RESEARCH PAPER

Evaluation of the phytochemical and antidiabetic properties of nano-based materials derived from Coccinia grandis, Luffa acutangula, and Syzygium cumini

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ABSTRACT

The aim of present research is to study, compares and examines the antidiabetic properties of aqueous extracts from Syzygium cumini, Luffa acutangula, and Coccinia grandis, as well as their mechanisms of action. The conventional procedures for phytochemical screening, which include alkaloids, phenols, carbohydrates, flavanoids, saponins, amino acids, and steroids, were applied to the three plant samples. Syzygium cumini exhibited the highest concentration of quantitative test results. Aqueous extract sample's antioxidant activity was measured utilizing the DPPH and Phosphomolybdenum Reduction Assay methods. Based on the comparison of the antioxidant results of DPPH and Phosphomolbdenum, Coccinia grandis and Syzygium cumini were mixed and an antidiabetic assay was conducted. Using the glucose uptake assay, antidiabetic studies on cell lines have demonstrated a higher percentage of insulin absence.

Keywords: Diabetics, phytochemical analysis, antioxidant activity, antidiabetic assay, Good Health and Well-Being

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1. INTRODUCTION

Diabetes was a chronic illness that affects rates of morbidity and death globally. A loss and reduced of cellular responsiveness to hormones of an endocrine system of metabolic illness. It has a long-term metabolic disease marked by elevated blood glucose levels [1]. This condition can be brought on by insufficient pancreatic insulin synthesis or by poor cell response to insulin. Diabetes comes in various forms, such as type 1 diabetes, type 2 diabetes, and gestational diabetes. Serious consequences include heart disease, stroke, kidney failure, blindness, and nerve damage can result from uncontrolled diabetes [2]. The goal of managing diabetes is to keep blood sugar levels within a safe range by taking medication, making lifestyle modifications, and routinely checking blood sugar levels. Urbanization, aging, obesity, physical inactivity, and population expansion were the factors contributing to the rise in the prevalence of diabetes [3]. There are many oral antidiabetic medications, including glibenclamide, insulin, metformin, and acarbose, that used as antidiabetic therapy. These medications came with a lot of negative side effects, were quite costly, and some were not readily available [4]. Even though there were a number of antidiabetic medicines available, including oral pharmaceuticals but they can often be expensive, had a lot of negative side effects, or not be accessible at all. Because of this, a sizable segment of the populace in underdeveloped and developing nations now used herbal and traditional remedies [5]. Due to their accessibility locally, affordability, and perceived safety, these treatments were preferred. Growing popularity as health assistance are medicinal plants, which recognized for having active components that help treat illnesses and reduce pain. For many years, Indian medical systems, for instance used herbal treatments to treat a variety of illnesses, including diabetes. Research in this field had the potential to advance the conventional medical practices and result in the creation of innovative treatments [6]. The most common type of diabetes was type 2, which defined by abnormalities in insulin resistance and secretion. The

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main objective of the research was to experimentally analyse Luffa acutangula, Syzygium cumini, and Coccinia grandis were the three distinct plant samples and compared an in vitro cell line investigation to ascertain the antidiabetic study. The method used to identify and measure the chemical components found in plants called phytochemical analysis. These substances which was also known as phytochemicals, diverse group of compounds that comprise, among other things, phenols, terpenoids, alkaloids, and saponins. Antioxidants were the substances that shield cells from harm caused by DPPH (diphenyl-1-picrythydrazyl) free radicals, which were unstable molecules; it was determined by Phosphomolybdenum Assay [7]. The α-amylase assay method was utilized to evaluate the antidiabetic assay, the examination of cell line-based invitro cytotoxicity proceeded. This experiment was carried out in accordance with Vero well-defined protocol for calculating invitro cytotoxicity by cell line analysis. This assay was carried out in accordance with Cirillo's clearly defined protocol to ascertain the antidiabetic properties through an invitro-cell line investigation.

2. MATERIALS AND METHODS

Preparation of aqueous extract from the sample

Each plant sample's aqueous extract was made by soaking 10g of the powdered samples in 150 ml of distilled water and letting it boil for half an hour. Next, the extracts are filtered via Whattman or filter paper.

Qualitative phytochemical analysis

Using the conventional procedures, a qualitative phytochemical analysis of the aqueous extract of Coccinia gran- dis, Syzygium cumini, and Luffa acutangula was carried out [8]. A preliminary phytochemical investigation (color reactions) using plant extracts and fractions was carried out to ascertain the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, flavanoides, terpenoids, steroids, phenolic, and sugars. In photochemical analysis the detection of alkaloid was done by the following methods Wagner’s Test, Dragendorff's test, detection of carbohydrates was processed by Fehling's solution, Analysis of glycosides was performed by Borntrager's test, Detection of saponins done by Foam test, Determination of proteins and amino acids was performed by Biorer test, identification of phenolic compounds done by ferric chloride test and also determination of flavonoid, terpenoids and steroids was completed [9].

Quantitative phytochemical analysis

Several chemical compositions were identified using quantitative phytochemical analysis, such as total amino acid content, total phenol, total flavanoids, total alkaloids, total carbs, and total saponins.

Antioxidant Activity

A variety of assays have been developed to assess the antioxidant activity of plants and food ingredients. Thus, selecting a suitable assay based on the chemical or chemicals of interest is essential to examining the antioxidant activity of the chemical or chemicals. Two common test types are frequently employed in various antioxidant researches [10]. A technique intended to extract the highest amount of the sample's antioxidant capacity was used to assess the antioxidant activity of meals. This study was subjected to the DPPH and Phophomolybdenum assay for antioxidant activity.

DPPH radical scavenging assay

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is purple in color and has a significant absorption maximum at 517 nm (IC50 = μg/mL). Syzygium cumini, Luffa acutangula, and Coccinia grandis aqueous extracts were evaluated for their antioxidant potential using the stable 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity as a benchmark. In order to separate the polar and non-polar components of the extracts, suitably diluted stock solutions were spotted on precoated silica gel TLC plates. The plates were then developed in solvent systems of various polarities (polar, medium polar, and non-polar). The plates were sprayed with 0.02% DPPH in methanol and allowed to dry at room temperature. The resolved band was used to bleach DPPH, and after 10 minutes, the color changes—yellow on a purple background were noticed.

Phosphomolybedenum Assay

The phosphomolybdenum assay was used to determine the overall antioxidant activity. The PM test relies on the sample reducing phosphate-Mo(VI) to phosphate Mo (V) and then forming a blue green phosphate/Mo(V) complex at an acid pH. In the lab, the phosphomolybdenum technique is frequently used to assess plant extracts' overall antioxidant potential. A quantitative technique for determining the rate of reduction reaction between an oxidant, antioxidant, and molybdenum ligand is the PM test. It entails producing auto-oxidation thermally over an extended length of time at a higher temperature [11]. It provides an accurate assessment of the antioxidant's capacity to reduce. αtocopherol was used as the standard in the phosphomolybdate procedure to measure the fractions' overall antioxidant capacity.

Antidiabetic Assay

The aqueous extracts of Coccinia grandis and Syzygium cumini plants were quickly evaluated for in vitro antidiabetic activity based on their capacity to block the enzyme α amylase and yeast cells' uptake of glucose [12]. All extracts exhibited dose-dependent inhibition, although the ethanolic extract had the lowest IC50 value, suggesting that the antidiabetic principle was the main one being used in all subsequent in vivo tests.

Invitro Cytotoxity

Cell Culture Maintenance

The National Centre for Cell Sciences (NCCS), located in Pune, India, provided the Vero (African green monkey kidney normal cell line). Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin kept the cells in the

logarithmic phase of growth [13]. They were kept in an incubator with 95% air humidified at 37°C and 5% CO_2.

Cytotoxicity effect

The sample's cytotoxic effect was evaluated using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay) on Vero cell line. The cells were seeded onto 96-well micro plates (1 x 106 cells/well), and they were allowed to develop to 70–80% confluence in an incubator with 5% CO_2 for 48 hours at 37°C. After that, the medium was changed, and the cells were exposed to various sample concentrations (20, 40, 60, 80, and 100 µg/mL) before being incubated for a full day (Okigbo, Eme, Ogbogu, 2008). After 24 hours, the morphological differences between the untreated (control) and treated cells were examined using a digital inverted microscope with a 40X magnification and captured on camera. After that, the cells were cleaned with phosphate-buffer saline (PBS, pH 7.4), and each well received 20 µL of the (MTT) solution (5 mg/mL in PBS). After that, the plates were left in the dark at 37ºC for two hours [14], After dissolving the formazan crystals in 100 µL DMSO, the absorbance was measured at 570 nm using spectrometry.

Antidiabetic activity by Cell Line Study

Differentiated Vero cells were used to measure the sample's in vitro glucose uptake activity. To put it briefly, the 24-hour cell cultures that were 70–80% confluent in 96-well titre plates were permitted to differentiate for four days by being kept in DMEM with 5% FBS [15]. Cell multinucleation was used to determine the degree of differentiation [16]. Following an overnight serum starvation, the differentiated cells were once again washed in phosphate buffer saline (PBS) and treated with 0.1% BSA in PBS for 30 minutes at 37 °C. Different concentrations of the material (20, 40, and 60 µg/ml) were applied to the cells for 30 minutes at 37°C, accompanied by negative controls [17]. After adding the D-glucose solution to each well concurrently, the wells were incubated for 30 minutes at 37°C. Following incubation, solutions from the wells were aspirated, and three rounds of ice-cold PBS washing were performed to stop the uptake of glucose. After losing the cells in a 0.1M NaOH solution, the glucose levels were determined using an aliquot of the lysates [18]. The percentage increase in glucose uptake above controls was calculated using the experimental values in duplicates.

3. RESULTS AND DISCUSSION

A qualitative phytochemical analysis of the aqueous extract of Syzygium cumini, Luffa acutangula, and Coccinia gran- dis was conducted using standard techniques. The findings show which supporting elements are present in the plant species and which ones are absent. According to the analysis of alkaloids, it was found in all three species of plant kinds. According to this study, three plant species contain carbohydrates, which are necessary for all living things. Glycosides was one of the components examined, and the results showed that it was absent from all three types of species. The next element was saponins, which showed that Syzygium cumini and Coccinia grandis lacked them but Luffa acutangula possessed them. Three species of pant variations lacked the protein component, however Coccinia grandis and Syzygium cumini have the basic protein components known as amino acids. Luffa acutangula and Syzygium cumini both contained phenolic molecules; in Syzygium cumini, only flavanoids were detected; in the other two species, flavanoids were lacking. Three other plant species did not contain the other two terpenoids and steroids components.

Table.1 Qualitative screening of Coccinia grandis, Syzygium cumini and Luffa Acutangula

QualitativeScreening	**Coccinia Grandis**	**Syzygium cumini**	**Luffa acutangula**
Alkaloids	+	+	+
Carbohydrates	+	+	+
Glycosides	-	-	-
Saponins	-	-	+
Proteins	-	-	-
Amino Acid	+	-	+
Phenolic	-	+	+
Flavanoids	-	+	-
Terpenoids	-	-	-
Steroids	-	-	-

Using the supporting organic chemical components, phytochemical analysis was used to do the quantitative screening. The following tests were performed on Luffa acutangula, Syzygium cumini, and Coccinia grandis: phenolic, flavonoid, alkaloids, saponin, and amino acid tests. In Syzygium cumini, the range of phenolic compounds was 1.449µg/ml, in Luffa acutangula, it was 1.162µg/ml and in Coccinia grandis, there were no phenolic traces. The flavonoid range was not detected in Luffa acutangula, but it was present in Syzygium cumini at a level of 1.632µg/ml and absent in Coccinia grandis. The range of alkaloids in the Luffa acutangula, Syzygium cumini, and Coccinia grandis series was 0.398, 1.911, and 0.385 (µg/ml). The carbohydrate content was 0.682µg/ml in Luffa acutangula, 0.844µg/ml in Syzygium cumini, and 0.468 µg/ml in Coccinia grandis. Luffa acutangula had a saponin level of 0.987µg/ml, In Coccinia grandis, the amino acid range was between 0.592µg/ml and 1.280µg/ml. There were no amino acid traces found in Syzygium cumini.

Table.2 Quantitative screening of Coccinia grandis, Syzygium cumini and Luffaacutangula (Concentration - µg/ml)

Quantitative Screening	PhenolicTest µg/ml	Flavanoids Test µg/ml	Alkaloids µg/ml	Carbohydrates µg/ml	Saponins µg/ml	AminoAcids µg/ml
Luffa acutangula	1.162	-	0.398	0.682	0.987	0.592
Syzygium cumini	1.449	1.632	1.911	0.844	-	-
Coccinia grandis	-	-	0.385	0.468	-	1.280

Three species antioxidant activity was assessed by stable DPPH free radicals. The assay, which included five distinct concentrations and a constant DPPH level, revealed the coccinia grandis antioxidant level at 10 (µl/ml) to be 8.636, the luffa acutangula antioxidant level at 7.75µl/ml, and the high Syzygium cumini antioxidant level at 72.375µl/ml. Coccinia grandis had an antioxidant level of 13.215µl/ml, luffa acutangula had an antioxidant level of 8.528µl/ml, and Syzygium cumini had a high level of 73.190µl/ml. Syzygium cumini had a high level of antioxidants at 77.879 µl/ml. For coccinia grandis, the level was 26.326µl/ml, whereas for luffa acutangula, it was 9.808. For Coccinia grandis, the antioxidant level for 80 was 50.260µl/ml; for Luffa acutangula, it was 13.006, and for Syzygium cumini, it was 79.306µl/ml. Coccinia grandis had an antioxidant level of 72.840µl/ml, luffa acutangula had a level of 27.931 µl/ml, and Syzygium cumini had a high level of 80.020µl/ml. Table 3 and Figure 1clearly show that Syzygium cumini had higher antioxidant activity than other species, such as Coccinia grandis and Luffa acutangula, at all five sample concentrations.

Table.3 Antioxidant Activity of Coccinia grandis, Syzygium cumini and Luffa acutangula by DPPH Inhibition Percentage

TestTubes	Sample Concentration (µl/ml)	DPPH (µl/ml)	Coccinia Grandis (µl/ml)	Luffa Acutangula (µl/ml)	Syzygium Cumini (µl/ml)
C	-	2	-	-	-
T1	10	2	8.636	7.675	72.375
T2	20	2	13.215	8.528	73.190
T3	40	2	26.326	9.808	77.879
T4	80	2	50.260	13.006	79.306
T5	160	2	72.840	27.931	80.020

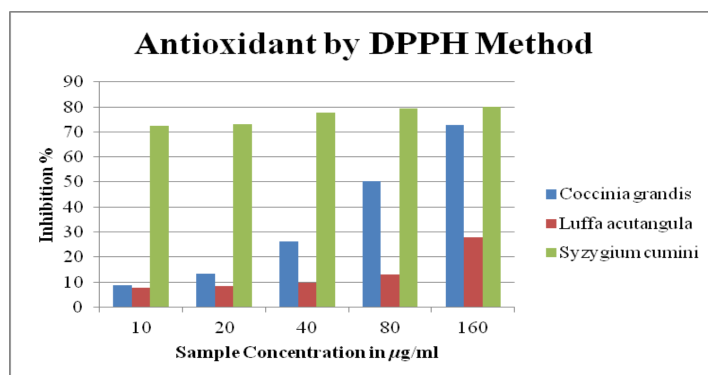

Figure.1 Antioxidant activity by DPPH method

The total antioxidant activity of three species was ascertained using the phosphomolybdenum reduction assay. The study examined the antioxidant levels of coccinia grandis at 10µl/ml and luffa acutangula at 42µl/ml and Syzygium cumini at 18.539 µl/ml. The test was conducted in five different concentrations while maintaining a constant level of DPPH. Coccinia grandis had an antioxidant level of 49.715µl/ml, luffa acutangula had a level of 54.545 µl/ml, and Syzygium cumini had a level of 29.268 µl/ml. For Coccinia grandis, the antioxidant level was 56.403µl/ml, whereas for Luffa acutangula, it was 60.597 µl/ml and for Syzygium cumini, it was 58.092 µl/ml. Coccinia grandis had an antioxidant level of 81.938µl/ml, luffa acutangula had a level of 65.311 µl/ml, and Syzygium cumini had a level of 72.537 µl/ml. For Coccinia grandis, the antioxidant level was 90.549µl/ml, but for Luffa acutangula, it was 70.588 µl/ml and for Syzygium cumini, it was 83.198 µl/ml. Table 4 and Figure 2 clearly show that Syzygium cumini had lower antioxidant activity than other species, such as Coccinia grandis and Luffa acutangula, at all five sample concentrations.

Table.4 Antioxidant Activity of Coccinia grandis, Syzygium cumini and Luffa acutangula by Phosphomolybdenum Reduction Assay

TestTubes	**Sample Concentration** (µl/ml)	**DPPH** (µl/ml)	**Coccinia Grandis** (µl/ml)	**Luffa Acutangula** (µl/ml)	**Syzygium Cumini** (µl/ml)
C	-	2	-	-	-
T1	10	2	18.055	42	18.539
T2	20	2	49.715	54.545	29.268
T3	40	2	56.403	60.597	58.092
T4	80	2	81.938	65.311	72.537
T5	160	2	90.549	70.588	83.198

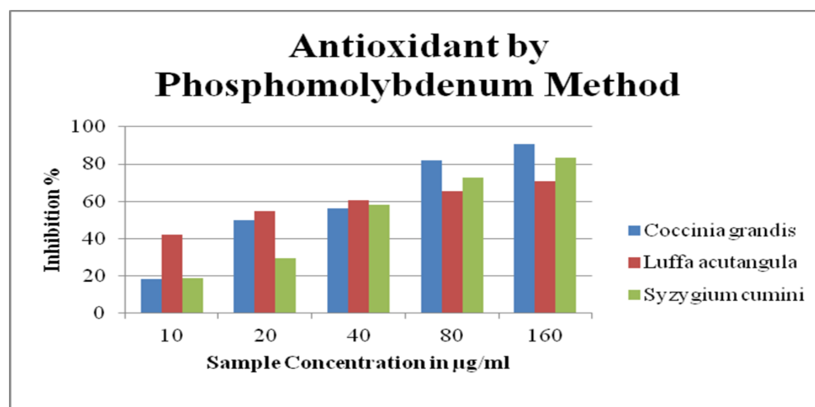

Figure.2 Antioxidant activity by Phosphomolybdenum method

Three species' inhibitory percentage activity was determined using an α-amylase assay. The test used 0.25 milliliters of α-amylase support, a 0.5 starch solution range, and a 0.2 uniform DNS solution level. Different concentrations of 10, 20, 40, 80, and 160µl/ml were used in the experiment, and the results showed that the inhibition percentages were 0.592, 0.697, 0.743, 0.776, and 0.784 µl/ml . Figure 3 and Table 5 show a clearly visible progressive increase as the concentration climbed.

Table.5 Antidiabetic activity of combination of Coccinia Grandis and Syzyguim Cumini by α amylase Assay

Test Tube	**Sample Concentration (µl/ml)**	**α amylase (ml)**	**Starch Solution (ml)**	**DNS**	**Inhibition %**
C	-	0.25	0.5	0.2	0.592
T1	10	0.25	0.5	0.2	0.697
T2	20	0.25	0.5	0.2	0.743
T3	40	0.25	0.5	0.2	0.743
T4	80	0.25	0.5	0.2	0.776
T5	160	0.25	0.5	0.2	0.784

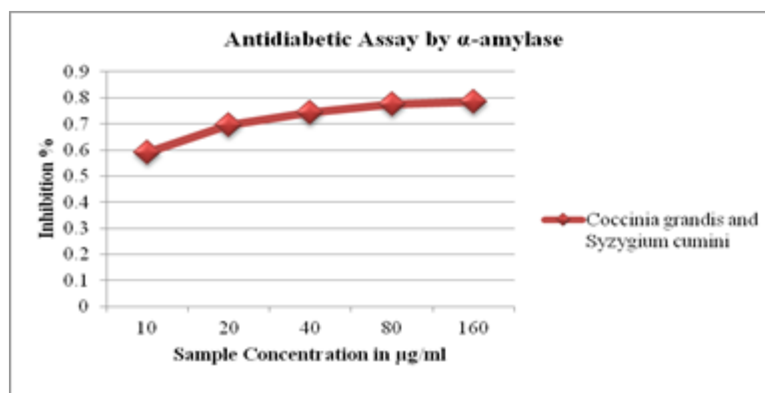

Figure.3 Antidiabetic activity of combination of Coccinia Grandis and Syzyguim Cumini by α amylase Assay

The species' cytotoxicity levels were calculated at five distinct concentrations and compared to one control sample. The absorbance was observed at concentrations ranging from 10 to 100, and the results indicated that the absorbance accuracy was higher at 20 µl/ml. The maximum absorbance was reached with an average of 0.93 µg/mL. Three species' levels of invitro cytotoxicity were also revealed by the cell viability. Cell vitality ranged from 98.77785% at the maximum concentration for the same 20µl/ml to 28.996% at the lowest concentration. This suggests that cell viability was highest at the optimal concentration level and declined as concentration increased. The surface bulging and roughening detected at the microscopically level at a concentration of 60 µl/ml (d) showed chemical alterations brought about by the reaction activity exhibited in the figure.4.

Table.6 Invitro Cytotoxicity effect by cell line study calculation

Concentrations(µg/mL)	Absorbance		Average	Cell Viability(%)
	I	II		
Control	0.946	0.937	0.9415	0
20	0.925	0.935	0.93	98.77854488
40	0.901	0.916	0.9085	96.49495486
60	0.735	0.741	0.738	78.38555497
80	0.503	0.496	0.4995	53.05363781
100	0.277	0.269	0.273	28.99628253

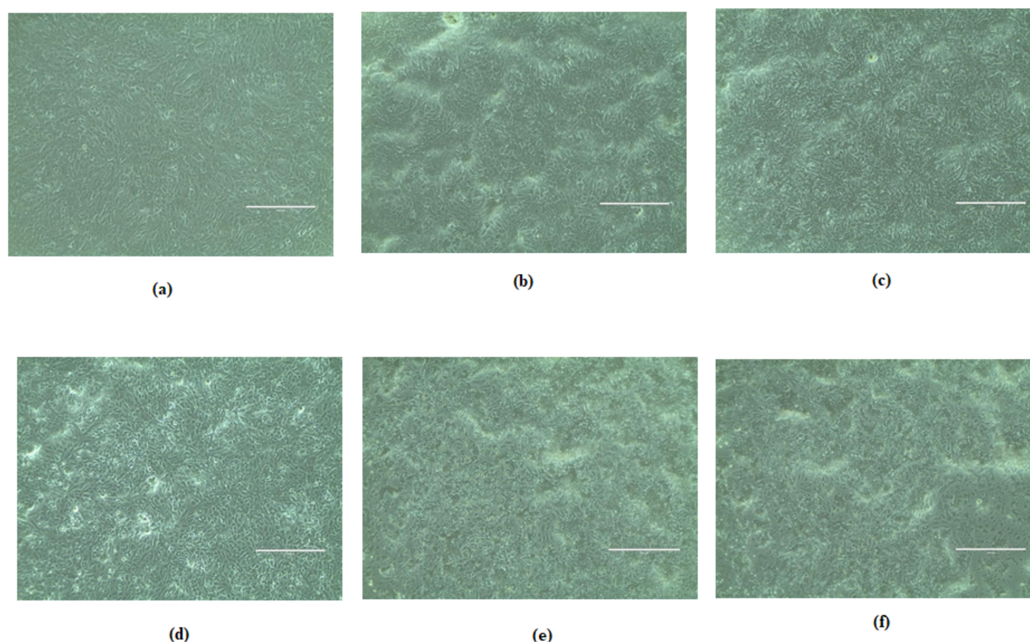

Figure.4 (a), (b), (c), (d), (e) and (f) Invitro cytotoxicity by vero cells with different concentration of the sample control, 20µl, 40 µl, 60 µl, 80 µl and 100 µl

The sample's in vitro glucose uptake activity was measured using differentiated Vero cells. The measurement of microscopical absorbance revealed the amount of glucose absorbed, which represented the amount of insulin that was present. The average absorbance ranged from 0.1925 to 0.3035 µg/mL, which is compared with the control absorbance of 0.1405 to examine the concentration of the material, which started at 20 to 60µg/ml. To make the presence of insulin more clear, the glucose uptake level was measured. The results of this experiment indicate that insulin is present in trace amounts ranging from 27.01 to 53.70, as shown in table 7.

Table.7 In vitro antidiabetic activity by cell line study with Glucose uptake assay tabulation

Concentrations(µg/mL)	Absorbance		Average	Glucose uptake (%) (Insulinabsent)
	I	II		
Control	0.136	0.145	0.1405	0
20	0.196	0.189	0.1925	27.01298701
40	0.207	0.216	0.2115	33.56973995
60	0.298	0.309	0.3035	53.70675453

4. CONCLUSION

The abundance of phytochemical compounds found in the plants was investigated in this study in order to determine their antioxidant activity and in vitro antidiabetic qualities. Both the DPPH technique and the phosphomolybdenum assay demonstrated strong antioxidant activity in Coccinia grandis and Syzygium cumini. These two samples were therefore undergoing additional processing and the Antidiabetic assay. By inhibiting the α-amylase enzyme, the plant extract mixture was also tested for its anti-diabetic properties. The results showed that the extract had a maximum inhibition at 160µg/ml (0.784%) when compared to other concentrations, but that the activity was moderate at the same concentration (82.27%) when compared to standard. This investigation used extracts from the nanoparticles that were prepared under various extraction conditions to determine the cytotoxicity of the particles in vitro. This method advances the objective of a comprehensive and methodical assessment of the plant sample's toxicity. Where the sample's cell viability was 98.77854488 at a 20µl/ml concentration.

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