciable improvements in serum lipid levels and decreased body weight and fat accumulation. These results align with previous studies showing the anti-inflammatory and antioxidant properties of the flavonoids and polyphenols found in *K. pinnata*, leading to better metabolic health. Additionally, histological findings showed that the extract could preserve the normal structure of tissues like the liver, kidneys, and pancreas. In addition to these effects, the saponins and glycosaponins present in the plant could inhibit fat absorption and enhance cholesterol excretion, further supporting these effects and enhancing the plant's potential as a natural agent for managing obesity [38, 39].

5. Limitations

This study evaluated the *in vitro* pancreatic lipase inhibitory activity and *in vivo* anti-obesity effects of *K. pinnata*, using Orlistat as a positive control, which adds relevance to the pharmacological evaluation. However, several limitations should be acknowledged. First, while some of the phytochemicals were determined quantitatively, no marker compounds were determined or standardized, which could impact the reproducibility and accuracy of future studies. Second, no comprehensive toxicity evaluation was performed. However, in the past, no toxicity was observed at the dosages used in this study. Moreover, although bioactive groups like flavonoids, saponins, and polyphenols were associated with the reported effects, mechanisms of action were not explicitly verified via molecular or biochemical assays. Lastly, observations from preclinical models need to be validated in the clinic to establish their translational significance. Future research should focus on

identifying marker compounds, conducting comprehensive toxicity assessments, and clarifying mechanisms to support the safe and effective use of *K. pinnata* in managing obesity.

6. Conclusion

The current study underscores the importance of studying plant-derived chemicals in combating obesity and its associated diseases and offers necessary new knowledge regarding the therapeutic value of *K. pinnata*. It examined the possible therapeutic effects of *K. pinnata* using its root-stem powder. Combining powdered root and stem is essential because this maximizes the plant's health benefits by ensuring a broader spectrum of bioactive compounds. The combination of unique phytochemical profiles present in both parts of the root and stem enhances the extract's overall effectiveness. It may also result in a synergistic effect, leading to better therapeutic results. Clinical trials will be required to confirm our findings in human populations and to develop anti-obesity therapies that enhance metabolic health and overall well-being.

Declarations

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Ethical Considerations

The Superior University of Lahore, Pakistan, Research and Ethics Committee granted animal ethical approval (reference number: SU/FoP/MSPCW/F22-07).

Competing Interests

The authors declare no conflict of interest.

Availability of Data and Materials

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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Abbreviations and Symbols

LFTs: Liver function tests

BMI: Body mass index

QoL: Quality of life

LDL: Low-density lipoprotein

VLDL: Very low-density lipoprotein

HDL: High-density lipoprotein

TGs: Triglycerides

IC50: Half maximal inhibitory concentration

AI Use Disclosure

The authors declare that no generative artificial intelligence (AI) tools were used in the writing, data analysis, figure generation, or editing of this manuscript.

Author Contributions

Concept or design of the work: MA, KA, MZA; Clinical studies and experimental studies: MA, KA;

Data acquisition, data analysis, and statistical analysis: MA, KA, MZA, SAS, AM, RH; Manuscript preparation, manuscript editing, manuscript review: MA, KA, MZA,

SAS, AM, RH; Final approval: MA, KA, MZA, SAS, AM, RH; Accountability for all aspects of the work: MA, KA, MZA, SAS, AM, RH.

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