Avicenna Journal of Phytomedicine (AJP)

*Original Research Article*

# Phytochemical profiling and anti-obesity potential of the methanol extract of *Carica papaya* ripe fruit peel

Rimsha Ahsan[1], Kanwal Ashiq[1,*], Shafiq Ali Shah[2], Muhammad Zaeem Ahsan[1], Shafiullah[1], Maryam Ashraf[1], Ishafaq Bukhari[3]

[1]*Faculty of Pharmacy, Superior University, Lahore, Pakistan*
[2]*Faculty of Pharmacy, Hamza College of Pharmacy and Allied Health Sciences, Lahore, Pakistan*
[3]*Department of Biomedical Sciences, Kentucky College of Osteopathic Medicine, University of Pikeville, Kentucky, USA*

***Article history:***
*Received: Feb 19, 2025*
*Received in revised form: Apr 19, 2025*
*Accepted: Apr 22, 2025*
*AJP, Vol. 16, No. 3, May-June 2026, 473-488.*
*https://dx.doi.org/10.22038/ajp.2025.26441*

**** Corresponding Author:***
*Tel: 04238103777*
*Fax: +92425330360*
*kanwal.ashiq@superior.edu.pk*

***Keywords:***
*Medicinal plant*
*Obesity*
*Carica papaya*
*Pancreatic lipase*
*Orlistat*

## Abstract

**Objective:** *Carica papaya* has been widely recognized for its medicinal benefits, particularly in treating metabolic disorders such as obesity. This study examines the weight loss effects of *C. papaya* fruit peel and determines its phytochemical composition.

**Materials and Methods:** Various standard procedures were utilized for the identification and quantification of phytochemicals. UV/Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography–Mass Spectrometry (GC/MS) were utilized to identify the functional groups and volatile compounds. *In vitro*, pancreatic lipase inhibition assay and *in vivo*, high-fat diet rat model were used to evaluate the anti-obesity activity of the methanol extract. Lipid profile, liver function tests (LFTs) and renal function tests (RFTs) were measured, and histopathological studies of the pancreas, liver, and kidneys were performed.

**Results:** The results of phytochemical analysis revealed the presence of various compounds such as flavonoids, polyphenols, and glycosaponins which are recognized for their anti-obesity properties. GC/mass analysis also confirmed the existence of volatile compounds. The methanol extract from *C. papaya* fruit peel significantly inhibited pancreatic lipase activity dose-dependently. *In vivo* studies showed that the methanol extract reduced the weight, improved the lipid profile, and restored the normal structure of the pancreas, liver, and kidneys. These effects were nearly identical to those of the standard reference drug, orlistat.

**Conclusion:** This study confirms the anti-obesity activity of *C. papaya* fruit peel. Further clinical studies are needed to validate its safety and efficacy, as well as to explore its underlying mechanisms of action.

*Please cite this paper as:*
Ahsan R, Ashiq K, Shah Sh.A, Ahsan M.Z, Shafiullah, Ashraf M, Bukhari I. Phytochemical profiling and anti-obesity potential of the methanol extract of *Carica papaya* ripe fruit peel. Avicenna J Phytomed, 2026; 16(3): 473-488.

## Introduction

Obesity is a complex, multifactorial disease that arises due to an imbalance between energy intake and expenditure and is caused by genetic, environmental, and hormonal factors. It affects males and females of all ages and ethnicities in various locations. The morbidity and mortality have significantly increased around the globe, especially in individuals with comorbid conditions like diabetes, coronary heart disease, cancer, atherosclerosis, and hypertension (Kokkorakis et al. 2024). According to the World Health Organization (WHO), the condition of overweight or obesity results from the excessive or abnormal deposition of fats in body tissue, which directly threatens well-being. The general body fitness is measured by a simple matrix called the body mass index (BMI). In addition, increased BMI is associated with many health problems resulting in deteriorating quality of life (QoL) (Hasani-Ranjbar et al. 2013; Hall et al. 2022).

Because obesity is linked to ill health and, therefore, costly medical treatment, it has to be the primary focus of cost-containment initiatives. To allow physicians to control obesity effectively, there has to be a rigorous evaluation of the patient's health factors influencing metabolism, energy intake, and expenditure (Ludwig et al. 2022; Westbury et al. 2023; Bakinowska et al. 2024).

Current treatments for obesity include lifestyle modifications, pharmacotherapy, and surgical interventions. The first-line approach continues to be dietary changes, increased physical activity, and behavioral therapy. Pharmacological options include glucagon-like peptide-1(GLP-1) receptor agonists like semaglutide and appetite suppressors like orlistat, notably impacting weight loss. Bariatric surgery, including gastric bypass and sleeve gastrectomy, is reserved for those with severe obesity and is effective in the long term for weight loss and metabolic health. Upcoming therapies include gut microbiota modulation, gene-based treatments, and new drugs targeting fatness-related energy expenditure pathways. Such innovative approaches seek to improve long-term weight management and mitigate obesity-related complications (Cornier 2022).

There is a new shift in the attitude of the public towards the health consequences of obesity and the social pressure to be thin and be satisfied with one's body. More research must be conducted to identify successful behavioral change strategies to be translated and applied to diverse groups (Blumberg 2024). Further research is also needed to develop safer and more effective drugs that will help obese people lose weight and maintain it in the long run.

Medicinal plants may provide a less expensive and safer method of treating obesity. Many medicinal plants have been used traditionally for weight management (Ashiq et al. 2021; Nabi et al. 2023; Salehsari et al. 2024). Humans have been utilizing plants for thousands of years for many purposes. Plants are crucial since they comprise over 250,000 species and contain metabolites that have pharmacological activities (Süntar 2020; Batool et al. 2024). There has been global research to verify their efficacy, and some findings have led to the development of plant-based drugs. The WHO reports that developing countries use naturally occurring substances for medical purposes. However, most countries rely mainly on plant-based medicine (Latif et al. 2020). Considering medicinal plants' relevance in managing and treating various disorders, the current paper shall focus on *Carica papaya*.

*Carica papaya*, often called papaya or pawpaw, is a fruit-bearing plant from the Caricaceae family, originating in Central America. Today, it is extensively grown in tropical and subtropical areas worldwide. Papaya is highly valued for its nutritional benefits, offering a rich supply of vitamins such as vitamin C, vitamin A, and folate, along with essential enzymes like papain which plays a vital role in digestion. Additionally, various parts of the papaya

tree including its leaves, seeds, and fruit skin, have been utilized in traditional medicine for their therapeutic properties, particularly in addressing infections, digestive problems, obesity, diabetes, dengue, malaria, inflammation, hypertension, and skin conditions. Furthermore, the papaya fruit contains different valuable phytochemicals including alkaloids, saponin, glycosides, ferulic acid, caffeic acid, flavonoids, polyphenols, carpine, and papain. It also has a range of vitamins, such as A, C, E, and B complexes (Ugbogu et al. 2023; Babalola et al. 2024).

*Carica papaya* has also come under the spotlight for its ability to control metabolic diseases such as obesity, since its high fibre level and enzyme activity are thought to aid digestion and facilitate weight maintenance. Studies indicate the plant's metabolites may regulate metabolic processes and lower fat storage in the body. Despite its historical applications and new scientific justification, additional investigation is necessary to fully understand its mechanisms of action and therapeutic effects, especially for its use in obesity and obesity-related metabolic disorders (Od-Ek et al. 2020). In traditional medicine, the plant's fruit and leaf juice is often used to treat obesity problems. Until now, various experiments have been conducted on seeds, leaves, and fruits to prove their beneficial effect in reducing body weight and treating obesity-related issues. Papaya peels can be another promising agent in weight loss that needs further investigation. Thus, it is crucial to validate the anti-obesity properties of *C. papaya* fruit peels through well-designed scientific experiments (Matsuane et al. 2023; Ugbogu et al. 2023).

Therefore, the present study is an initial study investigating ripened *C. papaya* fruit peel's phytochemical constituents and anti-obesity effects.

## Materials and Methods

### Chemicals

Quercetin, Acetic anhydride, Bovine serum albumin (BSA), Folin-Ciocalteu (FC) reagent, Carboxy methyl cellulose (CMC), Glucose, Aluminum chloride ($AlCl_3$), Sodium hydroxide (NaOH), Ethanol, n-Hexane, Chloroform, Sulfuric acid ($H_2SO_4$), Methanol, Gallic acid and Pancreatic lipase are some of the reagents employed in the process of the work and were acquired from Sigma and Merck.

### *Carica papaya* fruit collection, authentication, and drying

*Carica papaya* ripened fruit was collected from the local market of Lahore, Pakistan. The plant was identified by Prof. Dr Zaheer, botany faculty, Government College University (GCU), Lahore, Pakistan, under voucher 4056. The peels from the ripe fruit were separated and dried under share for 25 days. After drying the peels, they were ground into powder

### Extraction, drying and storage of the extracts

The *C. papaya* fruit peel powder was extracted in methanol using the maceration method. The powder was macerated in methanol for seven days. During maceration, the powder was often shaken. After seven days, it was filtered, and the solvent was evaporated using a rotary evaporator. Then, the extract was stored in a clean, dried and labelled vial at 4°C.

### Qualitative and quantitative assessment of the phytochemicals

Qualitative analysis was done based on the established methods. Quantitative estimation of primary metabolites (protein, carbohydrates and lipids) (Lowry et al. 1951; Shahidi and Wanasundara 2002; Yemm and Willis 1954) and secondary metabolites (flavonoids, glycosaponins and polyphenols) (Arvouet-Grand et al. 1994; Siddiqui et al. 2009; Singleton and Rossi 1965) was carried out based on published procedures.

## Analytical studies

### Fourier Transform Infrared Spectroscopy (FTIR) profiling

FTIR (Agilent, USA) scans of *C. papaya* fruit peel were taken by taking a minute quantity of the powder.

### UV/Vis scanning

The diluted solution of the extract (1 mg/ml) was prepared in methanol. Then, the solution was scanned from 200 nm to 800 nm for UV/Vis profiling.

### Gas Chromatography–Mass Spectrometry (GC/Mass) analysis

For analysis, the GCMS-QP2010 (Shimadzu, Tokyo, Japan) system was used. It set the injection temperature at 200°C, making possible injectable-type introduction with a split ratio. The sample was introduced to the column in the split mode at 0°C. The flow control mode was controlled through pressure regulation that maintained system pressure within 100 kPa. At a column flow of 1.69 ml/min, an overall flow rate of 165.04 ml/min was used so that the flow was perfectly in control and there was complete passage of analytes through the column. A 47.2 cm/sec linear velocity was established for the best possible separation efficiency. The ion source temperature was maintained at 200°C, and the GC and MS interface temperature was set to 250°C; it would take about three minutes in solvent cut time to avoid interference from the solvent peak. The results were interpreted using the NIST library.

## *In vitro* studies

### Pancreatic lipase inhibition assay

The protocol followed was already published. Briefly, dimethyl sulfoxide (DMSO) was used to dissolve the extract at a concentration of 1 mg/ml. Pancreatic lipase (50 µl) and reaction buffer (30 µl), along with either extract or standard solution at 1 mg/ml, were mixed together, and this mixture was continuously shaken and incubated at 37°C for 10 min. To initiate the enzymatic reaction, 100 µl of 1 mM 4-nitrophenyl phosphate (4-NPP) was added as a substrate. The appearance of a yellow color indicated the formation of 4-nitrophenol (4-NP), a product of substrate hydrolysis. Orlistat was selected as the standard reference drug for comparison. The experiment was done in triplicates, and absorbance was measured at 405 nm (Bae and Kim 2011; Stoytcheva et al. 2012). Percentage inhibition was calculated using the following formula:

$$\%\ inhibtion = \left(Abs\ of\ enzyme - Abs\ of\ the\ \frac{extract}{abs}\ of\ enzyme\right) x100$$

## *In vivo* studies

### Animal housing and diet

This work used male Sprague-Dawley rats with an age range of 4–5 weeks, body weights ranging from 150 to 200 g, and kept them in housing facilities at 22°C ± 2°C under controlled humidity, 40%–60% and controlled photoperiod for 12 ± 1 hr. Animals received free access to food and water. The high-fat diet (HFD) formulation contained 29.5 g of beef tallow, 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 a vitamin mixture, and 10.5 g of a mineral mixture.

### Ethical approval

The study obtained ethical clearance from the Research and Ethics Committee of Superior University, Lahore, Pakistan (Reference number: SU/FoP/MSPCW/F22-06).

### Experiment

After one week of accommodation, the rats were assigned random numbers. Apart from the normal control group, rats were fed an HFD for 4 weeks. The rats fed on HFD were found to have a visible increase in body fat after four weeks compared to the rats fed on a regular diet, thereby initiating the treatment protocol. The extract or the

positive control orlistat was administered orally five times a week by gavage in a solution containing 0.5% CMC for eight weeks. Normal control group rats were provided with an equal volume of 0.5% CMC. Their food intake and body weight were observed thrice a week. Before sacrifice, all the animals involved in the experiment were starved for the whole night. Animals were euthanized under deep anesthesia induced by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and their organs - liver, kidney, and pancreas- were histologically examined after taking blood samples from all the animals (Lim et al. 2013; Lee et al. 2018). The summary of each animal group (n=6) is as follows:

Group I (normal control group): Received standard diet only.

Group II (disease group): Rats were fed on a high-fat diet (HFD) to induce disease.

Group III (CPRFM/treatment group): Obese rats, induced by HFD were orally administered a 500 mg/kg dose of methanol extract of *Carica papaya* fruit peel (CPRFM) daily for 14 days.

Group IV (standard group): Standard treatment with orlistat (10 mg/kg) was administered orally once daily for 14 days following HFD-induced obesity.

### Statistical analysis

Statistical analysis was conducted with GraphPad Prism and Microsoft Excel. A regression analysis of phytochemicals was utilized with an *in vitro* study expressed as mean ± standard deviation (SD). The mean of data collected in an *in vivo* study used a one-way ANOVA coupled with post hoc Tukey's test to derivate the SEM. *P*-values less than 0.05 were considered statistically significant.

## Results

### Qualitative and quantitative assessment of the phytochemicals

The qualitative analysis of the phytochemicals was carried out using various established protocols. Any color change during the reaction was noted. The results of the qualitative analysis are presented in Table 1.

Table 1. Qualitative assessment of phytochemicals in methanol extract of *Carica papaya* fruit peel

| Test | Methanol extract |
|---|---|
| Proteins | ++ |
| Lipids | + |
| Carbohydrates | +++ |
| Flavonoids | +++ |
| Polyphenols | +++ |
| Saponins | ++ |

+ = weak reaction, ++ = moderate reaction, +++ = intense reaction

The results indicated the presence of proteins, carbohydrates, and lipids in the methanol extract, which means its nutritional value. In addition, the presence of polyphenols and flavonoids suggests the plant's medicinal value. The results of the estimation of primary metabolites are represented in Table 2. The content of total proteins was estimated through the BSA standard curve having $y = 0.0005x + 0.015$ and $R^2 = 0.9794$. The estimation of carbohydrates was done through a glucose calibration curve with $y = 0.0003x + 0.0138$ and $R^2 = 0.9897$. The estimation of primary metabolites proved the existence of carbohydrates (467±0.045 mg/g), proteins (106.55±0.076 mg/g) and lipids (45.79±0.095 mg/g), which proved to be rich in nutritional contents.

The results of the estimation of primary metabolites are represented in Table 3. Total polyphenol contents were determined based on the gallic acid standard curve: $y = 0.0028x + 0.0082$ $R^2 = 0.9832$. The total flavonoid contents were determined using a quercetin calibration curve with $y = 0.0023x + 0.0092$ and $R^2 = 0.9869$. All standard curves are provided in Supplementary File 1. The results of the present study established the existence of essential secondary metabolites, such as polyphenols (167.71±0.096 µg/g),

flavonoids (156.29±0.071 µg/g) and glycosaponins (109.34±0.083 µg/g).

## FTIR profiling

Figure 1 is the FTIR spectrum scan showing peaks corresponding to different functional groups. A strong, broad peak at 3310 $cm^{-1}$ corresponds to the O–H stretching vibrations indicative of hydroxyl groups overlapping with N–H stretching by amines or amides. Peaks from 2920 to 2850 $cm^{-1}$ signify C–H stretching of aliphatic –$CH_2$ and –$CH_3$ groups. A broad absorption at 1760 $cm^{-1}$ corresponds to C=O stretching, which is commonly found in esters or carboxylic acids. The band at 1610 $cm^{-1}$ is due to C=C stretching vibrations of aromatic rings or alkenes. Other peaks between 1400–1460 $cm^{-1}$ correspond to C–H bending and bands between 1200–1300 $cm^{-1}$ and 1000–1100 $cm^{-1}$ correspond to C–O and C–O–C stretching, respectively, indicating the presence of alcohols, esters, ethers, or polysaccharides. The fingerprint area of 600–900 $cm^{-1}$ displays out-of-plane C–H bending motions characteristic of aromatic compounds.

## UV/Vis scanning

For the UV/Vis spectroscopy profiling of the papaya ripe fruit peel's methanol extract, the result is as presented in Figure

2. The four major peaks recorded in the scan were 505.05 nm (peak 1), 267.90 nm (peak 2), 260.50 nm (peak 3) and 226.90 nm (peak 4).

## GC/Mass analysis

The results for GC/Mass spectroscopy are shown in Figure 3. The observations indicated the availability of some of the valuable compounds such as 2-Pentanone, 4-hydroxy-4-methyl, diacetone alcohol, docosanoic acid, octadecanoic acid, Oxirane, -epoxy tetradecane, 11,14-eicosadienoic acid and 1,2-benzenedicarboxylic acid (Table 4).

Table 2. Quantitative estimation of primary metabolites in methanol extract of *Carica papaya* fruit peel (mean ± SD, mg/g dry weight)

| Sample | Total proteins | Total carbohydrates | Total lipids |
|---|---|---|---|
| Methanol extract of *Carica papaya* ripe fruit peel | 106.55±0.076 | 467±0.045 | 45.79±0.095 |

Table 3. Quantitative estimation of primary metabolites in methanol extract of *Carica papaya* fruit peel (mean ± SD, µg/g dry weight)

| Sample | Total polyphenols | Total flavonoids | Glycosaponins |
|---|---|---|---|
| Methanol extract of *Carica papaya* ripe fruit peel | 167.71±0.096 | 156.29±0.071 | 109.34±0.083 |

Table 4. Identification of compounds in methanol extract of *Carica papaya* fruit peel using GC/Mass

| Compound | Retention Time | Area % | Molecular formula |
|---|---|---|---|
| 2-Pentanone, 4-hydroxy-4-methyl, diacetone alcohol | 3.118 | 9.19 | $C_6H_{12}O_2$ |
| Docosanoic acid | 3.47 | 5.29 | $C_{22}H_{44}O_2$ |
| Octadecanoic acid | 14.63 | 3.03 | $C_{18}H_{36}O_2$ |
| Phytol | 15.375 | 1.87 | $C_{20}H_{40}O$ |
| Eicosadienoic acid | 15.681 | 2.38 | $C_{20}H_{36}O_2$ |
| 1,2-benzene dicarboxylic acids | 17.83 | 78.25 | $C_8H_6O_4$ |

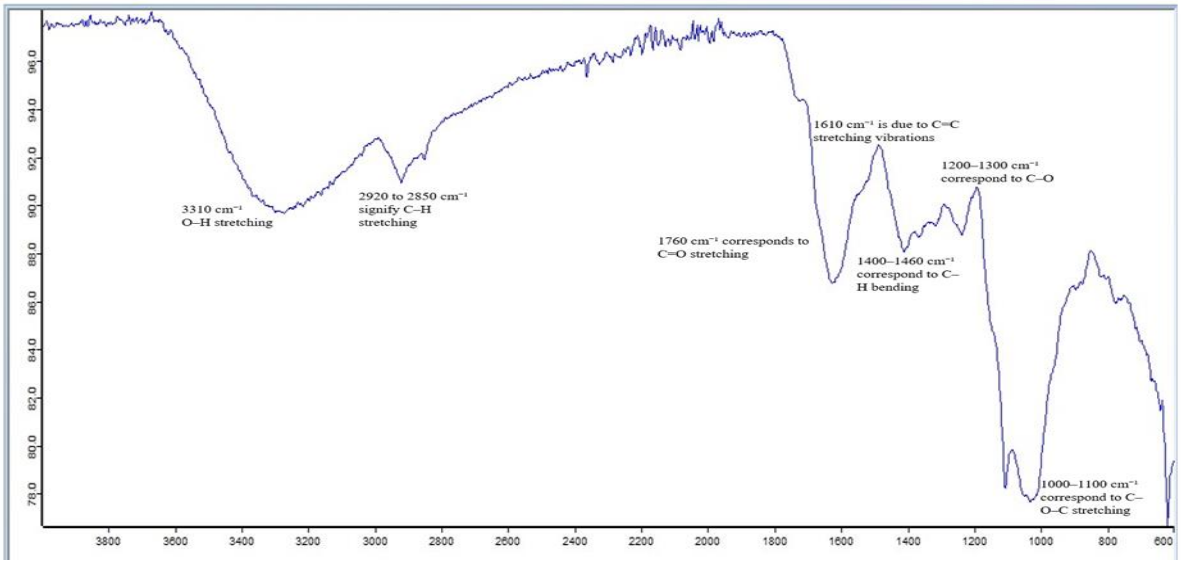



Figure 1. FTIR spectra of *Carica papaya* fruit peel powder

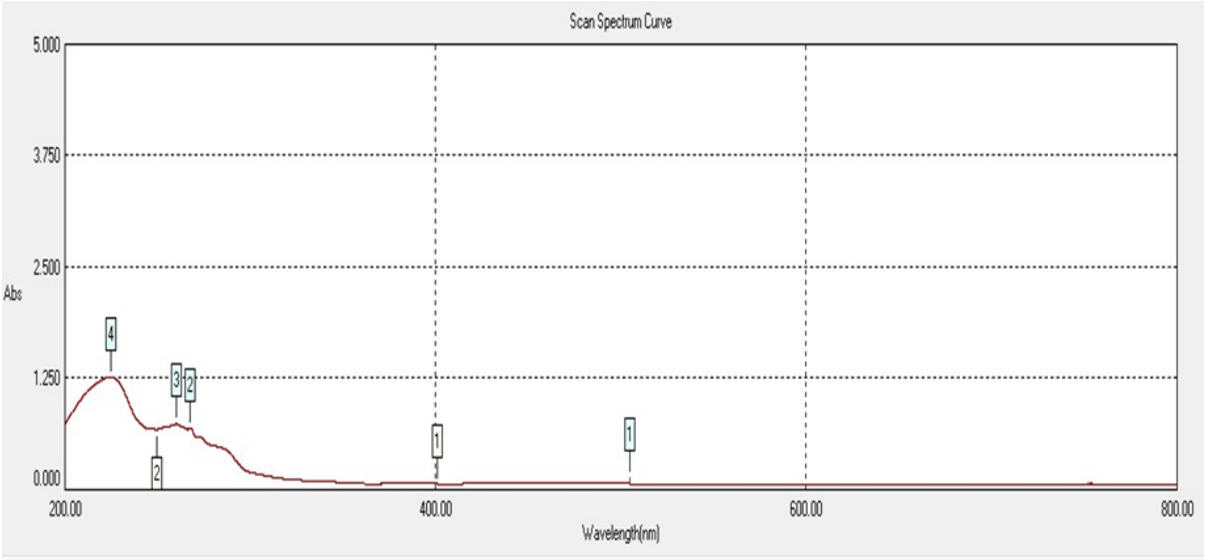



Figure 2. UV/Vis profiling of methanol extract of *Carica papaya* fruit peel

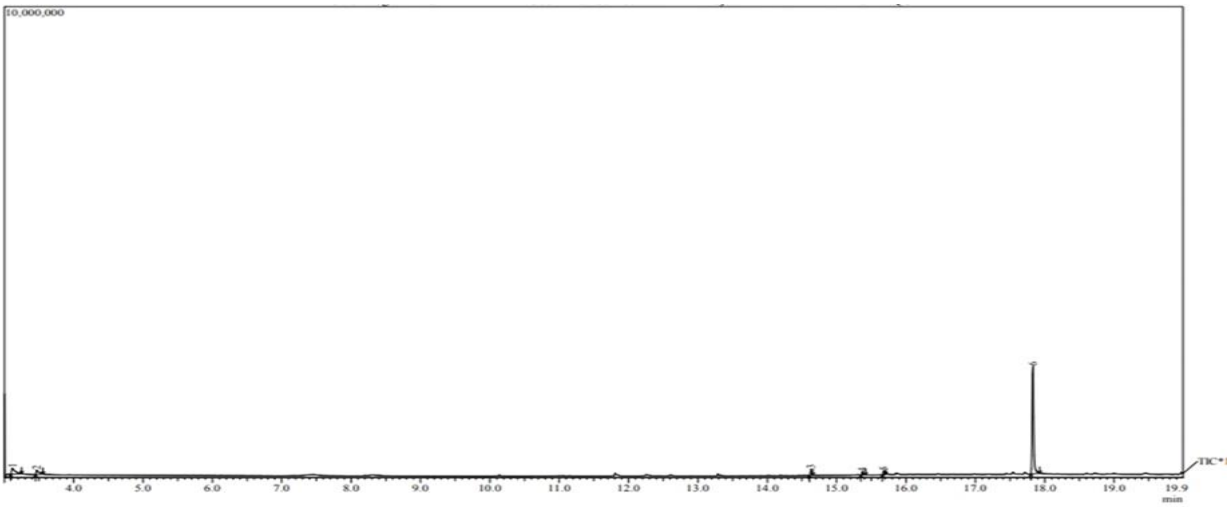


Figure 3. GC/Mass spectra of methanol extract of *Carica papaya* fruit peel

## *In vitro* studies

### Pancreatic lipase inhibition assay

Figure 4 illustrates the results of the pancreatic lipase inhibition assay. The extract and standard drug were evaluated at various doses to determine their ability to inhibit pancreatic enzyme pancreatic enzyme activity. According to the study findings, the extract and orlistat had a concentration-dependent effect. The IC50 of the methanol extract was found to be 510.48 µg/ml, and the IC50 of orlistat was estimated to be 440.70 µg/ml.

### Lipase inhibition assay

## *In vivo* studies

### Weight change

The results of the body weight changes are presented in Figure 5. The change in the study's results between the normal group and the disease group shows weight change is also different in these two groups by day 3 to day 14 at ($p<0.05$). However, extract and orlistat (standard) had a highly significant weight reduction compared to the disease group. They showed insignificant results compared to the control group from day 10 to day 14 ($p>0.05$).

### Biochemical parameters

The results of lipid profile, liver function tests (LFTs) and renal function tests (RFTs) are summarized in Tables 5-7. The findings illustrated that the methanol extract significantly improved the lipid profile, LFTs, and RFTs.

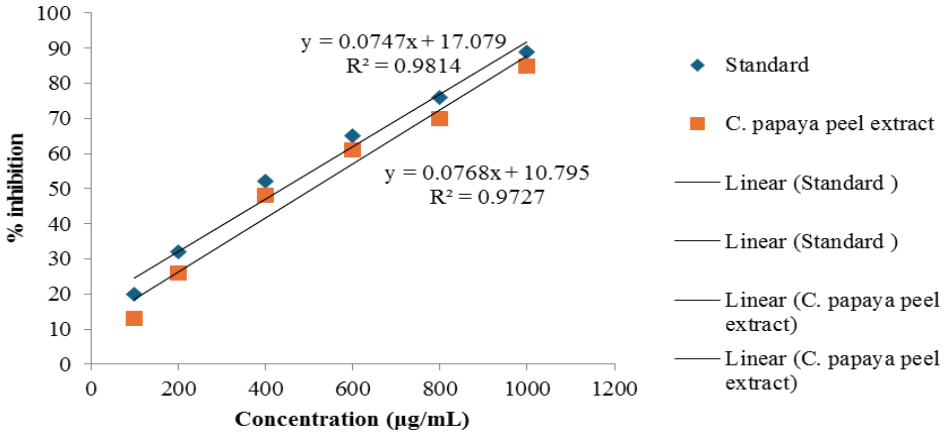



Figure 4. Concentration-dependent response of methanol extract of *Carica papaya* fruit peel and standard drug (orlistat) using pancreatic lipase assay

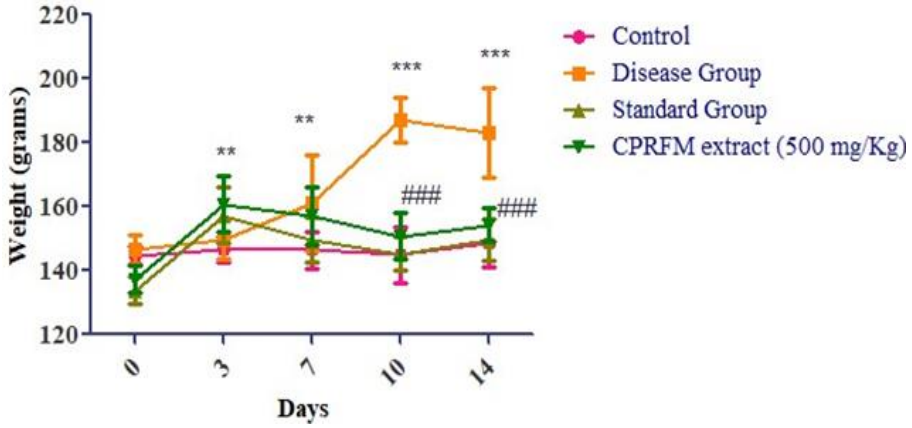



Figure 1. Change in weight after *in vivo* administration of methanol extract of *Carica papaya* fruit peel (CPRFM) and standard drug (orlistat) using high-fat diet-induced obesity rats. *Disease group compared to normal group, #*treatment* and standard groups compared to disease group. * and #$p<0.05$, ** and ##$p<0.01$, and *** and ### $p<0.001$,

Table 5. Effect of methanol extract of *Carica papaya* fruit peel on lipid profile in rats with high-fat diet-induced obesity

| Group | Total lipids (mg/dl) | Cholesterol (mg/dl) | TGs (mg/dl) | HDL cholesterol (mg/dl) | LDL cholesterol (mg/dl) | VLDL cholesterol (mg/dl) |
|---|---|---|---|---|---|---|
| Control | 230±0.67 | 67±0.32 | 85±0.56 | 27±0.68 | 63±0.47 | 16±0.43 |
| Disease | 267±0.04*** | 85±0.48** | 90±0.31* | 33±0.29* | 80±0.36*** | 25±0.55** |
| Standard | 230±0.10### | 72±0.09## | 81±0.34# | 28±0.53## | 65±0.07### | 19±0.31## |
| CPRFM (500 mg/kg) | 218±0.07### | 72±0.71## | 84±0.67# | 30±0.11# | 65±0.57### | 17±0.37### |

*Disease group compared to normal group, #methanol extract of *Carica papaya* fruit peel (CPRFM) and orlistat (standard) groups compared to disease group, * and # p<0.05, ** and ##p<0.01 , and *** and ### p<0.001. Triglycerides (TGs), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL)

Table 6. Effect of methanol extract of *Carica papaya* fruit peel on liver function tests (LFTs) in rats with high-fat diet-induced obesity

| Group | 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 | 0.64±0.23 | 0.32±0.28 | 0.32±0.07 | 50±0.61 | 60±0.43 | 90±0.35 | 6.62±0.25 | 4.62±0.05 | 2.00±0.11 |
| Disease | 0.70±0.08* | 0.34±0.46 | 0.36±0.54* | 65±0.06** | 109±0.48** | 157±0.41*** | 7.24±0.66* | 4.62±0.05 | 3.10±0.29** |
| Standard | 0.64±0.16# | 0.33±0.37 | 0.31±0.42# | 32±0.41### | 57±0.71### | 86±0.29### | 6.15±0.59## | 4.10±0.39 | 2.15±0.76## |
| CPRFM (500 mg/kg) | 0.68±0.15# | 0.32±0.32 | 0.38±0.32 | 56±0.65### | 85±0.05### | 127±0.27### | 6.85±0.71## | 4.33±0.48 | 2.52±0.28## |

*Disease group compared to normal group, #methanol extract of *Carica papaya* fruit peel (CPRFM) and orlistat (standard) groups compared to disease group. *, #p<0.05, **, ##p<0.01, and ***, ###p<0.001. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)

Table 7. Effect of methanol extract of *Carica papaya* fruit peel on renal function tests (RFTs) in rats with high-fat diet-induced obesity

| Group | Urea (mg/dl) | BUN (mg/dl) | Creatinine (umol/L) |
|---|---|---|---|
| Control | 21.00±0.54 | 10.59±0.32 | 53.00±0.16 |
| Disease | 32.00±0.44** | 14.50±0.52* | 75.00±0.41*** |
| Standard | 18.60±0.39### | 9.30±0.17## | 54.00±0.67### |
| CPRFM (500 mg/kg) | 24.80±0.27## | 12.40±0.41# | 55.00±0.58### |

*Disease group compared to normal group, # methanol extract of *Carica papaya* fruit peel (CPRFM) and orlistat (standard) groups compared to disease group. *, #p<0.05, **, ##p<0.01, and ***, ###p<0.001. Blood urea nitrogen (BUN)

## Histopathological findings

The histopathological findings of the pancreas, liver, and kidneys are shown in Figures 6-8. The tissue morphology was studied at 10x magnification using Hematoxylin and Eosin (H and E) staining. The study demonstrated significant differences among the untreated control, disease, standard, and *C. papaya* fruit peel extract-treated groups.

The pancreas of the disease group showed islet hyperplasia, increased inflammation, and oxidative stress markers that compromised insulin secretion. On the other hand, the control group had normal architecture of the pancreas, characterized by well-defined islets and healthy acinar cells. The standard treatment group showed significant protection with preserved islet integrity and reduced inflammation. The extract-treated group partially restored the pancreatic structure and improved β-cell function, showing moderate protection.

The liver of the disease group showed severe steatosis, ballooning degeneration, and inflammatory infiltrates, indicating

progression toward non-alcoholic fatty liver disease (NAFLD). However, the control group remained consistent in its hepatic structure without damaging hepatocytes. Both extract and standard treatment groups showed minimal steatosis and intact liver architecture, indicating protection against obesity-induced liver damage.

The kidneys of the disease group presented with glomerulosclerosis, tubular atrophy, and interstitial inflammation, which characterize obesity-related renal impairment. On the other hand, the control group showed normal morphology of the glomeruli and intact tubular epithelium. In the extract-treated group, there was marked preservation of tubular integrity and architecture of the glomeruli, indicating a protective activity against obesity-related kidney damage. Similarly, the standard treatment improved renal morphology by reducing the damage to the glomeruli, strengthening its therapeutic action.

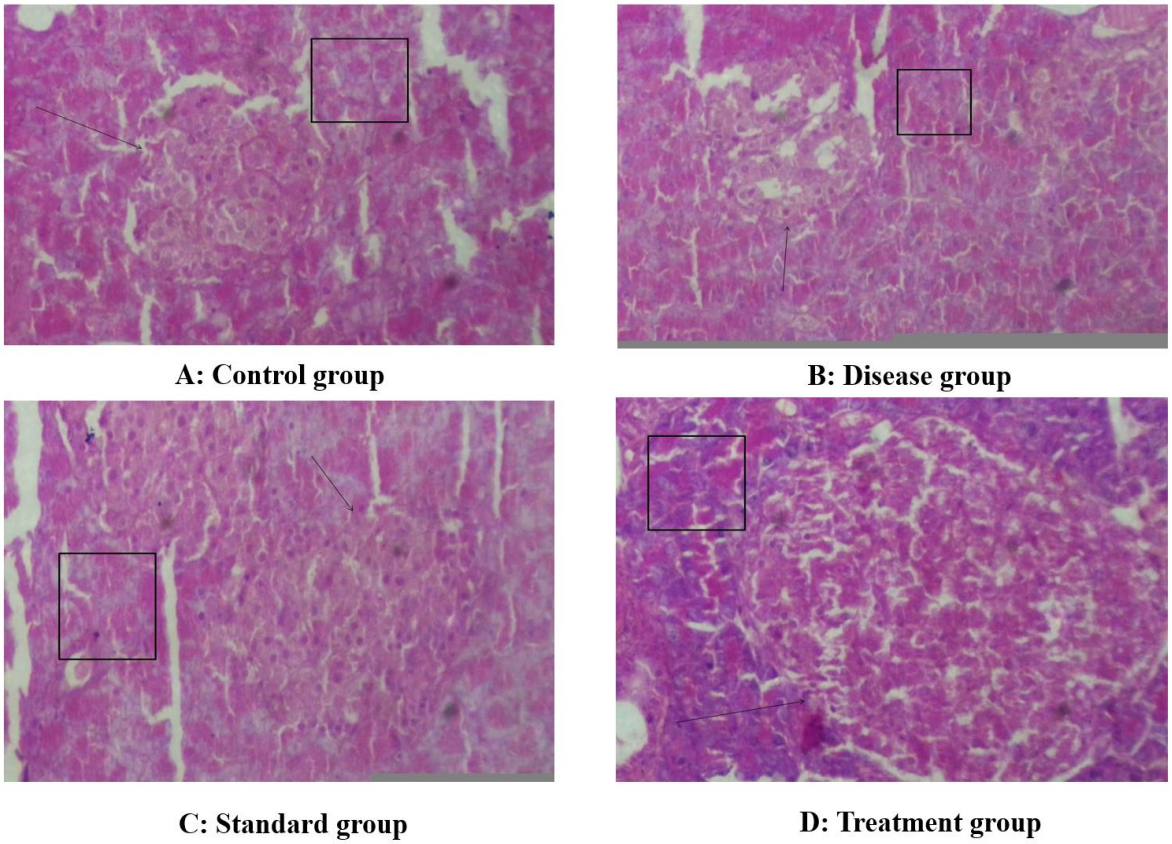



Figure 2. Effect of methanol extract of *Carica papaya* fruit peel on the pancreas histology in rats with high-fat diet-induced obesity (10x). Control group – Normal pancreatic architecture with well-preserved islets of Langerhans (indicated by the box), Disease group – Disruption of normal architecture and reduced islet density after HFD administration, Standard group (Orlistat 10 mg/kg) – Partial restoration of pancreatic structure with moderately preserved islets and Treatment group (methanol extract of *Carica papaya* fruit peel 500 mg/kg) – Improved architecture with visible islet regeneration, though less compact than the control.

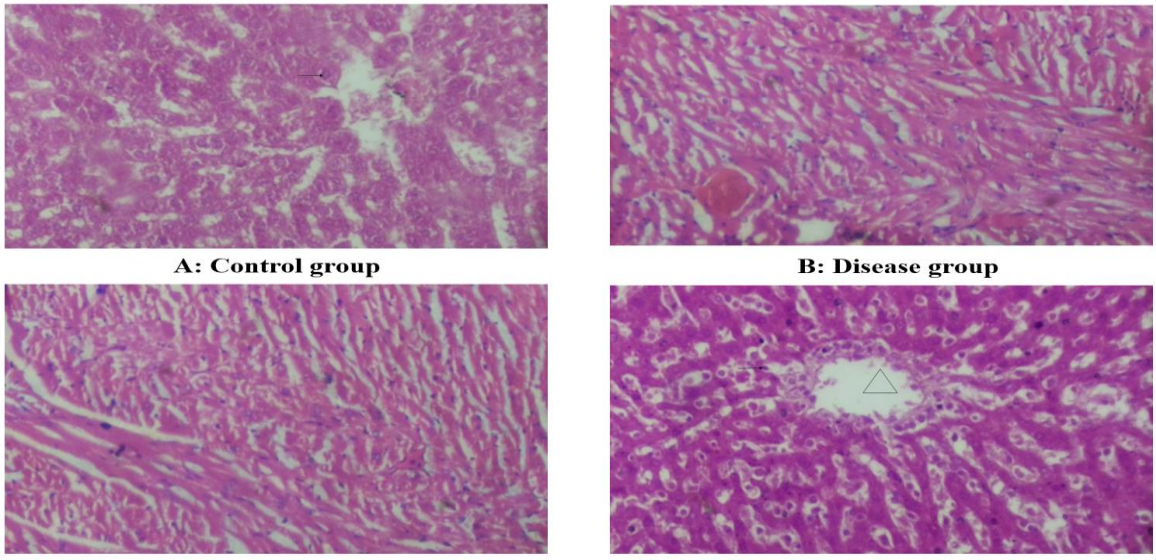



Figure 3. Effect of methanol extract of *Carica papaya* fruit peel on the liver histology in rats with high-fat diet-induced obesity (10x). The control group exhibited normal hepatic architecture with polygonal hepatocytes radiating around a central vein, indicating a well-preserved lobular structure. The disease group showed disrupted liver architecture with distorted polygonal shapes, hepatocellular degeneration, and dilated sinusoids following HFD administration. The standard group (Orlistat 10 mg/kg) demonstrated partial restoration of hepatic structure, with moderately preserved polygonal hepatocytes and improved cellular alignment. The treatment group (methanol extract of *Carica papaya* fruit peel 500 mg/kg) displayed improved liver architecture with regenerating polygonal hepatocytes and a more defined central vein region, though still less organized than the control as indicated by triangle.

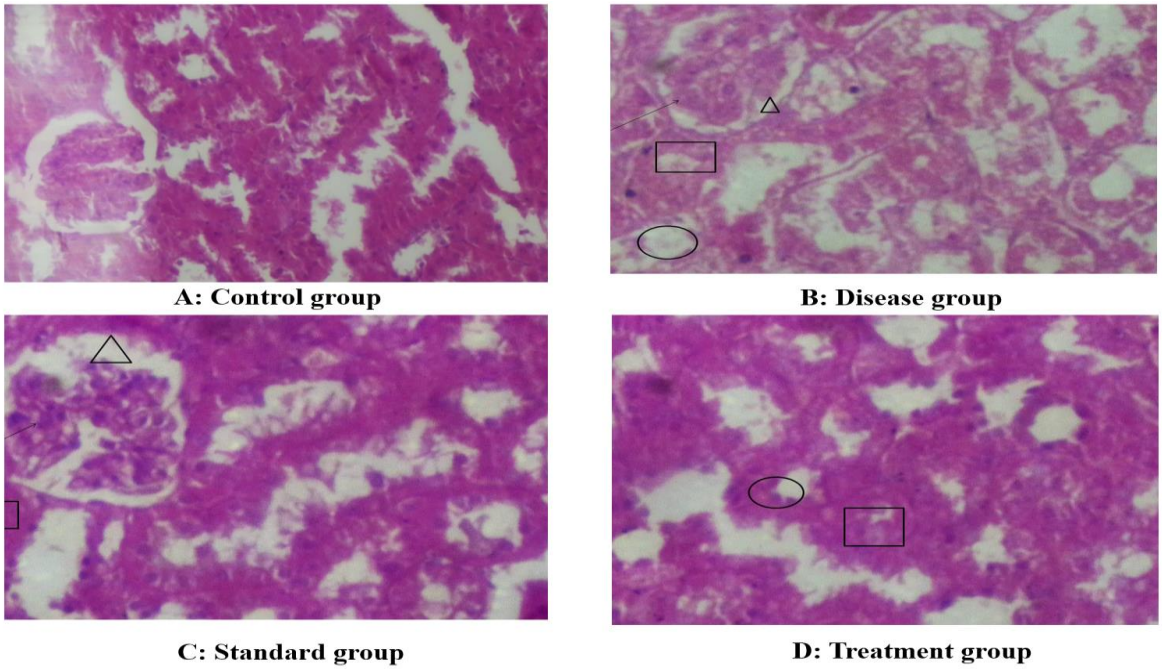



Figure 4. Effect of methanol extract of Carica papaya fruit peel on the kidney histology in rats with high-fat diet-induced obesity (10x). The control group displayed normal renal architecture with clearly defined glomeruli and intact Bowman's capsules, surrounded by uniform renal tubules. In the disease group, there was evident glomerular shrinkage (triangle) and tubular dilation (circle), along with disrupted tissue organization and inflammatory infiltration, indicating damage (box) due to HFD administration. The standard group (Orlistat 10 mg/kg) showed partial recovery with restored glomerular structures (triangle**)** and reduced tubular vacuolization. In the treatment group (methanol extract of Carica papaya fruit peel 500 mg/kg), noticeable improvement was observed, with glomerular regeneration (box) and relatively preserved tubules (circle), although the structure was still less compact compared to the control.

## Discussion

Obesity, defined as an excessive accumulation of body fat, is a worldwide health disorder leading to chronic diseases like type 2 diabetes, cardiovascular disease, and cancer. Its multifactorial aetiology encompasses physiological, behavioral, environmental, and genetic components (Elmaleh-Sachs et al. 2023). Its management involves behavioral therapy, dietary modification, exercise, and occasionally medication or surgery. Yet, nature-based alternatives such as medicinal plants are increasingly researched owing to fears of the side effects and expenses of drug treatments (Blüher et al. 2023).

Traditional medicine has long employed medicinal plants. Their bioactive compounds may modulate metabolic pathways, decrease fat absorption, improve fat metabolism, and inhibit appetite. Most medicinal plants possess alkaloids, saponins, flavonoids, and polyphenols, which have shown anti-obesity effects (Talib et al. 2020; Nyangono et al. 2022). Medicinal plants have fewer side effects compared to synthetic medicines and offer a more holistic method of treating obesity. Standardization of dosage, safety, and efficacy are, however, issues. Whereas the evidence supports their anti-obesity effects, clinical trials need to verify long-term efficacy and modes of action (Munir et al. 2022; Sun and Shahrajabian 2023). *Carica papaya* or papaya contains many bioactive constituents providing diverse therapeutic properties like better digestion, minimized inflammation, and antioxidant effects as a result of excessive levels of vitamins A and C. Its medicinal properties make it a desirable dietary ingredient and a promising drug discovery candidate (Ugbogu et al. 2023; Babalola et al. 2024).

*Carica papaya* is of great potential in anti-obesity treatment. Its high fibre and low-calorie content increase satiety and reduce calorie consumption, making it useful in weight control. Its antioxidants and anti-inflammatory substances can also inhibit obesity-induced chronic inflammation. Papain, a papaya enzyme, aids digestion, metabolism, and further control of obesity. Papaya has been found to regulate blood glucose and improve fat metabolism, possibly enhancing its purpose in weight regulation (Od-Ek et al. 2020).

The methanol extract from the peel of ripe *C. papaya* fruit was found to be rich in bioactive phytochemicals, including polyphenols, flavonoids, and glycosaponins. The polyphenol content indicated significant antioxidant activity. Polyphenols are known to influence lipid metabolism, reduce oxidative stress, and affect energy balance, making them relevant for obesity prevention and treatment. Similarly, the extract contained a high concentration of flavonoids, which are associated with anti-obesity effects through mechanisms such as inhibiting adipogenesis, enhancing lipid breakdown, and modulation of glucose uptake. Glycosaponins have been reported to curb appetite, inhibit pancreatic lipase activity, and reduce lipid absorption in the intestines, contributing to their anti-obesity effects. The abundance of these phytochemicals in ripened *C. papaya* fruit peel suggests their potential as natural remedies for obesity and related metabolic disorders. Further *in vivo* studies and clinical trials are needed to validate these effects and to explore their mechanisms in more detail (Martial-Didier et al. 2017; Od-Ek et al. 2020; Munir et al. 2022).

Additionally, the FTIR spectrum of the methanol extract showed several characteristic absorption peaks linked to various functional groups, confirming the presence of phytochemicals, such as polyphenols, flavonoids, terpenoids, and tannins. GC/mass spectroscopy of the methanol extract of the ripened *C. papaya* fruit peel identified several bioactive molecules. Among them are 2-pentanone, 4-hydroxy-4-methyl (diacetone alcohol), used as a solvent and antimicrobial agent, and phytol, a diterpene with antioxidant and lipid-lowering properties. Long-chain fatty acids such as docosanoic acid, octadecanoic

acid, and eicosadienoic acid which are involved in lipid metabolism and energy regulation, were also identified. The presence of 1,2-benzene dicarboxylic acids suggests their potential antioxidant and metabolic-modulating activities. These compounds contribute to the extract's potential effectiveness in managing obesity (Wrzosek et al. 2022; Yan et al. 2022).

The study demonstrated that papaya peel extract can inhibit pancreatic lipase activity, suggesting a potential mechanism for reducing fat absorption to manage obesity. Reducing fat absorption is crucial for weight control and promoting metabolic health (Rajan et al. 2020). This research supports the anti-obesity effectiveness of *C. papaya* ripe fruit peel, as evidenced by an *in vivo* rat study. After treatment with papaya peel extract, the groups receiving the extract exhibited significant reductions in body weight, fat accumulation, and serum lipid levels. A previous study found that papaya juice could inhibit pancreatic lipase activity, restore normal organ histology, elevate serum superoxide dismutase levels, and lower serum cytokine (interleukin 6) levels (Od-Ek et al. 2020). Another study showed similar results with ethanol extract of papaya leaf (Shaikh et al. 2025). The current findings align with earlier research indicating that plants rich in flavonoids and polyphenols enhance metabolic health (Saad 2023; Mohebbati et al. 2024). The study underscores the potential of incorporating papaya peels into weight loss programs and emphasizes the need for further research to evaluate their long-term effects and feasibility as a functional food product (Asyifah et al. 2014).

This research, however, is not without limitations. The small size of the rat sample might have implications for statistical power and result generalizability. The study period could be too short to establish the long-term impact of papaya peel extract on obesity. Fluctuations in flavonoid and polyphenol levels could have affected results. Although improvements in lipid profile and body weight were noted, the biochemical pathways are unknown, and extrapolation from animals to human beings is unclear. Clinical trials are thus warranted to establish safety and efficacy in human subjects.

In conclusion, the *C. papaya* fruit peel extract exhibited therapeutic potential in an anti-obesity model, as demonstrated by the pancreatic lipase assay and the high-fat diet model. The extract improved organ function, reduced inflammation, and provided protection against obesity-related damage. Further *in vivo* studies and clinical trials should be conducted to analyze its long-term efficacy and underlying mechanisms, which may lead to the development of novel anti-obesity therapies.

## Conflicts of interest

The authors have no conflicts of interest to declare.

## Ethical Committee Approval

The study obtained ethical approval from the Research and Ethics Committee of Superior University, Lahore, Pakistan (Reference number: SU/FoP/MSPCW/F22-06).

## Code of Ethics

All experimental procedures were carried out in accordance with the ethical guidelines approved by the Institutional Ethical Committee of Superior University. Animal handling and treatment complied with university policies to ensure humane care and use.

## Authors' Contributions

Concept: R.A., K.A., Design: K.A., S.A.S., Data Collection or Processing: R.A., M.Z.A., M.A., Analysis or Interpretation: K.A., S.A.S., S., I.B., Literature Search: R.A., M.Z.A., I.B., S., Writing: R.A., K.A., M.A.

## References


Arvouet-Grand A, Vennat B, Pourrat A, et al. (1994) Standardization of propolis extract and identification of principal constituents. J Pharm Belg, 49:462-468.

Ashiq K, Bajwa MA, Khokhar R, et al. (2021) A Cross-sectional analysis of knowledge, beliefs and practices regarding traditional medicine use among pharmacy students of different private and government universities of Lahore, Pakistan. Bangladesh J Med Sci, 21, 323–334.

Asyifah MR, Lu K, Ting HL, et al. (2014) Hidden potential of tropical fruit waste components as a useful source of remedy for obesity. J Agri Food Chem, 62:3505-3516.

Babalola BA, Akinwande AI, Otunba AA, et al. (2024) Therapeutic benefits of *Carica papaya* : A review on its pharmacological activities and characterization of papain. Arab J Chem, 17:105369.

Bae J-S, Kim T-H (2011) Pancreatic lipase inhibitory and antioxidant activities of Zingiber officinale extracts. Food Sci Preserv, 18:390-396.

Bakinowska E, Krompiewski M, Boboryko D, Kiełbowski K, et al (2024) The role of Inflammatory mediators in the pathogenesis of obesity. Nutr, 16:2822.

Batool A, Ashiq K, Khalid M, et al. (2024) An up-to-date review on the phytochemical and pharmacological aspects of the lawsonia inermis. J Anim Plant Sci, 34:22-23.

Blüher M, Aras M, Aronne LJ et al. (2023) New insights into the treatment of obesity. Diabetes Obes Met, 25:2058-72.

Blumberg B (2024) Do We Need a New Unifying Theory for Obesity? Endocrinol, 165:bqae029.

Cornier M-A (2022) A review of current guidelines for the treatment of obesity. Am J Manag Care, 28:88-96.

Elmaleh-Sachs A, Schwartz JL, Bramante CT, et al. (2023) Obesity management in adults: a review. JAMA, 330:2000-2015.

Hall KD, Farooqi IS, Friedman JM et al. (2022) The energy balance model of obesity: beyond calories in, calories out. Am J Clin Nutr, 115:1243-1254.

Hasani-Ranjbar S, Jouyandeh Z, Abdollahi M (2013) A systematic review of anti-obesity medicinal plants-an update. J Diabetes Metab Dis, 12:1-10.

Kokkorakis M, Chakhtoura M, Rhayem C, et al. (2024) Emerging pharmacotherapies for obesity: a systematic review. Pharmacol Rev,100002.

Latif A, Ashiq K, Ashiq S, et al. (2020) Phytochemical analysis and in vitro investigation of anti-inflammatory and xanthine oxidase inhibition potential of root extracts of bryophyllum pinnatum. J Anim Plant Sci, 30:219-228.

Lee J-J, Kim HA, Lee J (2018). The effects of Brassica juncea L. leaf extract on obesity and lipid profiles of rats fed a high-fat/high-cholesterol diet. Nutr Res Prac, 12:298-306.

Lim HH, Lee SO, Kim SY, et al. (2013) Anti-inflammatory and anti-obesity effects of mulberry leaf and fruit extract on high fat diet-induced obesity. Exper Biol Med, 238:1160-1169.

Lowry OH, Rosebrough NJ, Farr AL, et al. (1951) Protein measurement with the Folin phenol reagent. J biol Chem, 193:265-275.

Ludwig DS, Apovian CM, Aronne LJ, et al. (2022) Competing paradigms of obesity pathogenesis: energy balance versus carbohydrate-insulin models. Eur J Clin Nutr, 76:1209-21.

Martial-Didier AK, Hubert KK, Parfait KE, et al. (2017) Phytochemical properties and proximate composition of papaya (Carica papaya L. var solo 8) peels. Turkish J Agri Food Sci Technol, 5:676-80.

Matsuane C, Kiage BN, Karanja J, et al. (2023) Hypolipidaemic effects of papaya (*Carica papaya* L.) juice on rats fed on a high fat and fructose diet. J Nutr Sci, 12:e76.

Mohebbati R, Momeni-Moghaddam MA, Asghari R, et al. (2024). The comparison of the effects of nano-silymarin and silymarin on high-fat diet-induced fatty liver of adult male rats. Avicenna J Phytomed AJP, 14:365-375.

Munir MU, Saeed MA, Masood Z, et al. (2022) Analgesic and anti-diabetic potential of Roystonea regia. Int J Pharma Integ Health Sci, 3:03-14.

Nabi M, Latif A, Ashiq K, et al. (2023) Antioxidant and anti-inflammatory potential of Daucus carota L. seed extracts. J Anim Plant Sci, 33:220-228.

Nyangono N, Soppo L, Nko'o M, et al. (2022) Medicinal plants used by traditional practitioners for the treatment of diabetes,

obesity and arterial hypertension in the Dja and Lobo Department of Cameroon. Saudi J Med Pharm Sci, 8:704-719.

Od-Ek P, Deenin W, Malakul W, et al. (2020) Anti-obesity effect of *Carica papaya* in high-fat diet fed rats. Biomed Rep, 13:1-8.

Rajan L, Palaniswamy D, Mohankumar SK (2020) Targeting obesity with plant-derived pancreatic lipase inhibitors: A comprehensive review. Pharmacol Res, 155:104681.

Saad B (2023) A review of the anti-obesity effects of wild edible plants in the mediterranean diet and their active compounds: from traditional uses to action mechanisms and therapeutic targets. Int J Mol Sci, 24:12641.

Salehsari A, Rahbardar MG, Razavi BM, et al. (2024) Investigating the effect of zeaxanthin on olanzapine-induced metabolic disorders in rats. Avicenna J Phytomed AJP, 14:653-665.

Shahidi F, Wanasundara P (2002) Extraction and analysis of lipids. Food lip, 152-187.

Shaikh Khalil SS, Pathan AA, AR SA (2025) Protective efficacy of carica papaya leaf extracts in high fat diet-induced obesity: evaluation of anti-obesity activity. J Exp Zoo India, 228.

Siddiqui M, Ismail Z, Sahib H, et al. (2009) Analysis of total proteins, polysaccharides and glycosaponins contents of orthosiphon stamineus benth. in spray and freeze dried methanol: water (1: 1) extract and its contribution to cytotoxic and antiangiogenic activities. Pharmacogn Res, 1:320-326.

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vit, 16:144-158.

Stoytcheva M, Montero G, Zlatev R, et al. (2012) Analytical methods for lipases activity determination: a review. Curr Analyt Chem, 8:400-407.

Sun W, Shahrajabian MH (2023) Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Molecules, 28:1845.

Süntar I (2020) Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem Revi, 19:1199-1209.

Talib A, Batool A, Shah S (2020). Evaluation of medicinal activity of eclipta alba. Int J Pharma Integ Health Sci, 1:24-32.

Ugbogu EA, Dike ED, Uche ME, et al. (2023) Ethnomedicinal uses, nutritional composition, phytochemistry and potential health benefits of *Carica papaya* . Pharmacol Res Mod Chin Med, 7:100266.

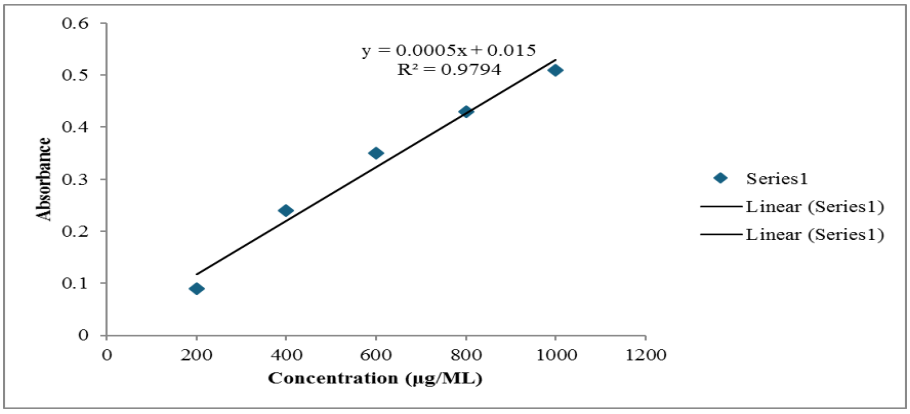
Westbury S, Oyebode O, Van Rens T, et al. (2023) Obesity stigma: causes, consequences, and potential solutions. Curr Obes Rep, 12:10-23.

Wrzosek M, Zawadzka Z, Sawicka A, et al. (2022) Impact of fatty acids on obesity-associated diseases and radical weight reduction. Obes Surg, 1:1-3.

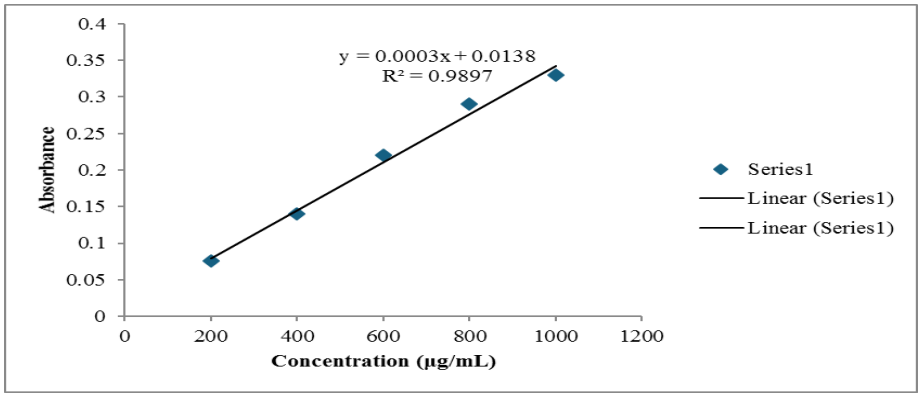
Yan X, Li L, Liu P, et al. (2022) Targeted metabolomics profiles serum fatty acids by HFD induced non-alcoholic fatty liver in mice based on GC-MS. J Pharm Biomed Anal, 211:114620.

Yemm E, Willis A (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J, 57:508-514.

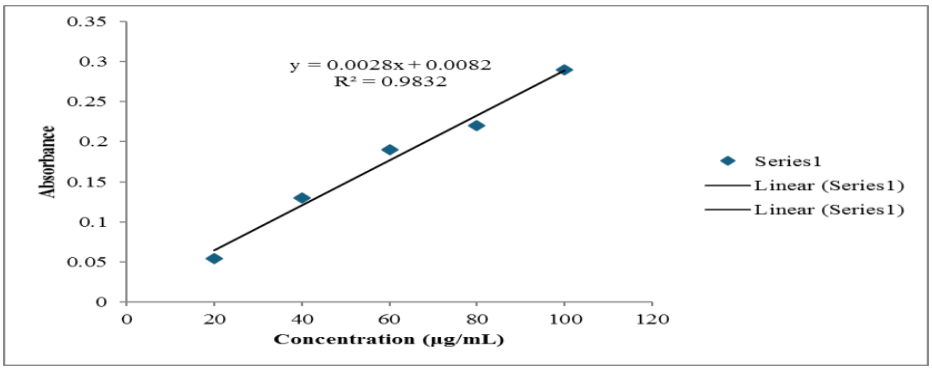
## Supplementary



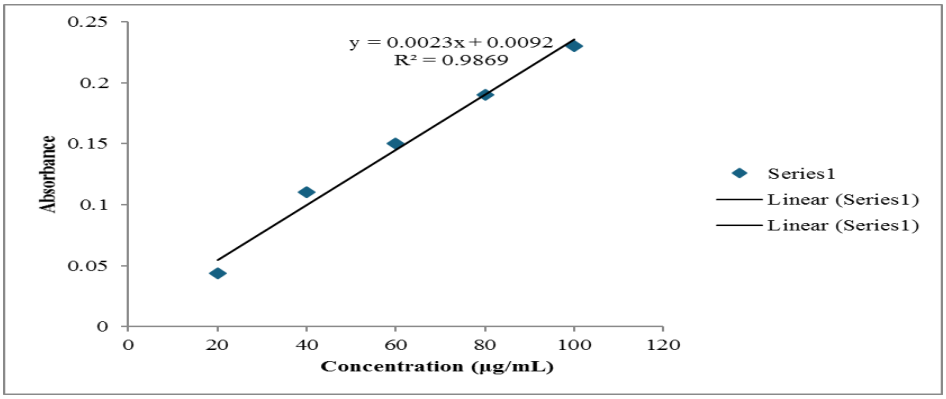

Supplementary Figure 5. Calibration curve of BSA for protein estimation




Supplementary Figure 6. Calibration curve of glucose for carbohydrates estimation




Supplementary Figure 7. Calibration curve of gallic acid for polyphenols estimation




Supplementary Figure 8. Calibration curve of quercetin for flavonoids estimation