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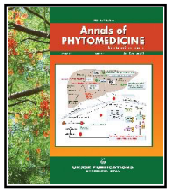

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Phytochemical analysis, antioxidant and antiobesity potentials of the ethanolic extracts of *Ziziphus mauritiana* Lam. fruits

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Abstract

Obesity causes metabolic comorbidities, which are serious health concerns in both industrialized and developing nations. The current study investigated characterize the phytochemical content of ethanolic extracts of *Ziziphus mauritiana* Lam. (Ber) fruit pulp and evaluate its antioxidant and antiobesity potentials using *in vitro* and *in vivo* studies. Qualitative phytochemical analysis of the ethanolic extract found the occurrence of flavonoids, tannins, glycosides, and saponins. Total phenolic content and total flavonoid content were shown that 45.21 ± 1.12 mg GAE/1000 mg and 36.25 ± 0.68 mg QE/1000 mg. The ethanolic extract was found 5.907 µg of quercetin by using HPTLC fingerprinting method. We have screened ethanolic extract for antioxidant effect on DPPH free radical scavenging and evaluated the inhibitory effect on alpha-glucosidase and pancreatic lipase inhibition assay. IC_{50} (Inhibitory concentration) values were calculated to be 92.11 µg/ml, 72.88 µg/ml, and 81.32 µg/ml, respectively. Obesity induces in rats by feeding cafeteria diets for 12 weeks after the end of the induction phase obese rats were administered two different doses of ethanolic extracts (200 mg/kg and 400 mg/kg, body weight) from the 12th to 18th weeks. At the end of the study, ethanolic extract-treated obese rats showed a momentous diminution (p<0.05) in the body weight, food intake, lee index, abdominal circumference, various biochemical parameters, organ weight, and triglyceride contents as compared to cafeteria diet fed negative control group obese rats. Ethanolic extract of *Z. mauritiana* fruits showed inhibition in the process of adipogenesis and reduction in the accumulation of lipid droplets similar to the standard drug (Orlistat) in 3T3-L1 preadipocyte cell lines. These results suggested that *in vitro* and *in vivo* screening of ethanolic extract of the *Z. mauritiana* fruits pulp has strong antiobesity potential and it may be the safe and efficient herbal approach for treating obesity and associated metabolic risk factors.

1. Introduction

Obesity has been characterized by mild level inflammation because of an elevation in adipose tissue volume, which results in hypoxia and the release of cytokines that are pro-inflammatory mediators (Bluher *et al*., 2019). Obesity decreases the life expectancy up to 5-20 years, depending on its comorbidity risk factors such as insulin resistance, dyslipdemia, hypertension, and disturbances in metabolic homeostasis (Abdelaal *et al*., 2017; Bauer *et al*., 2014). Medicines that include such as sympathomimetics, serotonergic agonists, lipase inhibitors, cannabinoid receptor antagonists, and gastrointestinal-derived peptides are all being investigated for their antiobesity potential. However, we still fail to fully comprehend the adverse consequences of their prolonged usage (Rebello *et al*., 2020; Muller *et al*., 2022). Consequently, there is a critical need for the creation of innovative and safe antiobesity medications. Furthermore, several preclinical and clinical researches, as well as several medicinal herbs, have shown significant gains in body weight management with only moderate side effects (Kawser *et al*., 2016). To create effective and secure antiobesity and antidiabetes medications, natural plants are, therefore, a promising alternative strategy. *Z. mauritiana*, belongs to the Rhamnaceae family and is commonly referred to as Ber or Indian Jujube. The jujube fruit has been cultivated for 400 years in China and India, and people have been eating it (Prakash *et al*., 2022). *Z. mauritiana* plant produced remarkable therapeutic effects due to their rich phytoconstituents likes alkaloids, flavonoids, terpenoids, saponin, and pectin are only a few of the many secondary metabolites (Hossain *et al*., 2019). The active phytochemical components of this plant, such as berberine, quercetin, kaempferol, sitosterol, stigmasterol, lanosterol, and others, have all been identified. Studies investigating the antioxidants, antimicrobials, antidiarrheal, antidepressants, immunomodulators, hepatoprotective, neuroprotective, antidiabetic, astringent, and antityphoid medications have been published in the literature (Akanda *et al*., 2021). The plant also has substances that regulate total cholesterol and the metabolism of carbohydrates.

The following research was done taking into account the nutritional and medicinal benefits of *Z. mauritiana* fruits:

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i. Ethanolic pull out of *Z. mauritiana* (EEZM) fruits pulp was subjected to qualitative and quantitative phytoanalysis.

ii. To assess the antioxidant, α-glucosidase inhibition, and pancreatic lipase embarrassment activities of EEZM fruits pulp.

iii. To investigate the antiobesity potential of EEZM fruit pulp in cafeteria diet-fed obese rats and 3T3-L1 preadipocytes cell lines.

2. Materials and Methods

2.1 Chemicals

DPPH free radical, Folin-Ciocateu reagent, sodium carbonate, sodium acetate, aluminum chloride hexadydrate, quercetin, 4-nitro phenyl palmitate, p-nitrophenyl α-D-glucoside (pNPG), α-glucosidase, pancreatic lipase, MTT reagent, Oil Red O Isopropanol, DMEM with high glucose and Fetal bovine serum were purchased from Sigma Aldrich. cholesterol and Glucose POD kit procured from Erba Mannheim Ltd, India.

2.2 Animals for experiments

According to the study protocol, healthy male Wistar rats (80-100 g) and weighing 7-8 weeks, were employed in the investigation. The animal house facilities at the AIPER in Indore served as the site of the studies and animals is kept in cages amid free admittance to water, normal pellet food, and a cafeteria diet at room temperature and humidity of 25 ± 5°C and 55 ± 5%, respectively. The Institutional Animal Ethics Committee, referenced by Ref. No. AIPER/IAEC/2021/001, gave its approval to all of the study protocols.

2.3 Plant material

Z. mauritiana ripe fresh fruits were procured from a nearby market in Indore (M.P.) and cleaned to eliminate dirt and debris. The fruits were washed, dried for 25 days in the shade, had the pulp peeled, and were then milled into a fine powder. The plant was verified at the Janta PG College Department of Botany in Rewa, (M.P.), and a voucher with the Accession No. J/Bot/ZMFP-020 was deposited in the herbarium.

2.3.1 Preparation of the extract

Z. mauritiana coarse dried fruits pulp powder was kept in a Soxhlet apparatus. By using the appropriate solvent ethanol extract was produced. Ethanol considers an extraction solvent due to its many advantages compared to other solvents because it was relatively safer, less toxic, and resulting large extraction quantities.

2.4 Phytochemical screening

2.4.1 Qualitative phytochemical screening

To determine the phytochemical compositions of plant material for phenols, sugars, flavonoids, protein, glycosides, alkaloids, terpenoids, saponins, and tannins, for example, conventional phytochemical testing techniques were employed (Mukherjee *et al.*, 2007; Kokate *et al.*, 2004).

2.4.2 Quantitative phytochemical screening

2.4.2.1 Determination of total phenolic content (TPC)

The modified folin-Ciocalteu method was used to calculate the extract's total phenolic content. Numerous portions of 10-50 µg/ml were generated after 10 ml of methanol was used to dissolve 10 mg of gallic acid. 10 mg of extract that was dried were given 10 ml of methanol before filtration. It used two ccs of this extract (1 mg/ml) to calculate the phenol content. 1 ml of the folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5 g/l) of sodium carbonate were combined with 2 ml of the extract and 1 ml of each standard. The mixture went through vortexes for 15 seconds before being left to stand for 10 min for establishing the color.

2.4.2.2 The measurement of the total flavonoid content (TFC)

The total flavonoid amount was calculated using the aluminum chloride method. 10 mg of quercetin was mixed in 10 ml of methanol, and various aliquots with 5-25 µg/ml were created. Before filtration, 10 mg of dried extract received 10 ml of methanol. The concentration of flavonoids was determined using this extract, 3 ml (1 mg/ml). A 2% $AlCl_3$ solution was added to 3 ml of each standard's extract, which was then permitted 15 min to stand at the ambient temperature. Then, absorbance was determined at 420 nm (Parkhe *et al.*, 2019).

2.4.2.3 Analysis of HPTLC fingerprints

Standard and extract sample preparations were diluted in 1 ml of chromatographic-grade methanol before being applied on a 60F 254 aluminum sheet for HPTLC plates. Samples were applied using a Linomat 5 applicator connected to a CAMAG TLC scanner to aluminum sheets. The chromatogram was generated in a Twin trough glass chamber 10 × 10 cm, saturated with the solvent's toluene, ethyl acetate, and formic acid (5:4:1 v/v/v), for 20 min after the application of the sample. White light, UV at λ 254 and λ 366 nm, as well as with and without staining with 10% H_2SO_4 solution, were used to observe the air-dried plates. CAMAG Visualizer: 180103 scanned the chromatogram. The occurrence of quercetin in the extract was measured using the R_f (Retention factor) value and fingerprint data (Adhikari *et al.,* 2023).

2.5 DPPH radical scavenging activity

The UV-Visible spectrophotometer at 517 nm was used to gauge the antioxidant activity of the pulp of EEZM fruits. It makes use of the fact that an antioxidant is a hydrogen donor and is a frequently used assay for antioxidant evaluation of natural products. The hydrogen is accepted by DPPH, a tool for evaluating a compound's capacity to scavenge free radicals. A first absorbance of 1.5 ml in 1.5 ml methanol was obtained from the stock solution (6 mg in 100 ml methanol). In a succession of volumetric flasks, 1.5 ml of DPPH, 1.5 ml of the standard and test sample at a mixture of concentrations, and 1.5 ml of methanol were added to compose the final volume of 3 ml. The same methods were used to collect and process three test samples. The average was then determined. The absorbance of DPPH at 517 nm after 15 min demonstrated that a final reduction was present in the extract (Parkhe *et al.*, 2019).

2.6 α-glucosidase inhibition assay

0.1 U/ml of the enzyme (1 mg of α-glucosidase dissolved in 100 ml of phosphate buffer pH 6.8) and 1.25 mM pNPG in the absence or the presence of extracts (10 µg/ml) in a final reaction volume of 200 µl at 37°C were used to measure the inhibition of α-glucosidase. After a 5 min pre-incubation at 37°C, 250 µl of α-glucosidase (0.15 unit/ml) was added, and then there was a 15 min brooding

period. α-glucosidase activity was assessed by observed the extent of *p*-nitrophenol generated from *p*-NPG and measuring absorbance at 410 nm with a spectrophotometer. The amount of a pull out obligatory to restrain 50% of the activity of α-glucosidase under test circumstances is known as its IC_{50} value. (Youn *et al*., 2004).

2.7 Pancreatic lipase inhibition assay

The formation of *p*-nitrophenol was measured to estimate lipase activity. After being mixed in response buffer (10 mg/ml), crude lipase was centrifuged at 7000 rpm for 10 min. Utilizing a 200 µl reaction volume, lipase assays were carried out. With a substrate of *p*NPP and a response buffer of 50 mM sodium phosphate, 5 mM sodium deoxycholate, and 10% iso-propranolol at pH 8.0, the reaction was carried out. Then, Orlistat and ethanolic extracts at escalating concentrations were combined with 20 µl of the enzyme buffer and incubated at 37^0C for 15 min with 5 µl of the substrate solution (10 mM *p*NPP in dimethyl formamide). At 37^0C, the enzymatic processes were permitted to continue for 30 min. By monitoring the hydrolysis of *p*NPP to *p*-nitrophenol at 400 nm, lipase activity was identified. The IC_{50} value was determined to be the amount of extract needed to restrain 50% of pancreatic lipase activity beneath the assessed circumstances (Senapaty *et al*., 2014).

2.8 Cell culture and differentiation

Preadipocytes of type 3T3-L1 were procured from NCCS Pune. On standard tissue culture-treated polystyrene culture plates, all cells were developed. Preadipocytes were maintained in DMEM at 37^0C with 5% CO_2. Every 2-3 days, when 80% confluence was obtained, cultures were passed. Preadipocytes were planted in 6-well plates and kept in regular growth conditions for differentiation. By switching the growth media for a differentiating medium four days after confluence, differentiation was induced. This induction medium was switched out for differentiation medium after two days, and this process was repeated every two days after that.

2.8.1 Cell viability/MTT assay

Cell concentration 24 h was spent incubating 1×10^4 cells/ml in culture media at 37^0C and 5% CO_2. We employed tissue culture standard cells in 96-well microplates. In 100 µl of culture medium, cells be inserted at a compactness of 104 cells per glowing (100 µl), and samples were added at a rate of 20, 40, 60, 80, and 100 µg/ml as necessary. In the control wells, the cell line and DMSO (0.2% in PBS) were incubated. Each sample was replicated three times for culture. Controls were maintained to calculate the fraction of living cells following culture and to monitor cell endurance. For 24 h, cell cultures were maintained at 37^0C and 5% CO_2 in a CO_2 incubator. After the medium had been entirely unconcerned from the incubation chamber, 20 µl of MTT reagent (5 mg/min PBS) was added. After the adding together of MTT, cells were grown in an incubator containing CO_2 for 4 h at 37^0C. The wells were then inspected under a microscope to check for the formation of formazan crystals. Live cells only changed the yellowish MTT into a dark-colored formazan after the media had been eliminated. It was then covered with aluminum foil and incubated at 37^0C for 10 min after adding 200 µl of DMSO. The triplicate samples were evaluated using a microplate reader utilizing the absorbance of all sections at a 550 nm wavelength (Senthilraja *et al*., 2015).

2.8.2 Oil red O staining for lipid accumulation determination

At each stage of adipocyte development, standards were introduced to the DMEM with 10% FBS and 50 µg/ml of EEZM fruit pulp. After distinction, phosphate-buffered saline (PBS) is used to gently wash the cells twice after removing the media. Give the cells 10% formalin and let them sit for 30-60 min. Throw away the formalin, then use water to wash the cells twice. The cells should be given 60% isopropanol before 5 min has passed. After discarding the 60% isopropanol, uniformly distribute the oil red O working solution over the cells. Incubate for 10 to 20 min while rotating the plate or dish. Throw away the oil-red O solution, then rinse the cells with water between two and five times to remove any remaining stain. Give the cells hematoxylin and let them sit for a minute. After washing the cells 2 to 5 times with water, discard the hematoxylin. View the cells under a microscope after covering them with water. Nuclei appear blue and lipid droplets seem red (Fathima *et al*., 2018).

2.9 Acute toxicity studies

Before investigation in animals, the acute oral toxicity test of EEZM fruit pulp was conducted by OEC 420 strategy. The EEZM fruit pulp was given to Wistar rats at a solitary dose of 2000 mg/kg body weight. The treated animals were monitored for transience, clinical symptoms, and signs for 14 days (Ecobichon *et al*., 1997).

2.10 Pharmacological evaluation of antiobesity potential

2.10.1 Collection of blood and serum samples for biochemical analysis

According to the study's protocols, rats be fasted for 12 to 14 h before blood was drawn from the ketamine-anesthetized animals *via* the retro-orbital plexus with a capillary tube. After allowing the blood to coagulate for 30 min at area heat, the serum was obtained using a straightforward tabletop centrifuge by centrifuging the mixture for 10 min at 3500 rpm. Using a clean, dry Pasteur pipette, the clear, nonhemolyzed supernatant was separated and kept at 40^0C. Using a semi-auto analyzer (Microlab RX50V) and common commercial test kits for the determination of various serum biochemical parameters.

2.10.2 Orientation of obesity in the rats

By feeding a cafeteria diet for 12 weeks, obesity was created. Water and cafeteria food were freely accessible for the animals. The cafeteria diet is composed in the following manners:

Diet 1: Bread (g) and condensed milk (g)

Diet 2: Dried coconut (g), biscuits (g), and chocolate (g)

Diet 3: Boiling a potato and adding cheese or vegetable oil (g)

Individual rats were given the three diets on days 1, 2, and 3, respectively, and the same succession was repeated for 12 weeks. The laboratory-standard pellet diet for the normal control group was continued *ad libitum*. After twelve weeks of exposure to a cafeteria diet (apart from the normal control). By scrutinizing factors associated with obesity, such as body weight, anthropometrical parameters, *etc*., the animals were evaluated for the induction of obesity. Signaling the completion of the induction phase for obesity and further use in the study (Dias *et al*., 2021; Harris *et al.,* 1993; Naim *et al*., 1985).

2.10.3 Experimental design

Four groups of six rats each were formed at random from the obese animals. In this study, one group of healthy rats served as a normal control group and was provided a normal pellet diet.

Group I: Normal pellet diet (standard control)

Group II: Cafeteria diet (Negative control)

Group III: Cafeteria diet + Orlistat, 30 mg/kg/day (Positive control)

Group IV: Cafeteria diet + EEZM fruits pulp, 200 mg/kg/day (Experiment groups)

Group V: Cafeteria diet + EEZM fruits pulp, 400 mg/kg/day (Experiment groups)

All of the treatments were given for 12th to 18th weeks. All of the treated rats were kept on a cafeteria diet for the duration of the study period. Throughout the study, rats received water *ad libitum*.

2.11 Calculation of body weight and food ingestion

During the study period, the rat's body weight was determined every week in grams (g) by using weigh equilibrium. The rats' daily food consumption was determined in the morning. The amount of food consumed was calculated by deducting from the deliberate quantity of foodstuff provide the preceding amount of foodstuff remains in each cage.

2.12 Anthropometric measurements

The morphological and anthropometric measurements were made once a week. The Lee index was unwavering using the Lee formula:

Lee index (%) = Cube root of the Body weight (gm)/Body length (cm) × 1000

Every rat's body length (measured from snout to anus) was recorded on a weekly basis. Employing a non-extensible string and a precise ruler with a reading of 0.1 cm, body length was measured. Abdominal circumference of rats was measured to estimate the largest area of the rat's belly in front of the hind leg. All of the time, the rats had been maintained in a ventral situation (Arika *et al*., 2019).

2.13 Calculation of organ weights and organ triglyceride content

After the completion of 18 weeks, rats were sacrificed by a cervical disarticulation and abdominal opening made to examine their viscera. Liver and adipose tissue were removed from the fat and connective tissues that surrounded them. Liver and adipose tissue were washed and cleansed three to four times with ice-cold PBS solution, blotted with filter paper, weighed, and then rinsed in regular saline. After that, a different group compared the weights of the liver and adipose tissue. For estimation of organ TG content, organ tissues were extracted in heptane: isopropanol (3:2) at 40^{0}C. Organ tissues were first homogenized, and then the homogenate tissue was treated right away to determine the tissue TG content by using a semiauto biochemical analyzer and a commercial kit, the TG content was determined. The amount of TG in each gram of tissue was determined.

3. Results

The EEZM fruits pulp was subjected to qualitative and quantitative phytochemical characterization.

3.1 Phytochemical study

3.1.1 Qualitative phytochemical screening

Phytochemical analysis of EEZM fruits pulp exposed the presence of phenols, flavonoids, tannins, saponin, glycosides, protein and carbohydrate. Secondary metabolites of natural products play important role in various cellular mechanisms. Preliminary phytochemical composition of EEZM fruits pulp as follows:

Table 1: Phytochemical screening of the EEZM fruits pulp

Phytoconstituents	EEZM fruits pulp
Flavonoids	Present
Phenolic	Present
Tannins	Present
Terpenoids	Absent
Saponins	Present
Alkaloids	Absent
Glycoside	Present
Carbohydrate	Present
Protein	Present
Sterols	Absent

3.1.2 Quantitative phytochemical screening

3.1.2.1 Total phenolic content

Total phenolic content (TPC) was calculated as mg/g of the equivalent of gallic acid of the dry extract sample using the calibration curve. The following formula: y = 0.014x - 0.013, R2 = 0.999, where X is the gallic acid equal (GAE) and Y is the absorbance. The pulp of the EEZM fruits was calculated to have a total phenolic content of 45.21 ± 1.12 mg GAE/1000 mg of dry extract.

3.1.2.2 Total flavonoid content

The using the equation, the total quantity of flavonoid was converted to quercetin equivalent.: y = 0.036x + 0.015, R_2 = 0.999, where X is the quercetin equal (QE) and Y is the absorbance (mg/g). According to calculations, the pulp of EEZM fruits contains 36.25 ± 0.68 mg QE of flavonoids per 1000 mg of dry extract.

3.1.2.3 Estimation of quercetin by using HPTLC fingerprinting

The results of the HPTLC fingerprinting of EEZM fruits pulp at 254 and 366 nm are given in Figure 1. At a sample volume of 2µl, the EEZM fruits pulp displayed, R_f value (Retention factor) found to be 0.82. The single spot at R_f = 0.82 of the quercetin peaks from the EEZM fruits pulp with that obtained by chromatography same as the standard quercetin R_f value was 0.82. The quercetin content in EEZM fruits pulp was calculated by using the standard curve of quercetin (y = 29.42x + 123, R^2 = 0.987). EEZM fruits pulp was found 5.907 µg of quercetin.

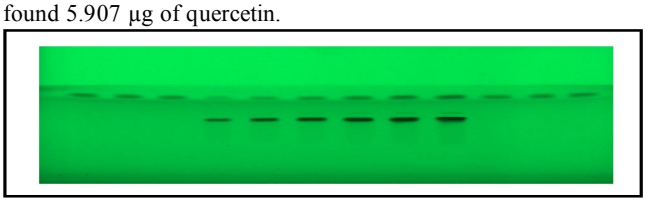


Figure 1: HPTLC fingerprinting analysis of quercetin in the EEZM fruits pulp.

3.2 Utilising the DPPH test, free radical scavenging activity

Using the DPPH radical scavenging assay technique, the antioxidant activity of the EEZM fruits pulp was examined. Comparing the EEZM fruits pulp's computed IC_{50} value to that of the orientation standard ascorbic acid, which had an IC_{50} value of 19.11 µg/ml, the EEZM fruits pulp's IC_{50} value was 92.11µg/ml.

Table 2: Absorbance and % inhibition of ascorbic acid and EEZM fruits pulp using DPPH method

S.No.	Concentration (µg/ml)	Absorbance			% Inhibition	
		Control	Ascorbic acid	Ethanolic extract *of Z. mauritiana*	Ascorbic acid	Ethanolic extract *of Z. mauritiana*
1	10	0.795	0.434	0.732	45.40	8.61
2	20		0.396	0.658	50.18	17.23
3	40		0.297	0.612	62.64	23.02
4	60		0.246	0.552	69.05	30.57
5	80		0.149	0.425	81.25	46.54
6	100		0.056	0.365	92.95	54.09
IC_{50} value (µg/ml)					**19.11**	**92.11**

3.3 α-glucosidase inhibition assay

We examined the inhibitory action in this study of EEZM fruit pulp against the α-glucosidase inhibition assay. The IC_{50} value for the pulp of EEZM fruits was determined to be 72.88 µg/ml for the inhibition of α-glucosidase; this finding demonstrated the hypoglycaemic potential of EEZM fruits pulp. As a benchmark for comparison, acarbose is employed as the standard α-glucosidase inhibitor.

3.4 Pancreatic lipase assay

Assay for pancreatic lipase *p*-nitro phenyl palmitate was used as the substrate, and Orlistat was used as the reference medication, to calculate the pancreatic lipase inhibitor activity. With an increase in the concentration of EEZM fruits pulp. According to our findings, EEZM fruit pulp exhibited an IC_{50} value of 81.32 µg/ml, a high inhibitory efficacy against pancreatic lipase.

3.5 Acute toxicity

According to the OECD 420 guidelines, EEZM fruit pulp was deemed safe at 2000 mg/kg. Until the end of the trial, no animal displayed any clinical signs of intoxication, and no deaths were noted.

3.6 Body weights and food intake

The rats' average weekly body weights during the research. In comparison to the rats fed the standard pellet chow (Group I), the cafeteria diet led to a substantial ($p<0.05$) improvement in body mass and food consumption after six weeks of consumption. Rats treated with the reference medication Orlistat and the two dosages of extract experienced a significant reduction in body weight and food eating from the 12^{th} to 18^{th} weeks during entire dosing periods.

The rats' weekly body weights in each group over the route of the study. At the end of the study, cafeteria diet-fed obese rats (Group II) led to a momentous ($p<0.05$) rise in body weight compared to rats eating normal pellet chow (Group I). Obese rats treated with the Orlistat and the ethanolic extracts (200 and 400 mg/kg), experienced a considerable decrease in body weight and food intake from the 12^{th} to the 18^{th} week throughout the full dosing period. Overall, obese rats treated with Orlistat and two ethanolic extracts showed substantial significant reduction ($p<0.05$) in body weight and % change in body weight gain as compared with the cafeteria diet-fed obese rats (Group II).

Table 3: Effect of six weeks of oral EEZM fruit pulp treatment on the body-weight of obese rats fed a cafeteria diet

Groups/time points	Body weights of rats (g) weekly basis						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group I	132.5 0 ± 1.06	134.83 ± 1.12	137.33 ± 1.27	138.50 ± 1.3	141.00 ± 1.21	143.17 ± 0.99	146.83 ± 1.08
Group II	203.50 ± 3.29**	214.83 ± 2.41**	221.83 ± 2.28**	230.00 ± 2.48**	238.00 ± 2.21**	245.67 ± 1.96**	249.00 ± 1.99**
Group III	205.50 ± 1.64	198.50 ± 2.12	191.17 ± 1.97	184.50 ± 1.89	177.5 ± 1.60##	171.00 ± 1.59##	163.00 ± 1.23##
Group IV	207.50 ± 1.97	202.83 ± 1.75	197.50 ± 1.66	193.17 ± 1.55	189.50 ± 1.43##	185.00 ± 1.56##	180.17 ± 1.42##
Group V	207.67 ± 2.07	202.00 ± 1.86	195.50 ± 1.80	190.00 ± 1.50	181.83 ± 1.61##	175.17 ± 1.16##	165.50 ± 0.65##

The data are shown as mean ± SEM, One-way ANOVA was performed on the data, by Tukey's Multiple Comparison at ($p<0.05$). Both # and * in the same row significantly deviate from the control (Group I) and the negative control (Group II), respectively.

The % modify in body weight of the rats fed the cafeteria diet was 22.35 ± 1.88%, whereas rats fed with the normal pellet diet 10.82 ± 3.8%. The % change in body weight of extract treated rats and positive control group were shown significant reduction ($p<0.05$) as compare to obese rat of negative control group. EEZM fruits pulp treated obese rats with both low (200 mg/kg) and high (400 mg/kg) doses shown the % change in body weight by –13.87 ± 0.70 and – 20.26 ± 0.76%, respectively (Table 4).

Table 4: Effect of six weeks of oral EEZM fruit pulp treatment on the % change in body weight of overweight rats fed a cafeteria diet

Groups/time points	Change in body weights of rats						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group I	0.00	1.76 ± 0.13	3.65 ± 0.29	4.53 ± 0.55	6.42 ± 0.47	8.05 ± 0.46	10.82 ± 0.38
Group II	0.00	5.57 ± 0.70	9.01 ± 1.05	13.12 ± 1.18	16.95 ± 1.55	20.72 ± 1.63	22.36 ± 1.88##
Group III	0.00	-3.41 ± 0.36	– 6.97 ± 0.42	–10.22 ± 0.43	– 13.63 ± 0.49	– 16.79 ± 0.45	– 20.68 ± 0.36**
Group IV	0.00	-2.24 ± 0.29	– 4.80 ± 0.57	– 6.88 ± 0.72	– 8.64 ± 0.83	– 10.82 ± 0.73	– 13.14 ± 0.72**
Group V	0.00	-2.72 ± 0.28	– 5.84 ± 0.51	– 8.49 ± 0.49	– 12.43 ± 0.29	– 15.63 ± 0.43	– 20.26 ± 0.76**

The data are shown as mean ± SEM.

3.7 Anthropometric measures

In general, anthropometric measurements changed after cafeteria diet-induced obese rats were treated with EEZM fruit pulp. At the end of the study, cafeteria diet-fed obese rats (Group II) showed momentous (p<0.05) elevation in Lee's index and abdominal circumference as compared to normal dietfed rats (Group I). Meanwhile, obese rats administered with the orlistat, and ethanolic extracts (200 and 400 mg/kg) observed a momentous diminution (p<0.05) in Lee index and abdominal circumference compared to cafeteria diet fed obese rats (Group II). In addition, data observed that both the normal control group and the negative control group obese rats increased abdomen circumference consistently throughout the study.

Table 5: Effect of six weeks of oral EEZM fruit pulp treatment on abdominal circumference and Lee index of obese rats fed a cafeteria diet

Groups/parameters	End of the study (week 6)	
	Abdominal circumference	Lee index
Group I	13.42 ± 0.05	307.32 ± 1.08
Group II	17.85 ± 0.11##	347.26 ± 1.26#
Group III	13.47± 0.05**	322.90 ± 0.90**
Group IV	14.18 ± 0.11**	329.13 ± 1.18**
Group V	13.65 ± 0.07**	319.87 ± 1.56**

The data are shown as mean ± SEM, One-way ANOVA was performed on the data, by Tukey's Multiple Comparison at (p<0.05). Both # and * in the same row significantly deviate from the control (Group I) and the negative control (Group II), respectively.

3.8 Serum biochemical parameters

After the trial, obese rats given the cafeteria diet showed a significant spike (p<0.05) in blood triglycerides, total cholesterol, and LDL compared to rats on a normal diet. Additionally, results showed that obese rats fed a cafeteria diet showed a substantial reduction (p<0.05) in blood lipid profile and glucose levels after receiving Orlistat and EEZM fruit pulp. Interestingly, both low and high doses of EEZM fruit pulp showed momentous reduction (p<0.05) in serum triglyceride levels as compared to cafeteria diet-fed obese rats (Group II). Results indicated that ethanolic extract doses and Orlistat administered to obese rats not shown significant elevation in serum HDL as compared to cafeteria diet-fed obese rats. Findings indicated that obese rats given the cafeteria diet had a significantly higher overnight blood glucose level than rats fed the usual diet (Group I). Orlistat and the pulp of EEZM fruits both showed a substantial decrease (p<0.05). In serum glucose level as compared to cafeteria diet-fed obese rats at the end of the study. However, obese rats treated with EEZM fruit pulp (200 and 400 mg/kg) show a momentous decrease (p<0.05) in triglyceride, LDL, total cholesterol, and glucose indicating hypotriglyceridaemic potential.

Table 6: Result of oral administration of EEZM fruits pulp after six weeks on serum biochemical investigation of cafeteria diet fed overweight rats

Groups/ parameter	Serum biochemical analysis				
	TG	TC	HDL	LDL	Glucose
Group I	68.83 ± 2.13	95.33 ± 2.43	24.67 ± 0.81	51.67 ±1.41	97.25 ± 1.44
Group II	103.33 ± 3.08**	126.17 ± 1.68**	24.17 ± 0.55	100.83 ±1.91**	137.58 ± 2.05**
Group III	75.67 ± 2.30##	107.83 ± 1.14##	30.67 ± 0.75##	68.67 ± 0.75##	123.25 ± 2.15##
Group IV	87.70 ± 0.91##	117.27 ± 0.94##	25.36 ± 0.56	79.51 ± 1.41##	130.27 ± 0.83#
Group V	77.74 ± 1.31##	111.32 ± 1.54##	27.06 ± 0.60#	71.63 ± 0.75##	126.11 ± 1.06##

The data are shown as mean ± SEM, One-way ANOVA was performed on the data, by Tukey's Multiple Comparison at (p<0.05). Both # and * in the same row significantly deviate from the control (Group I) and the negative control (Group II), respectively.

3.9 Organ weights and tissue triglyceride (TG) levels

At the end of the study, liver tissues and adipose tissue weight and TG content varied across each group of rats**.** The result showed that obese rats fed with a cafeteria diet had significantly higher liver and adipose tissue weight. In the experimental groups, EEZM fruit pulp (200 and 400 mg/kg) and Orlistat administered to obese rats showed that momentous diminish ($p<0.05$) in the liver and adipose tissues weight as compared to cafeteria diet-fed obese rats (Group II). In addition, EEZM fruits pulp and Orlistat also showed significant reduction ($p<0.05$) in the triglycerides content in the liver and adipose tissue as compared to cafeteria diet fed obese rats.

Table 7: Result of oral administration of EEZM fruits pulp after six weeks on organ weight, organ TG content and adiposity index cafeteria diet fed overweight rats

Groups/ parameters	Liver weight	Adipose tissue weight	Adiposity index	Liver TGcontent (mg/gm)	Adipose TG content (mg/gm)
Group I	11.04 ± 0.26	7.55 ± 0.11	5.14 ± 0.04	8.49 ± 0.32	28.48 ± 1.17
Group II	21.54 ± 0.44**	17.78 ± 0.19**	7.14 ± 0.10**	37.95 ± 2.53**	38.73 ± 1.23**
Group III	12.38 ± 0.39##	9.13 ± 0.16##	5.60 ± 0.11##	18.41 ± 0.76##	27.72 ± 10.6##
Group IV	16.77 ± 0.38##	10.54 ± 0.20##	5.90 ± 0.11##	23.03 ± 0.99##	30.95 ± 0.87##
Group V	13.97 ± 0.22##	9.21 ± 0.24##	5.47 ± 0.17##	20.15 ± 0.74##	28.72 ± 1.04##

The data are shown as mean ± SEM, One-way ANOVA was performed on the data, by Tukey's Multiple Comparison at ($p<0.05$). Both # and * in the same row significantly deviate from the control (Group I) and the negative control (Group II), respectively.

3.10 3T3-L1 adipocyte cell lines

3.10.1 Cell viability (MTT assay)

For the reason that it differentiates from a preadipocyte to a grown-up adipocyte that is particularly in the formation of lipid droplets after management with the cocktail of insulin, dexamethasone, IBMX, and Fetal bovine serum, the 3T3-L1 adipocyte cell line is used to investigate antiobesity potential as an *in vitro* model. For 72 h, 3T3-L1 cells were exposed to various doses of EEZM fruit pulp and standard orlistat (20, 40, 60, 80, and 100 µg/ml). Premature adipocyte feasibility was decreased by 2.05%, 7.40%, 8.54%, 11.1%, and 11.8%, respectively, when EEZM fruits pulp was administered at doses of 20, 40, 60, 80, and 100 µg/ml (Figure 2). Our findings revealed that EEZM fruits pulp was significantly reduced to the extent where cell survival rates and demonstrated prevention of adipogenesis were comparable to Orlistat after 72 h.

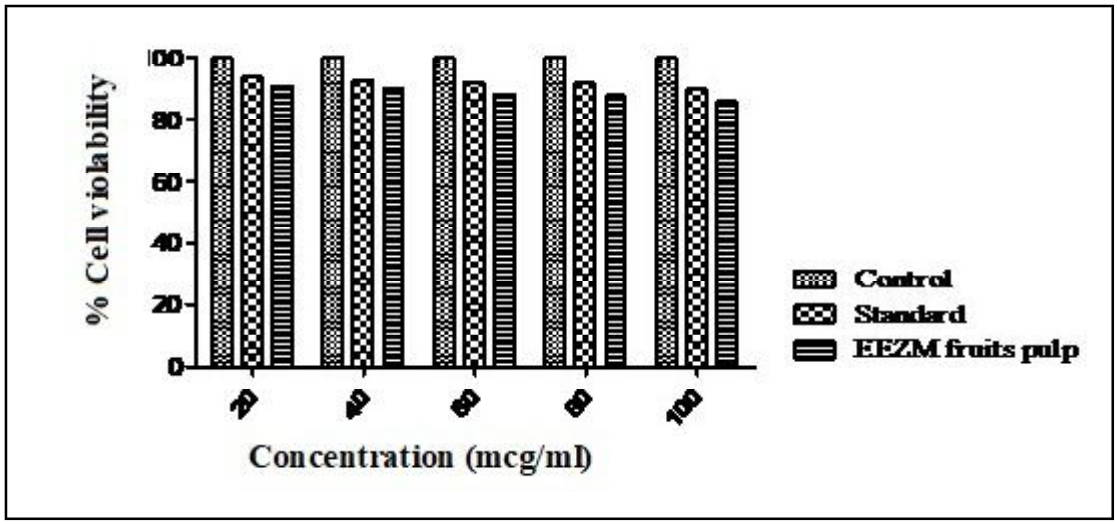
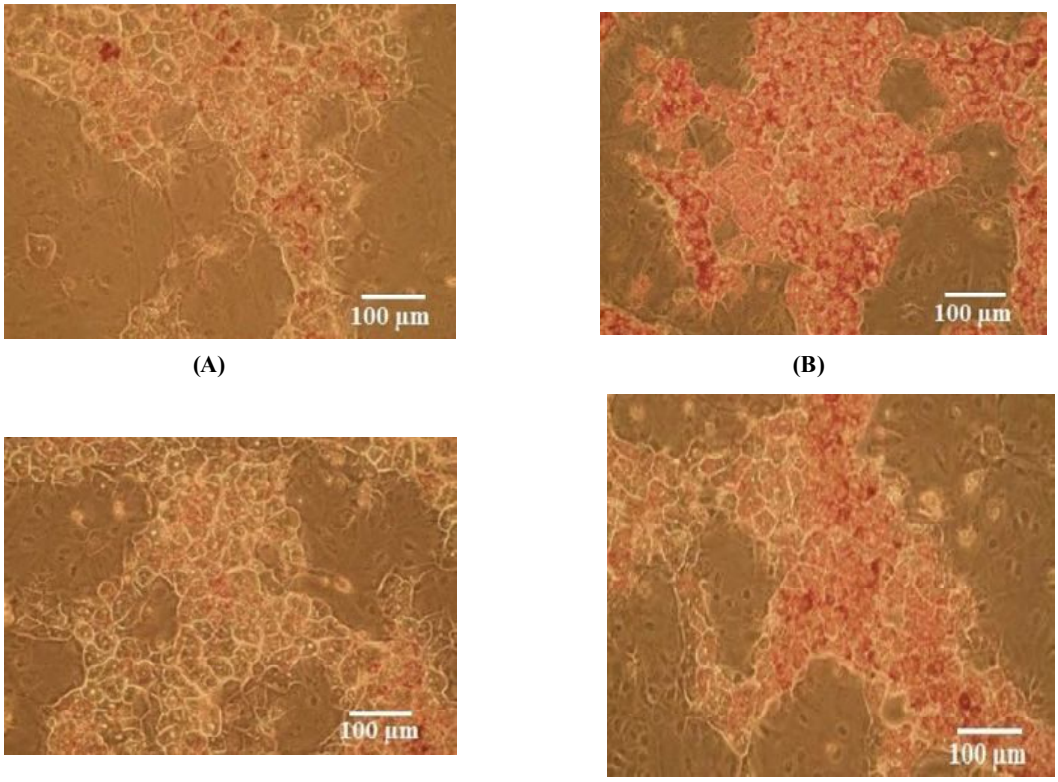


Figure 2: Effect of EEZM fruits pulp on cell viability in 3T3-L1 preadipocyte cell line by using MTT assay.

3.10.2 Lipid gathering (Oil red O staining)

The lipid drop accretion in the 3T3 L1 cell line has been demonstrated by Oil red O discoloration. 3T3-L1 cells were treated with normal control, positive control, negative control, and EEZM fruit pulp (50 µg/ml). Oil red O staining was used to examine intercellular lipid accretion after 10 days as a measure of the level of adipogenesis. The number of identifiable stained droplets was shown by microscopic pictures of EEZM and orlistat-treated 3T3-L1 preadipocyte cells taken after Oil red O staining, as exposed in Figure 3. Our results indicated that it showed inhibition of lipid droplet accumulation and promoted lipolysis as compared to positive control and showed similar manner reduction as compared to Orlistat.



(A) (B)

(C) (D)

Figure 3: Effect of EEZM fruits pulp on lipid droplet accumulation in 3T3-L1 preadipocyte cell lines. Cells were fixed and stained with Oil red O dye after 10 days. Use Microscope for captured the images of representative cells (100 x magnification), A: control, B: positive control, C: negative control, D: EEZM fruits pulp 50 µg/ml.

4. Discussion

Obesity and overweight are healthcare conditions that indicate an excessive accumulation of body fat and are dangerous to health (Dai *et al.,* 2020). The pathophysiology of obesity includes extensive research on the hereditary, and biological (Muller *et al*., 2022; Kawser *et al.,* 2016). In many underdeveloped nations, herbal remedies are the go-to form of treatment for almost all minor illnesses. They are a gift from nature to humans that will relieve diseases for healthy existence (Arika *et al*., 2019). An indigenous plant called *Z. mauritiana* has excellent therapeutic potentials, which are ascribed to a variety of secondary metabolites like phenolics, flavonoids, saponins, terpenoids, and tannins were the main components identified in the plant. Earlier studies also revealed that *Z. mauritiana* was assessed as rich in total phenolics, flavonoids, and antioxidant potentials (Hossain *et al*., 2016; Mbahi *et al*., 2018). The pulp of *Z. mauritiana* fruits was ethanolically extracted, and the results revealed TPC and TFC values of 45.2 1± 1.12 GAE/g and 36.25 ± 0.68 mg QE/g of dry extract. For the quantitative analysis of herbal extracts, HPTLC is a highly helpful approach for guaranteeing product quality, purity, and stability as well as for identifying or validating the complicated composition. In this investigation, construct the chromatographic fingerprint profile of the EEZM fruits pulp by utilizing mobile phase toluene, ethyl acetate, and formic acid (5:4:1 v/v/v). The fingerprint profile pattern discovered in this study can be applied to the quality assurance of plant samples. The identified compound from the chromatogram of this plant is quercetin, having an R_f value 0.82. Quantification of quercetin was also done by calculating the amount of quercetin per gram of plant extract. The amount of quercetin was found to be 5.907 µg in EEZM fruit pulp through HPTLC fingerprint analysis. The HPTLC analysis of the EEZM fruit pulp generally revealed the presence of quercetin and which was previously found (Adhikari *et al*., 2023).

The results of DDPH radical scavenging activity show that the *Z. mauritiana* fruits pulp has outstanding antioxidant capacity when extracted using ethanol. As a result, the pulp extract can reduce the release of oxidative species produced by the body. The antioxidant activity of the EEZM fruits pulp may be prompted by the hydrogen-donating ability of fruits pulp extract secondary metabolites like flavonoid and phenolic, which act as powerful natural free radical scavengers and affect the nature of the DPPH radicals in the reaction. This EEZM fruit pulp also may have multiple therapeutic uses due to presence of the numerous active phytochemicals. Evidence from recent studies suggests that flavonoids may prevent obesity and its co-morbidities by reducing oxidative stress, free radical damage, and associated inflammatory disorders (Gentile *et al*., 2018; Duan *et al*., 2018). The EEZM fruit pulp observed estimated phenolic content, a moderate amount of flavonoid content, and antioxidant capability on DPPH free radical scavenger assay. Only after being subjected to pancreatic lipase action is dietary fat directly absorbed

by the gut. Clinical studies revealed that Orlistat, which has been approved by the FDA, reduces dietary fat absorption and increases fat excretion in the feces, hence preventing obesity and hyperlipidemia. Therefore, pancreatic lipase and alpha-glucosidase are the most widely used methods for figuring out whether a natural product has antiobesity and antidiabetic potential. In our investigations, the EEZM fruit pulp significantly abridged the activity of the pancreatic lipase and alpha-glucosidase enzymes in a dose-dependent manner.

Studies revealed that numerous polyphenols, together with flavones, flavonols, tannins, and chalcones, have demonstrated a pancreatic lipase-inhibiting effect (Birari *et al*., 2007). Additionally, flavonoids affect the absorption of triacylglycerol by preventing the production of micelles in the small intestine and inhibiting the activity of alpha-glucosidase. Phenolic and flavonoid molecules were discovered to have extremely positive relationships with antioxidant activity, alpha-glucosidase activity, and pancreatic lipase activity, demonstrating that these chemicals were principally responsible for the hypoglycemic and antiobesity potential (Parkhe *et al*., 2018). Therefore, in this research work using acute and chronic animal models, the impact of multiple phytoconstituents-rich EEZM fruit pulp on preventing obesity was further observed. The 200 and 400 mg/kg doses were used to assess the antiobesity and hypoglycemic potential because the EEZM fruit pulp did not show any toxic symptoms of mortality at the 2000 mg/kg dose level in rats. As a result, the ethanolic extracts were deemed safe for further pharmacological screening.

Hence, the current investigations have been carried out to assess the antiobesity potential of an EEZM fruit pulp in cafeteria diet-fed obese rats. The results revealed that rats fed an assortment of highly appetizing, calorie-dense, cafeteria foods produced obesity-like conditions, a significantly elevate (p<0.05). Researchers investigated that cafeteria diets have been attributed to the development of obesity in both humans and animals (Dias *et al*., 2021). The cafeteria diet-induced obese animal model is well known for its rapid body weight gain accompanied by an increase in energy intake, which in turn leads to a reduction in metabolic efficiency and insulin resistance (Rolls *et al*.,1983). Findings revealed that feeding calorically dense diets to rats increased belly fat storage and decreased diet-induced thermogenesis or resting metabolic rate. Therefore, adipocyte numbers and sizes increased as a result of diet-induced obesity. In addition, the diversity and composition of cafeteria foods have a cumulative impact on the induction of obesity (Naim *et al*., 1985). At the end of the study, EEZM fruits pulp administered to obese rats show noteworthy diminution (p<0.05) in body weight and serum biochemical constraints such as triglycerides, LDL, and total cholesterol, as compared to cafeteria diet-fed obese rats (Group II). The researcher suggested numerous secondary metabolites like polyphenols, flavonoids, terpenoids, and tannins have capable decrease blood sugar, triglyceride, and LDL cholesterol levels, enhance energy expenditure, and decrease body weight and adiposity (Birari *et al*., 2007; Seyedan *et al*., 2015).

According to our investigation, obese rats administered with EEZM fruit pulp reduced their Lee index and abdominal circumference as compared to cafeteria diet-fed obese rats. The EEZM fruits pulp treated group of obese rats showed significant inhibition in liver weight, peritoneal adipose weight, and liver TG content, indicating a protective effect against obesity and related metabolic complications such as cardiovascular diseases, dyslipidemia, cancer, and insulin resistance, among others. Rats in the positive control group received Orlistat and observed an equivalent effect. The noted reduction in these values may be a consequence of the involved phytochemicals' individual, additive, or synergistic effects. These effects boost lipid metabolism and energy expenditure while lowering lipid and carbohydrate absorption (Seyedan *et al*., 2015). The primary phytochemicals contained in these plants are saponins, polyphenols, flavonoids, and caffeine. While *Hibiscus sabdariffa* aqueous extract is rich in anthocyanins (Zhao *et al*., 2005). Researchers discovered that the ethanolic extract of papaya seeds includes flavonoids that have the same pancreatic lipase inhibitor properties as Orlistat, such as epicatechin, catechin, and epigallocatechin-3-gallate (Subandi *et al*., 2019). The hypoglycemic activity of *Z. mauritiana* fruit extracts in petroleum ether fractions and aqueous extracts alongside an alloxan-induced diabetic mock-up in glucose-overloaded hyperglycemic rats has been studied in the past (Jarald *et al*., 2009).

Results investigated cafeteria diet-fed obese rats showed a cumulative increase in food intake, in comparison to other experimental groups. This finding shows that EEZM fruit pulp's ability to reduce appetite in rats fed a cafeteria meal may be the cause of the extract's ability to reduce body weight. The potential for improved satiety signals to mediate reduced food intake. By lowering palatability, tannins in EEZM fruit pulp contribute to a decrease in food intake. Previous studies have demonstrated that the brain's reward system affects eating behavior *via* a mechanism that takes into consideration both the hedonic and cognitive rewards of eating as well as the homeostatic requirement to feed (Sri Bharathi *et al*., 2021; Morosanu *et al*., 2022).

The process of adipocyte proliferation and differentiation has been the subject of screening for antiobesity drugs because it is another characteristic of obesity that is frequently observed in research. Currently, 3T3-L1 preadipocyte cells are used *in vitro* to study obesity. IBMX and insulin cause preadipocytes to differentiate into adipocytes, and during this process, the cells accumulate lipids as lipid droplets (Youn *et al*., 2004; Sri bhuvaneswari *et al*., 2021). Results observed that when Orlistat and EEZM fruits pulp were added to the adipocyte cell line that showed inhibition of adipogenesis and also reduce triglyceride accumulation and promoted lipolysis (Venkatachalam, *et al*., 2021). In consequently, it is possible that quercetin can be suppressed, and the angiogenesis process will be linked to adipocyte differentiation. Quercetin is a flavonoid that has been linked to anti-inflammatory, antioxidant, immune-boosting, and antiobesity qualities. It is typically found in onion peels, wine, and tea, according to research (Batiha *et al*., 2020; Neri-Numa *et al*., 2020; Hong *et al*., 2021; Sethumathi *et al*., 2021). According to studies, tannins from Banaba extracts cause adipocytes to activate the insulin-mediated signaling system, which in turn causes glucose transfer. Tannins also suppress or change the expression of crucial genes involved in the process of adipogenesis, which further delays adipocyte differentiation (Liu *et al*., 2005). Quercetin has undergone extensive research and most recently found anticancer activities against several malignancy cell lines (Salehi *et al*., 2020).

5. Conclusion

Herbal plants demonstrated considerable therapeutic potentials, which have been utilized historically and in modern pharmacotherapy to treat a variety of ailments and also provided limitless prospects for the discovery of novel pharmaceuticals. The fruit of *Z. mauritiana* has been claimed by researchers to be extremely advantageous to human health because of its nutritional worth, and the plant's extracts may have a variety of pharmacological benefits. Numerous secondary metabolites, including flavonoids, tannins, and saponins have been found in EEZM fruit pulp that has been shown a significantly intriguing impact during our study. This research work also showed that pancreatic lipase and alpha glycosidase, two essential enzymes in the breakdown of fats and carbohydrates, are strongly inhibited by the EEZM fruit pulp. The most likely cause of its inhibitory effect is its phytochemicals, which include flavonoids and tannins. In terms of antioxidant properties, the pulp of the EEZM fruits showed good free radical scavenging capabilities that can be used as a natural external source of antioxidants in meals and/or supplements to assist in addressing the issue of oxidative stress. Thus, our research shows that EEZM fruit pulp is a blend of flavonoids, tannins, and saponins. Its multifunctional antiobesity and antidiabetic mechanisms include a decrease in the absorption of lipids and carbohydrates, maintenance of energy intake and expenditure, improved impaired glucose metabolism, and inhibition of the process of adipogenesis. The pulp of the EEZM fruits may have antioxidant, antidiabetic, and antiobesity properties that work in concert. Our findings imply that the EEZM fruit pulp inhibits adipogenesis and prevents the accumulation of lipid droplets in 3T3-L1 preadipocyte cell lines.

Overall, this suppressive effect of EEZM fruit pulp may be possibly mediated by the down-regulated expression of mRNA and the primary adipogenic transcription activators (PPAR-gamma, C/EBP alpha, and SREBP-1) of the adipogenesis pathway. Hence, the fruit of *Z. mauritiana* may offer a potential therapeutic lead for further research into the management of associated comorbidities and the prevention of obesity. To ascertain the effectiveness, safety, and precise cellular and molecular mechanisms fruit of *Z. mauritiana* for their antiobesity potential, additional clinical investigations are still required.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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