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ASSESSMENT OF ANTI-OBESITY POTENTIAL OF BANANA PEELS *(MUSA PARADISIACA L): IN-VITRO* STUDIES

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ABSTRACT

The current study performed was to understand the anti-obesity potential of raw and ripe banana peels (*Musa paradisiaca L.*). The *Musa paradisiaca L*. is well known as banana. The fruits and peels contain rich nutrients and dietary sources. The Raw and ripe banana was collected, dried and the peel were macerate by using solvent and further separated by using rotatory evaporator. Lipase inhibition assay was conducted at the absorbance 400nm to obtained inhibition of enzymes. α- amylase assay was conducted to determine the inhibition activity and anti-diabetic properties at the absorbance of 540nm.The lipid accumulation was determined at the absorbance of 510nm by the Oil red O method. An MTT assay was executed to determine whether the extracts were toxic or non-toxic to the 3T3 adipocyte cell line with an absorbance of 492nm and achieved cell viability. Lipase inhibition also exhibited anti-obesity property; methanol ripe extract showed 74.7% inhibition at the concentration 500µg/ml. The α- amylase inhibition assay exhibited that methanol raw and ripe both extracts showed 91.4 and 91% inhibition at the concentration of 250µg/ml. The isopropyl raw extract inhibited highest lipid accumulation and adipogenesis during adipocyte differentiation as compared with absorbance of control. The results obtained through MTT assay showed that the raw and ripe banana peel extract is harmless to the 3T3 cells at the concentration ranging from 20µg/ml to 100µg/ml. The percent of cell viability obtained was 97.06% to 63.07% the result of present study depicts the ant obesity potential of ripe and raw banana peels. The peel contains rich dietary sources and high content of phytochemicals.

Which are known to exhibit medicinal properties. Thus, the study indicates the potential of ripe isopropyl and raw and ripe peel methanol extract as ant obesity agents which can be further studied.

Keywords: *Musa paradisiaca L*, anti-obesity, banana peels, phytochemicals and peel methanol extract

INTRODUCTION

Obesity and Overweight are an unrestricted collection of fat that cause risk to the health. It is the worldwide health problem. It is also called as multifactorial for the reason there is no single cause for the obesity and also called as the risk factor for the development of many metabolic untidiness like adult-onset diabetes, cardiovascular diseases, atherosclerosis and systemic hypertension according to World Health Organization (WHO). Obesity can also be the cause for certain categories of tumor in mammary glands, prostate, liver, gall bladder, kidney etc. It is worldwide, resulting in crucial increase in mortality and morbidity related to metabolic disorders **[1]**. The generality of overweight children and adolescents who are aged five to nineteen years are growing more than four time globally in the year 1975 to 2016 from four to eight percentage. Many factors are accountable for the cause of the obesity such as one of which is eating fast food containing high calories, fat and cholesterol, sedentary life.

Obesity leads to the occurrence of heart failure, congestive heart disease, and death so that the big spiral in the high rate of prevalence of cardiovascular disease **[2]**. Obesity can be determined by BMI (Body Mass Index) that include from 25 to 30 that states obese and overweight. Obesity and overweight can be preventable. The bodily activity has been decreased due to the nature of many types of work, due to moving with transport, growth of development in cities which lead to the utilization excessive fat energy sources. Immersion of dietetics fat macromolecules in small intestine involves chemical breakdown of substances into saturated fats by pancreatic lipase enzyme **[3]**. On the other side, the immersion of carbohydrates in small intestine involves hydrolysis into simple sugars by amylase enzymes. Inhibition of these enzymes could be.

Musa is genus from Zingiberales and liliopsid family; it includes bananas and plantains. About seventy species of *Musa* have a wide variation of uses. *Musa paradisiaca* is a hybrid name linking wild banana (*Musa acuminata)* and (*Musa balbisiana)* plant. The peels of fruits or vegetables have importance in protecting many diseases **[4]**. Banana is highly consumed fruit due to its soft texture, pleasant aroma and present of high content of phytochemicals **[5]**. Banana peel also contains Vitamin A, Vitamin C, Gallo

catechin, dopamine, Vitamin E, Vitamin B6, a- sitosterol, malic acid, succinic Acid, palmitic acid, Magnesium, phosphorus, potassium, fiber, iron and fatty acid **[6]**. Peels from banana are also used for variety of industrial applications including biofuel manufacturing, removal of substance from biological material, mash and paper, beautifying, pesticide-free fertilizers, environmental clean-up, and biotechnology. Generally, the raw banana peel is 15% starch and 30% sugar, while the fully developed banana peel is 30% and 9%.

As banana peel mature, their chemical composition changes and they can be used to manufacture various products such as alcohol brewages, essence, plant food, soap and cellulose, nanofibers. Pectin is the gelling agent in banana peels, which contain biological catalysts such as phenolase. Fruit skin have been a useful source for keeping the human health alive. There are medicinal uses for the entire banana plant **[7]**: the flowers can be used to treat bronchitis, dysentery, ulcers, and diabetics, the sever plant fluid can be applied to cramps, Hansen's disease, fever, hemorrhages, and diarrhea, and on piles, bugs and bites, immature leaves are applied to burns and skin illness, the ashes of the undeveloped peel and the leaves are used in abdominal pain and used for treating virulent ulcers **[8]**; the rootlets are regulated in intestinal disorders, dysentery and banana seed mucilage in diarrheal cases in India. The ripe bananas peels contain antifungal and antibiotic properties **[9]**. Fruit peels and pulp of banana protect tomato plant against fungus diseases **[10]**. Ripe skin and pulp also contain noradrenaline, dopaminergic, and vasopressin **[11]** which affect many physical factors, blood pressure etc. Serotonin suppresses gastric juice secretion and stimulates intestinal smooth muscle. Various studies have been conducted on banana peels, including: antibacterial, antioxidant, anti-diabetes, anti-fungal, anti-ulcer, etc. Since then, every part of the banana has medicinal properties. Since, there is lack of literature reported for anti-obesity potential of banana peels. Therefore, in this study, the aim is to investigate Banana peels (*Musa paradisiaca. L*): An assessment of their anti-obesity potential *in vitro.*

MATERIAL AND METHODS

Sample collection

The raw and ripe bananas were procured from the local market of Belagavi city, Karnataka, India. The 3T3 cell line were purchased from National Centre for Cell Science, Pune, India. The banana peels were washed and shade dried before further analysis.

Preparation of Banana peel extract

Ripe and raw banana peels were dried and powdered and weighed. The powder was mixed with two different solvents i.e. 70%

Isopropyl Alcohol Extract and 70% Methanol Extract. These mixtures were kept at room temperature for about 72hrs and the liquid was filtered by using Whatman filter paper no. 40. followed by the use of Rotatory Evaporator for the effective separation of solvents from samples via distillation.

Lipase inhibition assay

Microplate of 96 well were used for the assay with two different solvents each for banana raw and ripe with six different concentrations followed by labelling the plates. To each well 25ul of peel solution was added. (Each sample was collected in triplicate with decreasing concentration). 50ul of enzyme solution followed by 100ul of buffer was added to each well. 25ul of PNPB substrate was added to microplate well and addition of enzyme, buffer and PNPB was kept as blank (triplicates). Standard was taken as 200µl of orlistat and the microplate was incubated at 37^0C for 30 mins**.** The plate was read in the ELISA reader at 400 nm absorbance **[12]**. Using the below formula % Inhibition activity was calculated.

$$\% \text{ inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times 100$$

Alpha Amylase Inhibitory Essay

In the Eppendorf tube, 0.5 ml of test sample at various concentrations (50µg/ml, 100, 150, 200, and 250µg/ml) was combined with 1 ml of PBS. 200ul of 0.5 mg/ml standard solution of α-amylase was added and by 200µl of 5mg/ml starch solution and incubated at ambient temperature for 10 mins. The controls were starch with and without amylase. After maturation, the reaction was halted by introducing of 400ul of DNS solution, which was warmed in an evaporating water bath for 5 minutes and then cooled. Metformin was taken as a standard, and at 540nm absorbance was measured **[13]**. The following formula was used to enumerate the percentage of enzyme inhibition:

$$\% \text{ of } \alpha\text{-amylase inhibition} = [(Ac - A_t)/A_t] \times 100$$

Where, Ac and A_t are the absorbance of control and sample, respectively

Oil Red staining

Day 1: About 1 ml cells was added to four wells of a 12 well plate. One well was seeded with a negative control and cultured in a CO_2 incubator overnight. On Day 2 and Day 3 1ml of plant extract was enumerated to each well of 12 well plate and kept for 48 hours in CO_2 incubator. On Day 4 Carefully the supernatant was removed, and PBS was used to rinse the wells. After that, 10% 200ul of formalin was added and the mixture was incubated for half hour. After incubation plate was washed with 500ul of PBS for one time and 1ml of ORO stain was enumerate to each of 12 wells and incubated for 30min. The plate was washed for two times with 1ml of dis. water and observed under microscope**.** To the wells 300ul of isopropyl

alcohol was added to each well and 100ul of the preparation was transfer to 96 well plates in triplicates and absorbance was determined using ELISA reader at 510nm **[14]**.

MTT Assay

Plant extract preparation: 1 mg of each of four samples was dissolved in 1ml of DMEM media

MTT Dye: In 1ml of PBS 5mg of dye was dissolved (immediately prepared before use). On Day 1 to determine the cell viability, Trypan blue assay was conducted using hemocytometer was used to observe and count the cells. Cells were planted in a microplate of 96 well, and the test was done in triplicates (78 well were seeded as there were four different extract solvents). 100ul of cell suspension and 50ul of DMEM media was added and final volume was made to 150ul and was cultivated overnight in CO_2 incubator. On Day 2, 100ul of this peel extract at six different concentrations was applied to a microplate of 96 well. Negative control was added with 100µl of DMEM media to microplate of 96 well. In a CO_2 incubator at 37^0 C in the presence of 5% CO_2 the microplate was incubated for 24 hours. On Day 3, 20ul of 5mg/ml of MTT dye was added to the plate after 24 hours and covered with aluminum foil and incubated for four hours at ambient temperature. Without disrupting the precipitated formazan crystals, 100ul of supernatant was discarded. To dissolve the generated crystals, 100ul of DMSO was applied to each well plate. The absorbance was taken using ELISA reader at 492nm **[15]**.

Absorbance of test /Absorbance of blank ×100

RESULTS

Total yield of Peel extract

The total yield of plant extract was found to be 8.08g, 9.9g and 8.5g and 9.6g from raw and ripe banana peels (*Musa paradisiaca L*) utilizing solvents such as isopropyl alcohol and methanol respectively.

Lipase Inhibition Assay

Lipase inhibition, which is based on the assumption that dietary fat would not be directly absorbed by the stomach until it has been treated with pancreatic lipase, is the most often researched activity for defining anti-obesity therapy. The extracts (raw and ripe isopropyl alcohol) and (ripe and raw methanol) were made at six concentrations the blank was taken as 3.72 and the absorbance was determined at 400nm in the ELISA reader. When compared to standard orlistat, methanol extract demonstrated the strongest lipase inhibition for both raw and ripe at 500µg/ml and 250g/ml, respectively, as shown in **(Figure 1)**, followed by isopropyl extract.

Alpha Amylase Inhibition Assay

In vitro, starch hydrolysis in the presence of -amylase enzyme can be used to test -amylase activity. This process was quantified using the DNS reagent, which

gives an orange-red color when coupled with starch. The reduced intensity of orange hue indicates enzyme-induced hydrolysis of starch into monosaccharides. If the substance/extract inhibits -amylase, the color intensity will be higher. The extracts were produced in five concentrations with the blank being taken at 1.28 and absorbance at 540nm being compared to standard metformin. Methanol raw showed the maximum amylase inhibition at 250g/ml, and ripe likewise showed the same **(Figure 2)**, followed by isopropyl alcohol for raw and ripe extract.

Oil Red O staining

Oil red O staining is used to show the presence of fat and lipids in tissue sections as well as to compare the degree of adipocyte differentiation in cell culture. For comparison, all four extracts were applied to a 12 well microplate along with a negative control, and results were collected after four days **(Figure 3 a, b, c, and d)** When compared to absorbance of control during adipocyte separation, isopropyl raw extract prevented the most lipid accumulation and adipogenesis **(Figure 4)**.

MTT Assay

The MTT Assay is a calorimetric test that is used to determine cell metabolic activity and cell viability. All the four extracts were added to 96 well microplate in triplicate form with five different concentrations. The standard was kept as orlistat drug. Isopropyl ripe possessed the highest cell rate of survival of 98.7% at a 20ug/ml concentration, while other extracts such as isopropyl raw, methanol raw, and ripe had lower survival rates of 67.59%, 64.1%, and 76.3% at 100g/ml concentration as shown in the **(Figure 5)**.

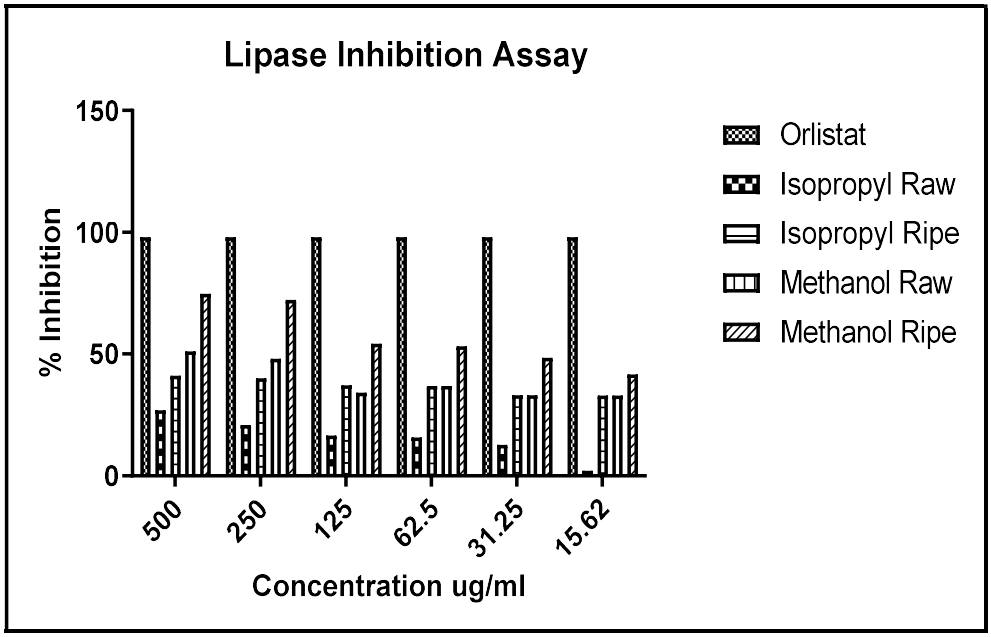


Figure 1: Lipase inhibition Assay concentration v/s percent inhibition

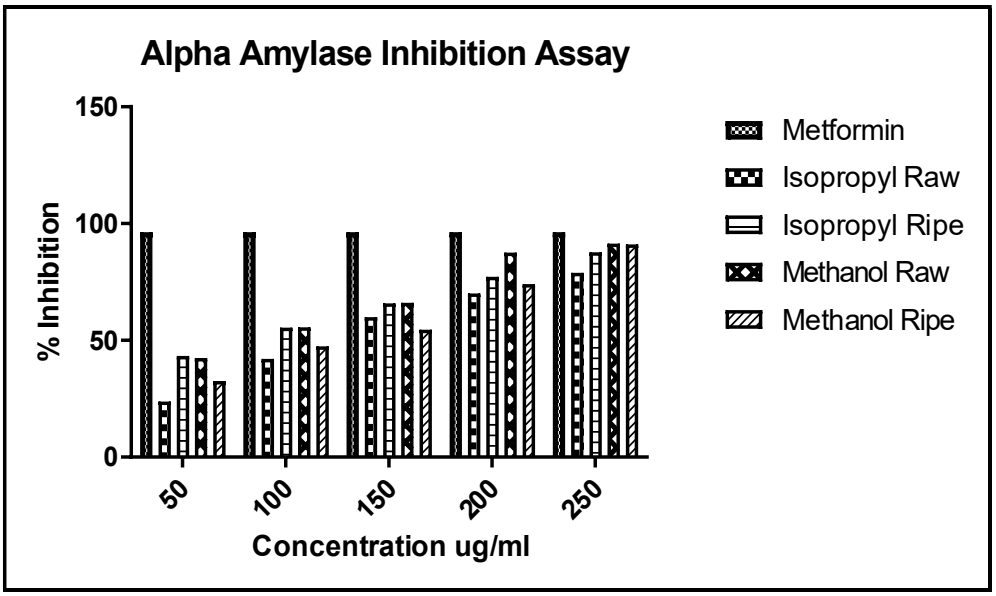
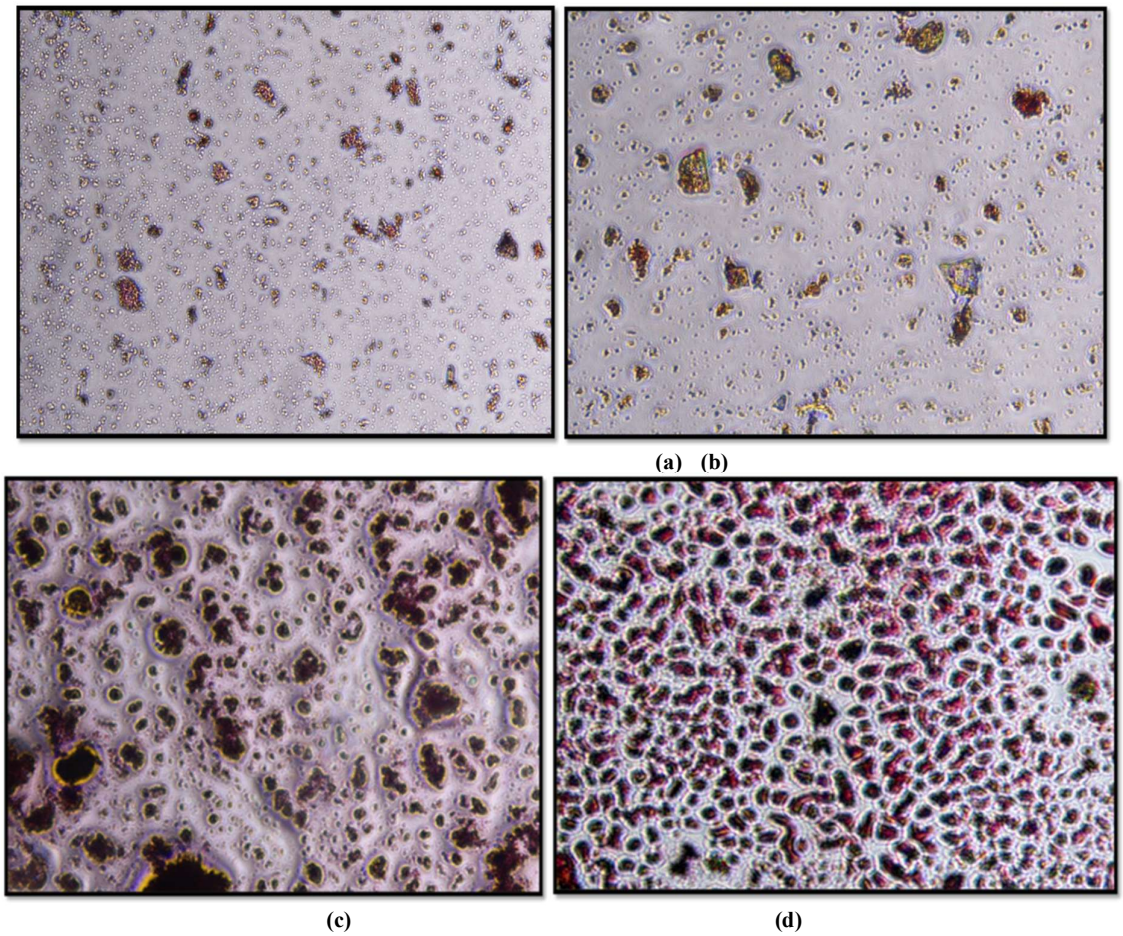


Figure 2: α- amylase inhibition assay concentration v/s percent inhibition



(a) (b)

(c) (d)

Figure 3: (a) and (b): Effect of Isopropyl raw and ripe extract on lipid accumulation in differentiated 3T3- L1 Cell Line (c) and (d): Effect of methanol raw and ripe on lipid accumulation in differentiated 3T3-L1 cell line

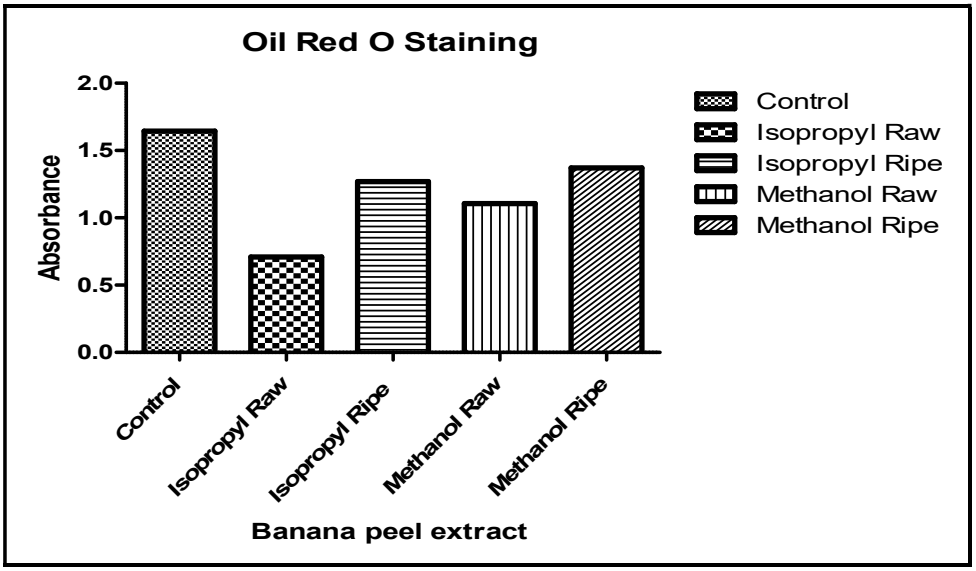


Figure 4: Oil Red O staining, banana peel extracts v/s absorbance

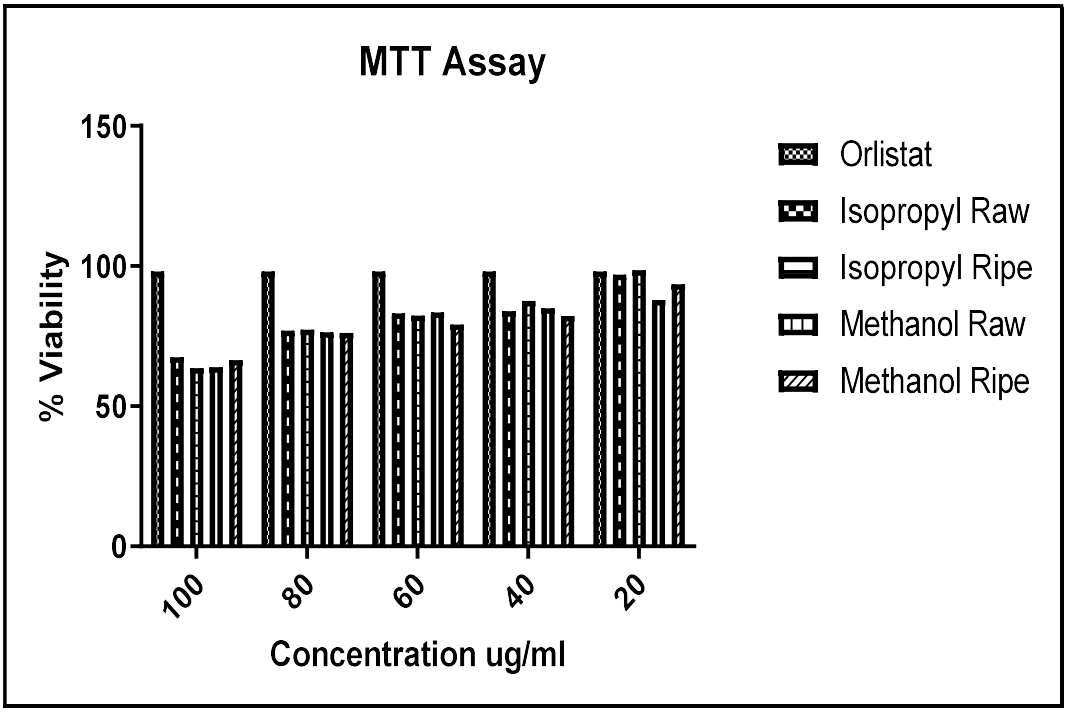


Figure 5: MTT assay concentration v/s percent viability

DISCUSSION

Obesity is the problem that is increasing over time. It is an abnormal condition that results in fat accumulation that mediate with normal health. Through food the great amount of fat enters the body in form of kilocalories that lead to cause metabolic disorders. It is worldwide, resulting in crucial increase in mortality and morbidity related to metabolic disorders World Health Organization (WHO). Numerous variables are responsible for the cause of the weight such as one of which is eating quick nourishment containing high calories, fat

and cholesterol, with stationary life. Diverse sources of common compounds have given security and improvement of pharmaceutical operators against obesity. *Musa paradisiaca* could be a crossbreed title between Musa balbisiana and Musa acuminate **[16]**. Fruits and vegetables peels have importance in protecting many diseases. Banana peel also contains Vitamin A, Vitamin C, Gallo catechin, dopamine, Vitamin E, Vitamin B6, â- sitosterol, malic acid, succinic Acid, palmitic acid, Magnesium, phosphorus, potassium, fiber, iron and fatty acid. The following analysis was to find out whether the plant extracts have anti-obesity potential, and the findings were correctly obtained. Lipase inhibition Assay This test is focused on to decrease the sum of body fat or fat weight. The Pancreatic inhibition assay is commonly used to investigate the mechanism and determine the antiobesity potential of a substance. Xenical (orlistat) is the exclusive drug approved by FDA for the acquirable medication for weight-loss. The sum of normally happening compounds for the treatment has effect on the investigation for recognizable proof of recently found pancreatic lipase inhibition that will need troublesome side impacts. In the current study methanol ripe exhibited highest lipase inhibition activity up to 74.7% at the concentration 500µg/ml as depicted in the above **Figure 2**. Similarly, Divya *et al*., in 2016, an investigation was made on aqueous dosage of 10 mg/ml, a banana flower extract showed lipase inhibitory action of up to 15.14% **[17]**. It was determined that *Musa paradisiaca* 's flower shows biological activities. Alpha amylase is responsible to cleave the starch during the digestive process which play important role in blood glucose level. It is a key protein in the body that is responsible for disintegration of starch (polysaccharide) into simpler sugars. Amylase inhibitors are moreover known as blockers of starch as they halt the ingestion of this dietary starch and decrease the glucose level. It has been detailed that the nearness of characteristic polyphenols inhibits carbohydrate hydrolyzing enzymes. This measure was utilized to decide percent inhibition of amylase utilizing peel extract. Standard was taken as metformin. The raw and ripe extracts of methanol showed the highest % inhibition, with 91.4 and 91%, respectively, at 250g/ml concentration. Similarly, within the study appeared that the stem portion of *Musa paradisiaca* with methanol extract shown efficient inhibition of α-amylase enzyme and hydroalcoholic was observed to be more dynamic against the chemical **[18]**. Moreover, in another study considered adult-onset diabetes utilizing Inflorescence phenolic from *Musa paradisiaca* showed that way better action in repressing the carbohydrate digestive enzymes **[19]**. Lipid accumulation is

completely separated cells by Oil red O Staining. To look at the lipid droplet-accumulated triglycerides in adipocytes a straightforward quantitative strategy is utilized that is Oil red O staining. The fat cells are recoloured with Oil red O stain that are commonly visualized microscopically within the shape of cell colonies from the culture of predispose cell line. It shows a considerable link between fat change and the amount of recolouring of 3T3 cells with Oil red stain. To see how banana peel extract affects lipid accumulation in the 3T3 cell line. The cells were refined for 48 hours after being treated with peel extract. After 48hrs accumulation of lipids were watched and it was stained with Oil red O stain to decide the nearness of lipid droplets that demonstrates the degree of adipogenesis. The solubilized stained lipid droplets' absorbance at 510nm was used to evaluate the results. Consequently, it was concluded that isopropyl raw extract inhibited highest lipid accumulation and adipogenesis during adipocyte separation as compared with absorbance of control. And other three extracts too inhibited lipid accumulation. Therefore, the results appear that raw and ripe banana peels inhibit lipid accumulation and may have potential anti-obesity impacts. Banana peels have a consequence on mouse adipocytes 3T3-L1 cell line, fibroblasts treated with all four-extract isopropyl raw and ripe, and methanol raw and ripe. This measure was performed to decide the intracellular cytotoxicity of all four extracts. All four extracts were given to doses of 3T3-L1 (Swiss albino) cells ranging from 20, 40, 60, 80, and 100ug/ml. After a 48-hrs period of maturation, the practicality of the cells was set to examine using the MTT method. 98 was used to quantify the rate of viable cells by determining viability of cells without treatment (control). Isopropyl ripe possessed the highest cell rate of survival of 98.7% at a 20ug/ml concentration, while other extracts such as isopropyl raw, methanol raw, and ripe had lower survival rates of 67.59%, 64.1%, and 76.3% at 100g/ml concentration. As a result, no extracts had an effect on the cells. Similarly, in earlier study, methanolic raw peel extract of M. paradisiaca flowers research showed that this tepal extract was non-toxic on cell line 3T3-L1 (Swiss albino) **[20]**.

CONCLUSION

The current focused on the anti-obesity potential of ripe and raw banana peel (*Musa paradisiaca L.*). Plants contribute too many medicinal compounds used today to treat diseases such as cancer, jaundice, diabetes and inflammation. Different sources of natural compounds have provided safety and development of pharmaceutical agents against obesity. Banana peel was used to determine anti-obesity property that could be used for further treatments and reduce any other side effects. Lipase inhibition

assay exhibited that methanol raw and ripe extract obtained significant inhibitory activity we can conclude that *Musa paradisiaca L* peel has a lipid-lowering effect. The results determined that α-amylase inhibition assay obtained anti-diabetic potential by significant inhibition of carbohydrates digestive enzymes. From this, it is concluded that the raw methanol extract and the ripe extract showed inhibitory activity. It is demonstrated that banana peel extracts suppressed adipogenesis in 3T3 cell line by inhibiting lipid accumulation and adipogenesis. The results obtained through MTT assay concluded that both raw and ripe peel extract was non-cytotoxic to the cells at the concentration range up to 20µg/ml to 100µg/ml and therefore, the extracts can be drawn for the further study and ministrations of obesity. We are able to conclude that all four extracts inhibit lipid accumulation, but the isopropyl raw extract showed the elevated lipid accumulation. Therefore, the results suggest that these all four extracts of raw and ripe banana peel (*Musa paradisiaca L*) could be useful for prevention of obesity.

Statements and Declarations

Financial interests: The authors declare they have no financial interests**.**

Competing Interests: Rubeen Nadaf, Manjula Kambi, Surthi Ravedar, Parveen Nadaf and Shridhar Ghagane declares that she/he has no conflicts of interest.

Authors' contributions: Rubeen Nadaf and Surthi Ravedar conducted experimental work and drafted manuscript, Manjula Kambi and Parveen Nadaf analysed data, and Shridhar Ghagane designed and reviewed manuscript. All authors commented on the manuscript and approved the final manuscript.

Ethics approval and consent to participate:

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication: Not applicable.

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