# INVITRO STUDY OF ANTIDIABETIC ACTIVITY OF METHANOLIC BARK EXTRACT ON SYZYGIUM CUMINI [B]

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**Abstract:**
*Syzygium cumini*, also called Jamun, is a member of the Myrtaceae family. *Syzygium cumini* seeds, a type of traditional remedy, were available on the market and were extensively used to control disease. The purpose of the present study is to investigate the immense effect that *Syzygium cumini* bark has upon diabetes. This study's objective is to confirm the antidiabetic activity. Using DPPH, Abts, Phosphomolybdenum, and Ferrous Reducing Power, the scavenging capability of methanolic extract and the standard (Ascorbic acid) was evaluated. The $IC_{50}$ was identified as 45.67% and 45.06%, whereas the $RC_{50}$ was identified as 46.18% and 56.45%. With an $IC_{50}$ value of 18.62%, the 3T3 L1 Adipocytes diabetic cell line revealed anti-diabetic action.

**Keywords:** Anti-oxidant activity, total phenol content, phytochemical screening, 3T3 L1 Adipocytes cell line and *Syzygium cumini* (*B*).

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## INTRODUCTION:

The use of herbal medications is tremendous nowadays, so while diabetes is a serious complication all around. The "Rasayana" collection of herbal remedies, which is part of the Indian traditional medical system (Ayurveda), includes a variety of medicinal plants. Since the beginning of human civilization, plants have been crucial to its development, serving as a remarkable source of natural medicine. The use of medicinal herbs was usually widespread at the beginning of the 19th century [1]. Diabetes has a gigantic detrimental role on a patient's quality of life, affecting their ability to work, sleep and be independent [2-3]. As a consequence of our current situation, more and more individuals are turning to using steroids to treat minor illnesses like lung distress. However, overusing these medications can lead to negative side effects like hyperglycemia. This could result in steroid-induced diabetes or a new onset of diabetes [3].Hence, this study was formulated to characterize the several active components procured in the bark and to examine the anti-oxidant activity of a methanolic extract of *Syzygium Cumini* using Dpph, Abts, Ferrous reducing power & Phosphomol-ybdenum assay and to estimate the activity against diabetes mellitus by using 3T3-L1 Adipocytes cell line.

## MATERIALS AND METHODS:

### Collection of plant material:

Mercantile obtainable dried bark of *Syzygium Cumini* was acquired from the orthodox herbal market, Karaikal district, Puducherry, India. Recognition was evaluated by a Botanist at Institute of natural science research (INSR), Theni, India.

### Composing of plant material:

- Dried bark was grounded to nominal mesh aperture of 180 microns powder with a grinder and then it was stored in an air tight container. The collected crude product was shade dried respectively and extracted by using Soxhlet apparatus. Extracted samples were kept in an Amber coloured container to prevent from the contamination.

### Chemicals and Reagents:

- Methanol, Distilled water, DPPH(Diphenyl-1-picrylhydrazyl), Ascorbic acid, Potassium persulfate, phosphate buffered saline, potassium ferricyanide, trichloroacetic acid, phosphomoly-bdenum reagent, dragendroff's reagent, chloroform, ferric chloride, sodium nitroprusside solution, conc. Nitric acid, sodium hydroxide, lead acetate solution, Ciocalteau reagent, sodium carbonate, sodium nitrite, aluminium chloride, neutral 5% ferric chloride, alcoholic alpha naphthol solution, lead acetate solution.

### Analysis:

- The phytochemical screening were done, Polyphenol content analyses were done by total phenol contents and total flavonoids, Anti-oxidant activities were done by 2,2-diphenyl-1-Picrylhydrazyl, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), Ferric reducing power assay, Phosphomolybdenum assay. Invitro anti-diabetic potential of *Syzygium cumini* was performed by using the cell line 3T3 L1 Adipocytes and confirmed Alpha amylase assay.

### Phytochemical Screening:

The sample was subjected to various phytochemical screening to determine the active chemical constituents.

**Antioxidant activity**: The anti-oxidant activity was confirmed by DPPH Radical scavenging activity, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid), ferric reducing power assay, phosphomolybdenum assay.

**DPPH$^{\cdot}$ radical scavenging assay:** The ability of the extract to scavenge free radicals was assessed using DPPH Radical Scavenging Activity (1, 1-diphenyl-2-picrylhydrazyl). One mL of 0.1 mM DPPH solution in methanol was combined with one mL of different material amounts (ranging from 50 to 300 g/mL). It was then allowed to remain in the dark for 30 minutes. 1 mL of methanol and 1 mL of DPPH solution were combined as a reference. The absorption started to drop at 517 nm. The mentioned example was ascorbic acid. (figure 1)

Calculating the percentage of inhibition used the following formulas:

$$\frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

**ABTS$^{+}$ radical cation scavenging assay:** After the procedure, the antioxidant potential were calculated as per Abts radical cation scavenging activity. The reaction of 2.45 mM $k^{+}$ persulfate and 7 mM Abts Stock solution produced ABTS, which was then allowed to stay at room temperature about 12 to 16 hrs before use. The ABTS solution, which is robust for a couple of days, has been mixed along with five millimetres of phosphate-buffered salt in order to attain an absorbance at 730 nm. (Potential of Hydrogen is 7.4). Following the addition of methanol extract at various concentrations (10–60 g/mL) to 1 mL of reduced ABTS solution, the uptake was assessed 10 minutes later. The samples

capability to scavenge radicals in accordance with ABTS was described. (figure 2)

**Ferric reducing power assay:** Applying antioxidants as reducing agents in a redox-linked colorimetric process, the ferric reducing antioxidant power assay method. It was mixed with a single milliliter of phosphorous solution (0.2 M, potential of Hydrogen was 6.6) and 1 mL of 1% K+ ferricyanide to generate various extract concentrations (20-120g/mL). For 20 minutes, the mixture was kept at 50° C. 10% trichloroacetic acid in a volume of 1 mL was mixed with the mixture. Later 1 mL of readily produced, 0.1% Ferric cl was mixed, and the final solution's absorbance was evaluated at 700 Nm. (figure 3)

**Phosphomolybdenum assay:** The reduction assay method, which is depends on the development of green phosphomolybdenum complex, was used to evaluate the antioxidant activity. Different extract concentrations (20–120 g/mL) were added with one mL of the Phosphomolybdenum reagent. The containers were sealed and incubated for 30 minutes at 95°C in a water bath. The specimen were frozen to the room temperature before measuring the absorption at 695 nm in comparison to a control sample. (figure 4)

**3T3 L1 ADIPOCYTES:** By using Invitro study, the anti-diabetic potential of *syzygium cumini* bark was performed by using the cell line 3T3 L1 Adipocytes and confirmed Alpha amylase assay. The cell line was incubated for 48 hours.

**Cytotoxicity and anti-diabetic activity:**

**Cell Culture:** 3T3-L1 adipocytes diabetic cell line was collected from National Centre For Cell Science research institute, Pune, Maharashtra and it was seeded in Rose well Park Memorial Institute medium, it was enriched with 10 percentage of foetal bovine serum, Penicillin /macrolide such as streptomycin (Two fifty U/mL), aminoglycosides such as gentamycin (One hundred µg/mL) and Polyenes such as amphotericin-B (One mg/mL) procured from Sigma Chemicals. Each cell's development was maintained at 37°C in a wet atmosphere with 5% CO2. Cells were allowed more than 24 hours for growth before use.

**Cell growth inhibition studies by MTT assay:** The standard MTT reduction test was applied to evaluate survival of cells. 3T3 L1 Adipocytes $5x10^3$ lymphocytes/well of each type were inoculated in 96-well dishes for one day in 200 L of the RPMI alongside ten percent of fetal bovine serum. Then, the cultured cells were removed and RPMI consisting of several concentrations (1.56-100 µg/mL) of methanolic extract of *syzygium cumini* bark was mixed and placed under incubation about 48 h. Later, the cells were placed under incubation with Mtt (10µL, 5mg/mL) at 37°C for 4 hours and then the same progress was done along with DMSO for one hour at ambient temperature. A scanning multi-developed spectrophotometer was used to analyse the samples at 595 nm. The resulting statistics were provided as mean values through three separate trials.[4]

**ALPHA AMYLASE ASSAY (Antidiabetic activity):**

- Alpha-amylase enzyme inhibition assay α-amylase enzyme inhibition assay was carried out based on the starch-iodine test . The total assay mixture was composed of various concentration (20-120 µg/mL) of extracts of , 20 µL of alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and incubated at 37°C for 10 min. Then, 200 µL of soluble starch (1%, w/v) was added to each reaction tube and incubated at 37°C for 60 min. One hundred µL of 1 M HCl was added to stop the enzymatic reaction and followed by 200 µL of iodine reagent (5 mM I2 and 5 mM KI) solution was added. The colour change was noted and the absorbance was read at 595 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. A dark-blue colour indicates the presence of starch; yellow colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extract, the starch added to the enzyme assay mixture is not degraded and gives a dark-blue colour complex, whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-amylase.

**RESULTS:**

**Phytochemical screening:** *Syzygium cumini* bark extract was examined using a phytochemical method, and the results are shown in Table 1. The analysis identified the presence of alkaloids, phenolic compounds, terpenoids, tannins, carbohydrates, flavonoids, quinone, steroids, and glycosides.

**Polyphenol content:** The polyphenol content analyses was confirmed by the total phenol content test and total Flavonoids test and the results are shown in the table 2.

**Antioxidant activity:** The results of the radical scavenging activity of the extract are shown in Table 4; the extract has an IC50 value of 45.67 and 45.06 using DPPH assay & ABTS assay respectively and RC50 value of 46.18 & 56.45 using ferric reducing power assay and phosphomolybdenum assay respectively.

**Cell viability using MTT assay:** The *S.cumini* bark extract showed positive results against 3T3 L1 Adipocytes cell line with an IC50 of **18.62 µg/ml.** The results were shown in the table 5.

**CONCLUSION:**

- Results from the present research revealed that the methanolic extract of *syzygium cumini* had a good anti-oxidant potential using DPPH, ABTS, Ferrous reducing power & Phosphomolybdenum assays and proved the anti-diabetic potential of the Methanolic extract of *syzygium cumini* using the 3T3-L1 adipocyte diabetic cell line**.**

Also extracts of *s.cumini* could be further evaluated and examined in detail for its mechanism of action in order to develop a less side effecting and more cost-effective, targeted anti-diabetic agent for the benefit of suffering humanity.

**TABLES:**

**Table 1:** Phytochemical screening

| S.No | Parameter | Results |
|---|---|---|
| 1. | Alkaloids (dragendroffs test) | + |
| 2. | Terpenoids (Salkowski test) | + |
| 3. | Phenolic compound (ferric chloride test) | + |
| 4. | Flavonoids (alkaline reagent test) | + |
| 5. | Tannins (lead acetate test) | + |
| 6. | Carbohydrates (Molisch test) | + |
| 7. | Saponins (foam test) | _ |
| 8. | Glycosides (legals test) | + |
| 9. | Quinone (sulphuric acid test) | + |
| 10. | Proteins (xanthoproteic test) | _ |
| 11. | Steroids (Liberman burchard test) | + |

**Table 2:** Polyphenol Content

| SI.NO. | TEST | OD AT 765NM | GAE (mcg/ml) |
|---|---|---|---|
| 1. | Estimation Of Total Phenol | 0.421 | 218.58 |
| 2. | Estimation Of Total Flavonoids | 0.452 | 80.93 |

**Table 3:** Antioxidant activity:

| S.No | Test | Conc (µg/ml) | percentage |
|---|---|---|---|
| 1. | DPPH Radical Scavenging Activity | 300 | 94.97 |
| 2. | ABTS Radical Cation Scavenging Assay | 60 | 97.26 |
| 3. | Ferric Reducing Power Assay | 120 | 67.54 |
| 4. | Phosphomolybdenum Assay | 120 | 93.22 |

**Table 4 : IC50 and RC50 of *S.cumini* bark extract.**

| S.No | Test | $IC_{50}$ | $RC_{50}$ |
|---|---|---|---|
| 1. | DPPH Radical Scavenging Activity | 45.67 | - |
| 2. | ABTS Radical Cation Scavenging Assay | 45.06 | - |
| 3. | Ferric Reducing Power Assay | - | 46.18 |
| 4. | Phosphomolybdenum Assay | - | 56.45 |

**Table 5:** Optical density of cell viability in MTT assay.

| Conc µg/mL | OD1 | OD2 | OD3 | % Cell Death | | | Mean | SD | SEM | % Live Cells |
|---|---|---|---|---|---|---|---|---|---|---|
| **100** | 0.04 | 0.08 | 0.045 | 94.59 | 89.19 | 93.92 | 92.57 | 2.95 | 1.70 | 7.43 |
| **50** | 0.15 | 0.14 | 0.18 | 79.73 | 81.08 | 75.68 | 78.83 | 2.81 | 1.62 | 21.17 |
| **25** | 0.21 | 0.24 | 0.28 | 71.62 | 67.57 | 62.16 | 67.12 | 4.75 | 2.74 | 32.88 |
| **12.5** | 0.32 | 0.3 | 0.39 | 56.76 | 59.46 | 47.30 | 54.50 | 6.39 | 3.69 | 45.50 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **6.25** | 0.42 | 0.46 | 0.48 | 43.24 | 37.84 | 35.14 | 38.74 | 4.13 | 2.38 | 61.26 |
| **3.125** | 0.52 | 0.53 | 0.52 | 29.73 | 28.38 | 29.73 | 29.28 | 0.78 | 0.45 | 70.72 |
| **1.56** | 0.62 | 0.61 | 0.63 | 16.22 | 17.57 | 14.86 | 16.22 | 1.35 | 0.78 | 83.78 |
| **Control** | 0.75 | 0.78 | 0.71 | | | | | | | 100 |
| **Control mean** | 0.746667 | | $IC_{50}$ :18.62 | | | | | | | |

The methanolic extract of *Syzygium cumini* bark at 10*0* µg/ml Concentration the cell death was found to be 92.57% and IC50 was to be **18.62 µg/ml**

| S.No. | Concentration | Absorbance at 595nm | % of Inhibition |
|---|---|---|---|
| **1.** | CONTROL | 0.406 | - |
| **2.** | 20 | 0.473 | 14.16 |
| **3.** | 40 | 0.528 | 23.1 |
| **4.** | 60 | 0.633 | 35.86 |
| **5.** | 80 | 0.690 | 41.15 |
| **6.** | 100 | 0.755 | 46.22 |
| **7.** | 120 | 0.930 | 56.34 |

**Alpha Amylase Assay:**

- The methanolic extract of *Syzygium cumini* bark at 100 µg/ml Concentration the inhibition activity was found to be 56.34%.

% of α- amylase enzyme inhibition = (sample-control/ sample) x 100

**FIGURES:**

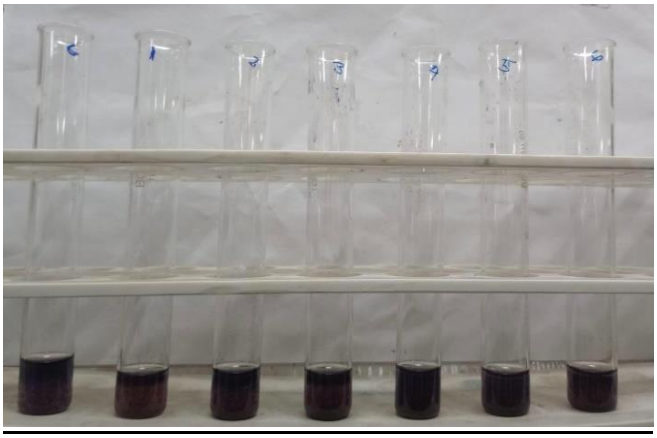


**Figure 1:** ANTIDIABETIC ACTIVITY OF EXTRACT USING ALPHA AMYLASE ASSAY

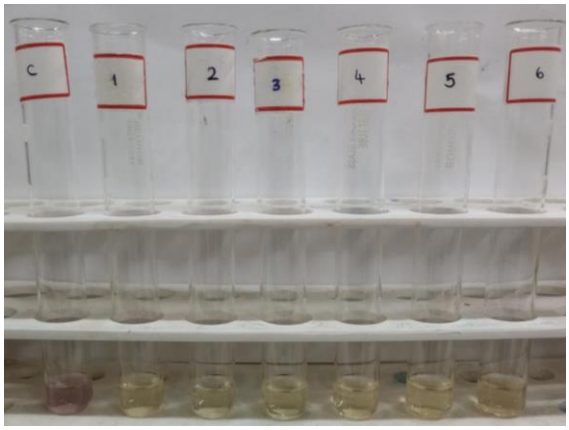



**Figure 2:** DPPH assay

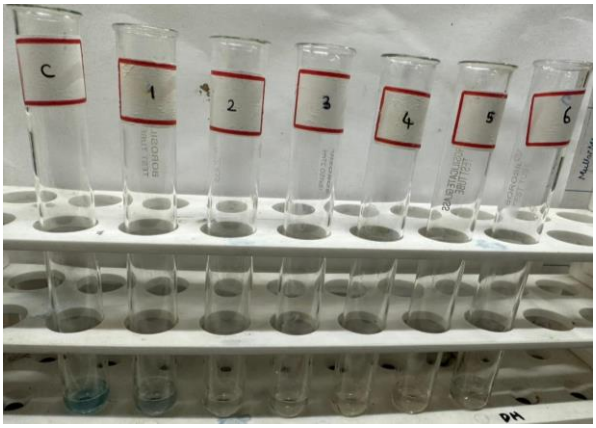



**Figure** 3: ABTS assay

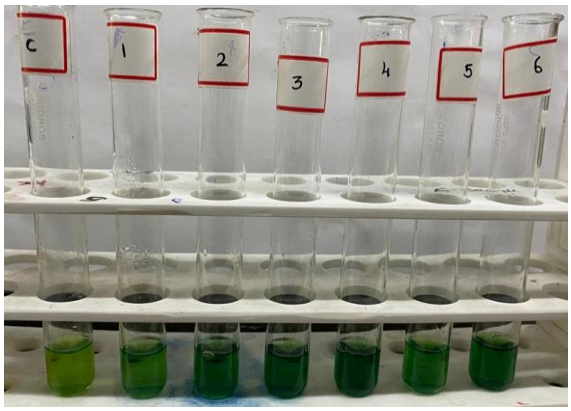



**Figure 4:** ferric reducing power assay

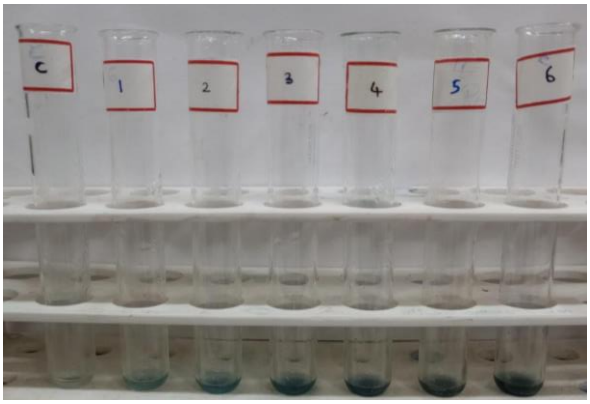



**Figure 5:** phosphomolybdenum assay

**Cell Viability:**

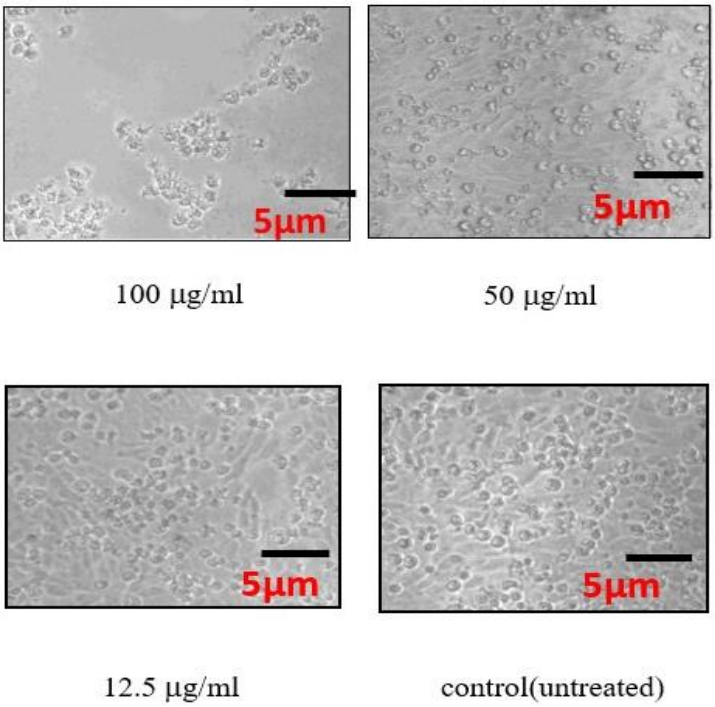
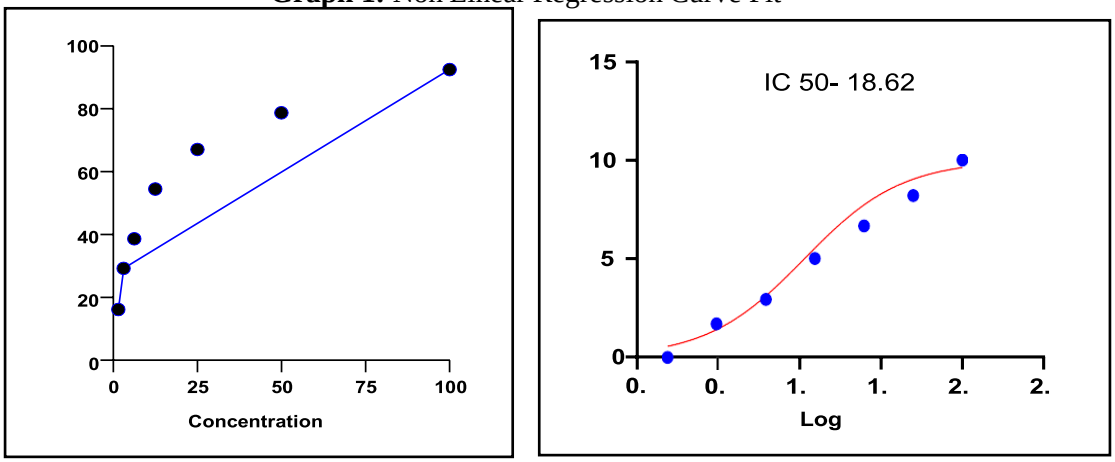
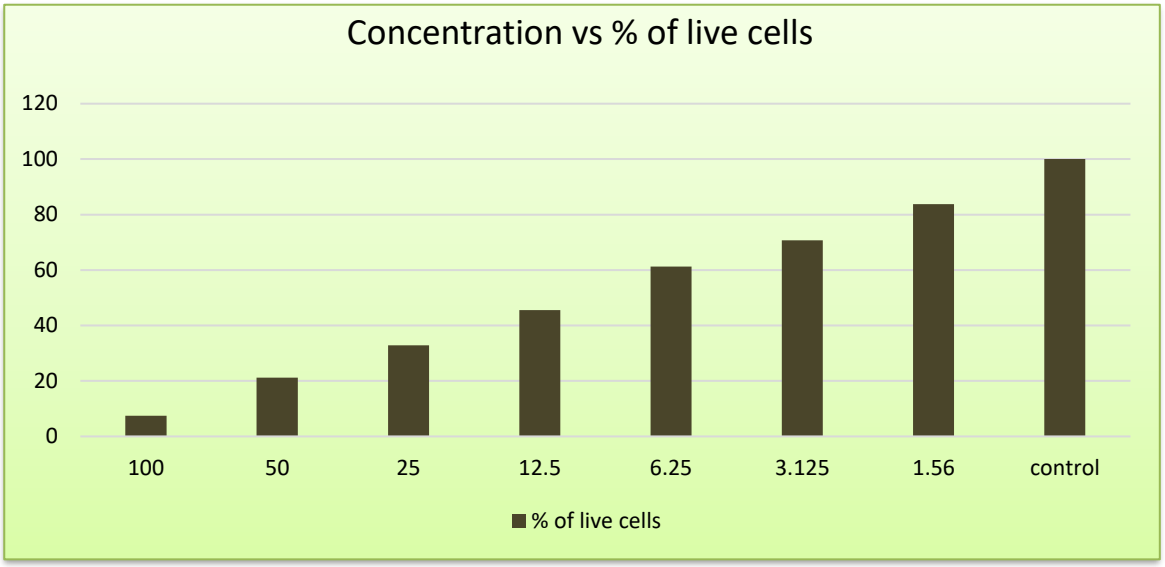



**Figure 5:** Microscopic observation of 3T3 L1 adiocyte cells treated with *S.cumini* bark extract

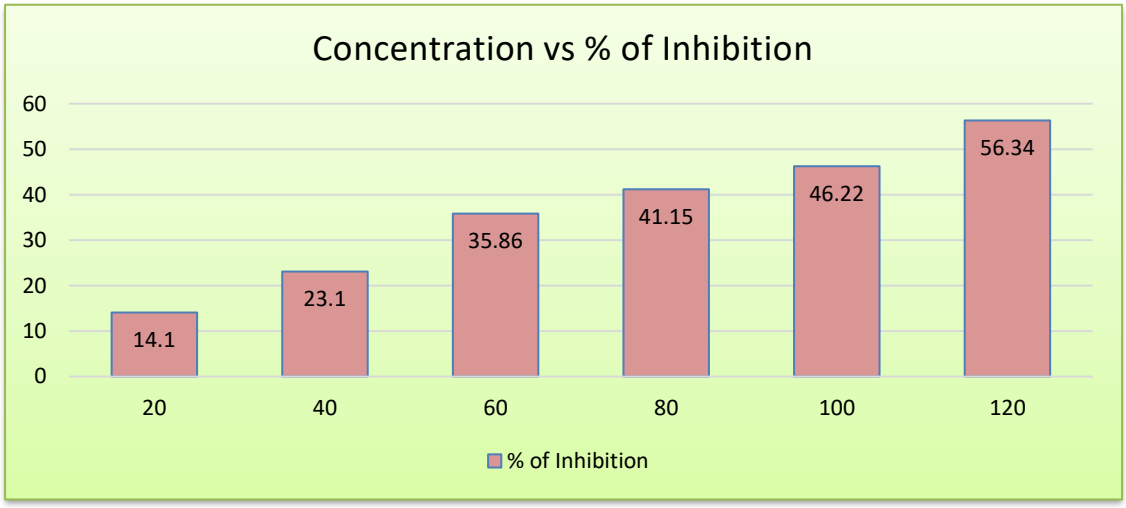
**Graph 1:** Non Linear Regression Curve Fit






**CYTOTOXIC ACTIVITY OF EXTRACT USING MTT ASSAY**

- The methanolic extract of *Syzygium cumini* bark at 100 µg/ml Concentration the cell death was found to be 94.59%_and IC50 was to be 18.62µg/ml




**ANTIDIABETIC ACTIVITY OF EXTRACT USING ALPHA AMYLASE ASSAY**

**CONFLICT OF INTEREST:** none

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