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Article

Phytochemical Screening and Antidiabetic Efficacy of *Balanites aegyptiaca* Seed Extract and Their Silver Nanoparticles on Muscle and Pancreatic Cell Lines

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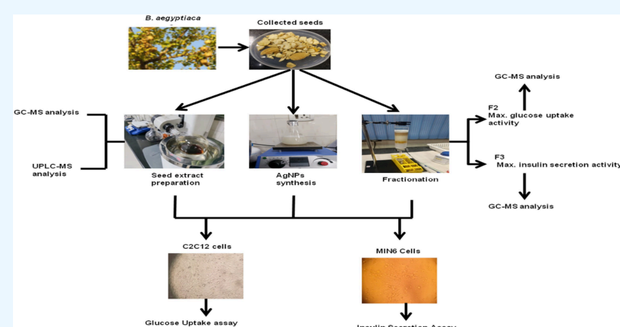
ABSTRACT: *Balanites aegyptiaca* (L.) Delile, a member of the Zygophyllaceae family, is commonly known as the desert date. This tree is famous for yielding edible fruits and is esteemed for its nutritional richness and diverse health advantages. The primary aim of this research was to assess the potential antidiabetic and cytotoxic effects of seed extracts from *B. aegyptiaca* and its AgNPs for the first time on C2C12 and MIN6 cells, focusing on glucose uptake and insulin secretion, respectively. Additionally, the seed extracts underwent column chromatography through different solvent systems, resulting in the isolation of five distinct fractions with a mixture of methanol and water as an eluting solvent in different ratios. Comprehensive characterization of the aqueous seed extract was carried out using GC-MS and UPLC-MS. The study determined that the aqueous seed extract exhibited no toxicity at any tested concentration (6.25−100 μg/mL) on both cell types. The calculated IC_{50} values were 206.00 and 140.44 μg/mL for C2C12 and MIN6 cells, respectively, for seeds of AgNPs. Additionally, the aqueous seed extract and their AgNPs significantly increased glucose uptake by 150.45% and 156.00% of the control in C2C12 cells at a concentration of 100 μg/mL. Insulin secretion was also notably enhanced by 3.47- and 3.92-fold of the control after administering seed extracts and AgNPs, respectively, at 100 μg/mL. GC-MS and UPLC-MS analyses identified various compounds across different categories. Notably, the F2 fraction (methanol and water in ratio of 80:20 as eluting solvent) exhibited the highest glucose uptake activity (156.81% of control), while the F3 fraction (methanol and water in ratio of 70:30 as eluting solvent) fraction demonstrated the highest insulin secretion activity (3.70 folds of the control) among all fractions at 100 μg/mL. GC-MS analysis was employed to characterize both fractions, aiming to identify the compounds contributing to their antidiabetic potential. The study's findings concluded that both seed extracts and their AgNPs possess significant antidiabetic properties, with elevated activity observed in the case of AgNPs in both assays. Various compounds, including diosgenin, oleic acid, linoleic acid and palmitic acid esters were detected in the seed extracts, known for their reported antidiabetic and hypoglycemic effects.



INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder influenced by a blend of genetic and environmental factors, leading to an abnormal increase in blood sugar levels.[1] Disruption of the normal metabolism of glucose and lipids in this condition results in oxidative stress, contributing to dysfunction in vital organs such as the liver, kidneys, and cardiorespiratory system, among other complications. Comparable to other pathogenic or toxic conditions, diabetes is linked to elevated serum concentrations of hepatic enzymes and urea, suggesting potential damage to the liver and kidneys.[2] Diabetes poses a substantial global public health challenge, impacting both developed and developing nations alike. The increase in diabetes is primarily linked to a contemporary sedentary lifestyle influenced by Westernization and modernization, heightened individual obesity, and specific genetic predispositions.[3] The main treatment for type I diabetes mellitus (DT1) involves insulin replacement therapy, while for type II diabetes mellitus (DT2), oral hyperglycemic medications, lifestyle modifications, and dietary changes can be beneficial in reducing blood glucose levels. DT2 affects around 462 million individuals globally, constituting over 90% of the total diabetic population.[4] An estimated 193 million people are believed to be living with undiagnosed diabetes.[5,6] Projections indicate a further increase to approximately 700 million by 2045.[7,8] According to WHO 2019, the diabetic population in

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Figure 1. Photographs showing the (a) fruits of *B. aegyptiaca*. (b) Extracted seeds from fruits.

South East Asia is expected to increase from 46 million to 119 million people, and in India, diabetes prevalence is anticipated to rise from 31 million to 79 million people.

Despite the existence of various therapeutic approaches for treating diabetes, none provides a definitive cure for diabetes and frequently entail significant side effects. Scientists are increasingly turning their attention to the medicinal components of plants due to their comparatively lower side effects and greater efficacy. In the last 30 years, there has been considerable interest in employing living plants, whole plant extracts, and biological materials to convert inorganic substances into nanoparticles.[9]

Balanites aegyptiaca is commonly known as a "desert date" or Hinghot. It persists in dry areas and shows growth in very low lying areas. It can grow in a wide variety of soils, sand to heavy clay. It belongs to the Zygophyllaceae family. It is commonly observed in northern and eastern Africa, including Egypt, and in India, its primary presence is in Rajasthan and near the Aravli Hills of the Haryana, India.. While it can adapt to various environments, it flourishes in low-lying, semidesert regions with deep sandy loam and unobstructed access to water, such as depressions, riverbanks, or the base of rocky hillsides. It demonstrates tolerance to a broad spectrum of temperatures and humidity levels.[10] Various secondary metabolites are present in Hinghot plants such as alkaloids, steroids, pregnane glycosides, saponins (furostanol, spirostanol and open-chain steroidal saponins) and polyphenols (flavonoids, coumarins, stilbenes, tannins lignins and phenolic acids) extracted out from the different plant tissue like stem bark, seeds, root, leaves fruits and galls.[11] Many nonflavonoid polyphenols isolated from *B. aegyptiaca* are cinnamic acid, benzoic acid, p-coumaric acid, gentisic acid, vanillic acid, ferulic acid, syringic acid, sinapic acid, 2-methoxy-4-vinylphenol, 2-methoxy-3(−2- propenyl)-phenol, 2,6-dimethoxyphenol, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone, 2-methoxy-4-(1-propenyl)-phenol, 2,4-di*tert*-butyl-phenol 2,6-di*tert*-butyl-phenol and caffeic acids etc.[12−14] All these phytochemicals described are responsible for several medicinal properties like antioxidant,[15−20] antimicrobial,[21−28] anticancerous activities[16,28−30] antidiabetic[16,31,32] and several other protective activities.

A myriad of plants and vegetables have been scrutinized for their antidiabetic activities in animal models, signifying a worldwide quest for novel antidiabetic agents sourced from plants with fewer side effects. The enduring tradition of herbal medicine knowledge and usage is deeply ingrained in various populations.[33] Despite challenges hindering the clinical applications of therapeutic plants, such as low oral uptake, limited systemic absorption and bioavailability, lack of consistency, and uncertain toxicity,[34] it has been acknowledged that employing nanoparticles provides a viable strategy to overcome these obstacles in medicinal herb utilization.[35] Recent advancements in antidiabetic research have centered on nanoscience, renowned for its unique properties like compact size, the ability to transport drugs through biomembranes, and bioadaptability.[36] This study explores the antidiabetic potential of seed extract from *B. aegyptiaca* and their corresponding AgNPs. Moreover, the current investigation seeks to identify the chemical compounds present in the seed extract that are responsible for its antidiabetic properties.

2. MATERIALS AND METHODOLOGY

2.1. Chemicals. All the chemicals of analytical grade like $AgNO_3$, Dulbecco's Modified Eagles Medium (DMEM) for cell culture, Fetal Bovine Serum (FBS), Phosphate buffer saline (PBS), Trypsin, Antibiotic solution, L-Glutamine, Normal phase sillica gel for column chromatography, Dimethyl sulfoxide (DMSO), (3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT), Glucose oxidase/peroxidase reagents, Insulin, Bovine Serum Albumin (BSA), Sodium Chloride (NaCl), HEPES, Calcium Chloride ($CaCl_2$), Potassium chloride (KCl), Sodium hydrogen carbonate ($NaHCO_3$), Magnesium sulfate ($MgSO_4$), Potassium monophosphate (KH_2PO_4) etc. were procured from HI-Media. Mouse INS1/insulin ELISA Kit was procured from Sigma-Aldrich.

2.2. Collection of Plant Material and Processing. The fruits of *Balanites aegyptiaca* were harvested from native plants in Kankapura village, Jaipur district, Rajasthan. The local community referred to this plant as Hinghot. To validate the authenticity of the plant parts, verification was conducted by the National Institute of Science Communication and Information Resources (NISCARE) in New Delhi, India. The extraction of fruit pulp involved the use of a sharp-edged knife, and the kernels with tough outer shells were acquired, as depicted in Figure 1. Following this, a hammer was employed to eliminate the outer

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hard shell, facilitating the preparation of the seeds' kernels for subsequent processing.

2.3. Seed Extract Preparation. The seeds were left to air-dry for a duration of 2 days and later pulverized with an electric grinder. The resulting powdered seeds underwent an extraction process using double-distilled water, following the infusion technique detailed by Zaky et al.[37] To generate the extract, 50 g of seed powder was combined with 500 mL of boiling distilled water for 15 min. Subsequently, the resulting mixture underwent filtration using Whatman filter paper No. 1, and the extract was obtained through a rotary evaporator.

2.4. Synthesis of Silver Nanoparticles. AgNPs synthesized were done by following the method described by Bar et al.[38] 10 mL of plant extract was mixed with 100 mL of 1 mM solution of silver nitrate ($AgNO_3$) in a conical flask and heated at 55 °C on a hot plate with continuous shaking with the help of magnetic stirrer for 3 h until the color was changed from cloudy white to intense brown. The synthesized AgNPs were collected after centrifugation at 15000 rpm for 15 min for further usage. The synthesized AgNPs underwent characterization through diverse techniques, including UV−visible spectroscopy, FTIR (Fourier transform infrared spectroscopy), XRD (X-ray Diffraction), SEM (Scanning Electron Microscopy), and TEM (Transmission Electron Microscopy), among others.

2.5. Cell Culture and Maintenance. The acquisition of C2C12 and MIN6 cells took place at the National Centre for Cell Sciences in Pune, Maharashtra, featuring passage numbers of 20 and 24, respectively. These cells were obtained in T25 culture flasks, which exhibited 50% confluency. Following this, the cells were cultured and sustained in a CO_2 incubator adjusted to 37 °C with 5% CO_2. In the culture of C2C12 and MIN6 cells, Dulbecco's Modified Eagles Medium (DMEM) was utilized. The medium formulation involved the addition of 10% Fetal Bovine Serum (FBS), 1% antibiotic solution, and 1% L-glutamine.

2.6. Column Chromatography. The seed extract was mixed with an equivalent amount of silica in distilled water, and any excess water was subsequently removed through evaporation to obtain a fine powder. At the end of the column, a cotton plug was attached and the silica slurry was poured into the column with caution to avoid bubbling and cracking. The column was stabilized within 15 min. A mixture of ethyl acetate and hexane was passed through the column to remove the impurities. Subsequently, the plant extract was introduced above the column, reaching a height of 1−1.5 cm. A solvent gradient composed of methanol and water (varying from 90:10 to 50:50), was prepared. Five fractions, labeled as F1 (90:10), F2 (80:20), F3 (70:30), F4 (60:40) and F5 (50:50), were collected.

2.7. GC-MS (Gas Chromatography/Mass Spectrophotometry). In order to identify the bioactive phytochemicals in seed extracts and their fractions, GC-MS analysis was conducted using a SCIO-SQ 436-GC. A 1 mg/mL solution of the samples (seed extract and its fractions F2 and F3) was introduced into the GC-MS through the injector with a flow rate of 1 mL/min. The injector temperature was kept constant at 400 °C. The oven temperature was initially set at 80 °C for 3 min and gradually increased to 250 °C at a rate of 8 °C per minute, completing a total run time of 49 min.[39]

2.8. UPLC-MS Analysis. The analysis was conducted using a UPLC-MS Spectrometer Model Q-ToF Micro Waters. For LC/MS, a Sunfire C18 analytical HPLC column (5 μm, 4.6 mm × 150 mm) was employed, utilizing a mobile phase gradient from 0% to 100% MeOH over 30 min at a flow rate of 1 mL min^{-1}. The accurate masses obtained for eluted peaks were used to deduce potential molecular formulas, which were subsequently cross-referenced in the Dictionary of Natural Products, CRC Press (online version), for corresponding chemical structures.[40]

2.9. MTT Assay. For the cytotoxicity assay, 100 μL of culture media containing 2.5×10^4 cells were introduced into each well of a 96-well plate and incubated for 24 h in a CO_2 incubator. On the subsequent day, the cells were subjected to different concentrations of seed extracts of *B. aegyptiaca* and their AgNPs (6.25−100 μg/mL) in triplicate, assessing their cytotoxic potential. 100 μM H_2O_2 and 0.1% DMSO were taken as positive and negative control, respectively. After a 24-h exposure, 10 μL of fresh medium containing 5 mg/mL MTT was introduced into each well, replacing the previous medium. The cells were then incubated for 4 to 8 h at 37 °C in the dark. The absorbance was measured at 570 nm, and the percent cell viability was calculated using the following formula:

$$\%\text{Cell Viability} = \frac{\text{Abs(Sample)}}{\text{Abs(Control)}} \times 100$$

2.10. Glucose Uptake Assay. Glucose uptake assay was performed by following the Glucose oxidase/peroxidase method.[41] C2C12 cells were plated in a 96-well plate at a concentration of 3×10^4 cells/ml. The cells were allowed to grow until reaching a confluency of 80−90%. For the treatment, a media solution was prepared by dissolving seed extracts (6.25−100 μg/mL), AgNPs seeds (6.25−100 μg/mL), and all of the fractions (100 μg/mL) in FBS-free media. Insulin was taken as positive control at 100 nM concentration. The growth medium was then replaced with the treatment medium and incubated for 24 h at 37 °C. All experiments were performed in triplicates.

Following the incubation period, the treatment media were removed, and each well was replaced with 100 μL of glucose oxidase/peroxidase reagent. The cells were then incubated for an additional 30 min, and the absorbance was measured at 505 nm. The percent glucose uptake was calculated by the formula given below.

$$\%\text{Glucose Uptake} = \frac{\text{Abs(Control)} - \text{Abs(Sample)}}{\text{Abs(Control)}} \times 100$$

2.11. Insulin Secretion Assay. Mid log Min6 cells (cells of a specific type that are in mid logarithmic phase of growth and actively dividing with high metabolic activity) were cultivated in a 24-well plate, with a density of 2×10^5 cells per well in 100 μL of 25 mM glucose media.[42] Following a 24 h incubation period, the cell culture media were replaced with 100 μL of various treatments, comprising a positive control (Metformin 100 μg/mL), seed extracts (6.25−100 μg/mL), standard insulin (6.25 μIU-100 μIU), biosynthesized AgNPs (6.25−100 μg/mL), and fractions (F1−F5) obtained from the seed extract. The cells were then incubated for an additional 24 h.

Subsequently, the cells were washed twice with washing buffer (KRB buffer) adjusted to pH 7.4 and supplemented with 0.5% w/v BSA. MIN6 cells were further incubated with 0.5% BSA, KRB, and 5 mM glucose for 2 h. Supernatants were collected from each well, briefly spun at 1500 rpm to remove debris, and utilized for subsequent experiments on insulin secretion. The insulin secretion assay was performed using a Mouse Ins1/insulin ELISA kit.

2.12. Statistical Analysis. All experiments underwent a minimum of three repetitions. The data are expressed as means

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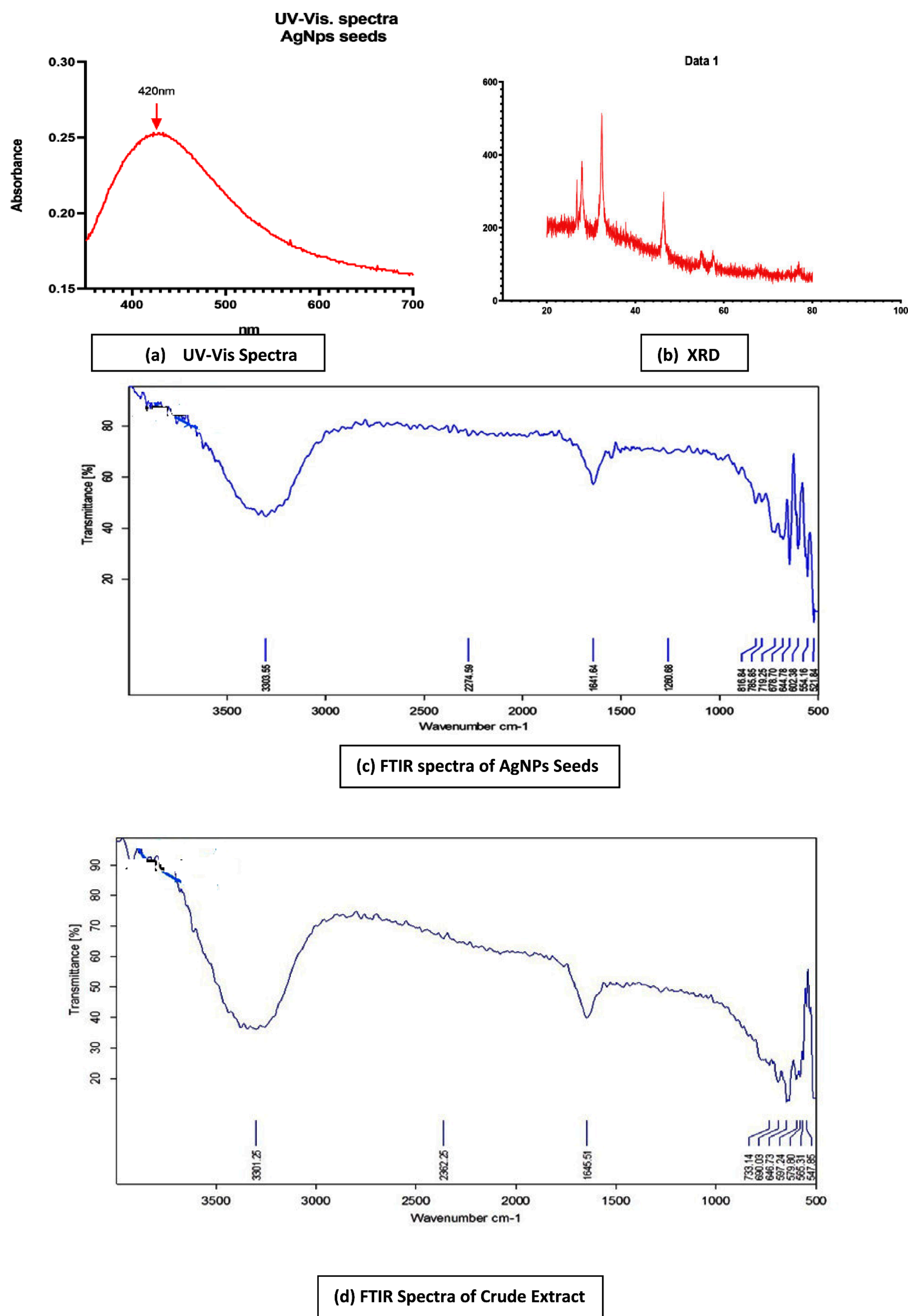

Figure 2. continued

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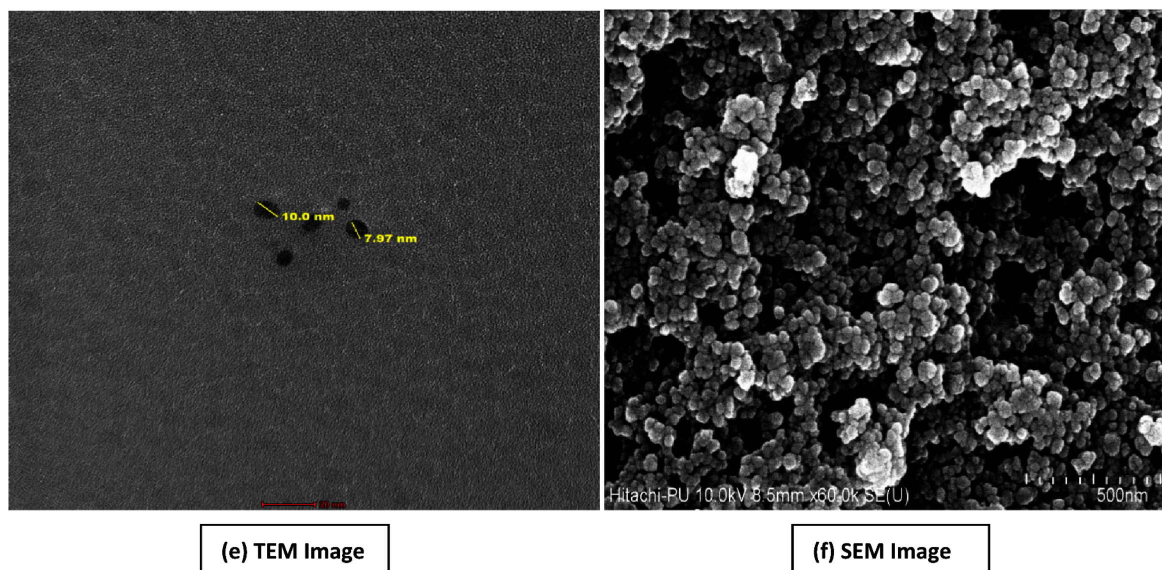

Figure 2. Characterization of biologically synthesized AgNPs (a) UV−vis Spectro (b) XRD spectrum, (c) FTIR spectrum of AgNPs seeds, (d) FTIR spectra of crude extract, (e) TEM image and (f) SEM image. Note: TEM images at a scale bar of 20 nm and SEM image at a scale bar of 500 nm

± the standard error of the mean (SEM) and were subjected to analysis using one-way analysis of variance. Multiple comparison was done by Tukey's post hoc analysis. Statistical significance was set at $p \leq 0.05$.

3. RESULTS

3.1. Seed Extracts and Fractionations. After extracting 50 g of seeds, 13.78 g of seed extract was obtained. Subsequently, 10 g of this extract underwent separation via normal phase silica gel chromatography, resulting in five fractions designated as F1, F2, F3, F4, and F5, with yields of 2.23 3.48, 2.81, 0.98, and 0.5 g, respectively.

3.2. AgNPs Synthesis. The absorbance peak at 420 nm in UV−vis spectrophotometric analysis confirmed the synthesis of AgNPs (Figure 2.a). The synthesized nanoparticles were exposed to XRD analysis for the confirmation of crystalline structure. Four peaks (Figure 2.b) present at 2θ = 37.648°, 43.467°, 69.752°, and 83.483° mainly correspond to Braggs reflection planes (111), (200), (220) and (311) respectively and reported the presence of face centered cubic lattice. The sharpness of the peaks indicated that the biosynthesized AgNPs have pure and nanocrystalline nature. The average crystalline size was calculated using the Scherer formula by fwhm (full width at half-maximum) of the diffraction peaks. The formula includes the following equation:

$$D = (K\lambda)/(\beta\cos\theta)$$

D = average Crystalline size
K = Scherrer's coefficient (0.9)
β = fwhm
θ = peak angle

The average size was calculated as depicted in Table 1.

The average calculated size was 10.35206 nm. Crystalline nature was confirmed using XRD analysis. The FTIR analysis of seed extracts displayed distinctive absorption peaks at specific wavenumbers, including 3303.55, 2274.59, 1641.64, 1260.68, and 719.25 cm^{-1} (as illustrated in Figure 2.c). The absorption peak at 3303.55 cm^{-1} primarily indicates the stretching of the −OH bonds, suggesting the presence of hydroxyl groups. At 2274.59 cm^{-1}, the observed peak corresponds to the stretching of isothiocyanate bonds (−N═C═O−), signifying the existence of isothiocyanate functional groups. The absorption band at 1641.64 cm^{-1} is attributed to the stretching of imine bonds (−C═N−), indicating the presence of compounds with imine groups. Lastly, the peak at 719.25 cm^{-1}, is primarily associated with the bending of aromatic (−C−H−) bonds, suggesting the presence of aromatic compounds in the seed extracts. The absorbance band in the FTIR spectrum of the crude seed extract at 3301.25, 2362.25, 1645.51, 733.14 cm^{-1} confirms the synthesis of AgNPs from the seed extract. The synthesied silver nanoparticles were of spherical shape and of size ranging from 7.97 to 10 nm in SEM and TEM analysis (Figure 2e,f respectively).

Table 1. Showing the Peak Positions and Average Size Calculated from the XRD Analysis of the Synthesized AgNPs from the Seed Extracts

Sample	Peak Position (2θ)	Peak Orientation (Miller indices)	fwhm	Crystal size (nm)
AgNPs seeds	37.648	110	0.4653	18.03827
	43.467	200	0.9875	8.660468
	69.752	220	1.2568	7.704913
Average size				10.35206

3.3. GC/MS Analysis of Seed extracts. A total of 20 compounds were diagnosed in the GC-chromatogram of seed extracts named as 5-(piperidine-1-ylmethyl)-3-pyridin-3-yl-5,6-dihydro-4H-1,2,4-Oxadiazine (5.235%), 2-cyclopenten-1-one 2-hydroxy-3-methyl (7.815%), 1H-imidazole-4-carboxylic acid methyl ester (4.447%), Pentadioic acid dihydrazide, N2,N2′-bis(2-furfuryldeno) (5.879%), Benzene-1-chloro-4-methoxy (13.973%), (1-Methoxypentyl)-cyclopropane (2.559%), histamine N-trifluoroacetyl-2-amino (3.040%), Cyclotrisiloxan, hexamethyl (1.478%), cyclopentanecarboxylic acid, dodec-9-ynyl ester (8.421%), octadecanoic acid 2-hydroxyl-1,3-propanediyl ester (9.231%), myo-inositol 2-C-methyl-(2.315%), Beta-D-Glucopyranose (4.569%), malonic acid bis(2-trimethylsilyl) ester (2.956%) Octadecanoic acid 9,10-dichloro methyl ester(3.742%), 4,7,10,13,16,19-docosahexaneoic acid methyl

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Table 2. Details of the Chemical Compounds Obtained after GC-MS Analysis of Seed Extracts of *B. aegyptiaca*

Sr. No.	Retention time	Compound name	Molecular Formula	Area	Molecular weight
1	3.213	5-(Piperidine-1-ylmethyl)-3-pyridin-3-yl-5,6-dihydro-4H-1,2,4-Oxadiazine	$C_{16}H_{18}ClN_3OS$	2.05×10^{08}	335
2	4.616	2-Cyclopenten-1-one, 2-hydroxy-3-methyl	$C_6H_8O_2$	3.06×10^{08}	112
3	5.588	1H-imidazole-4-carboxylic acid methyl ester	$C_5H_6N_2O_2$	1.74×10^{08}	126
4	6.065	Pentadioic acid, dihydrazide, N2, N2′-bis(2-furfuryldeno)	$C_{15}H_{16}N_4O_4$	2.30×10^{08}	316
5	7.024	Benzene-1-chloro-4-methoxy	C_7H_7ClO	5.47×10^{08}	142
6	7.383	(1-Methoxypentyl)-cyclopropane	$C_9H_{18}O$	1.00×10^{08}	142
7	7.836	Histamine N-trifluoroacetyl-2-amino	$C_7H_9F_3N_4O$	1.19×10^{08}	222
8	8.568	Cyclotrisiloxan, hexamethyl	$C_6H_{18}O_3Si_3$	5.79×10^{07}	222
9	9.434	Cyclopentanecarboxylic acid, dodec-9-ynyl ester	$C_{18}H_{30}O_2$	3.30×10^{08}	278
10	10.936	Octadecanoic acid, 2-hydroxyl-1,3-propanediyl ester	$C_{26}H_{36}O_{11}$	3.61×10^{08}	524
11	11.805	Myo-inositol, 2-C-methyl-	$C_7H_{14}O_6$	9.07×10^{07}	194
12	12.644	Beta-D-glucopyranose	$C_6H_{12}O_6$	1.06×10^{07}	180
13	14.170	Malonic acid, bis(2-trimethylsilyl) ester	$C_{13}H_{28}O_4Si_2$	1.16×10^{08}	304
14	18.115	Octadecanoic acid, 9,10-dichloro methyl ester	$C_{19}H_{36}C_{12}O_2$	1.47×10^{08}	366
15	18.556	4,7,10,13,16,19-Docosahexaneoic acid, methyl ester	$C_{23}H_{34}O_2$	1.77×10^{08}	342
16	20.103	13-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	4.82×10^{07}	296
17	20.168	9,12,15-Octadecatrienoic acid 2,3-bis [(trimethylsilyl)oxy] propyl ester	$C_{27}H_{52}O_4Si_2$	9.40×10^{07}	496
18	28.300	Diosgenin	$C_{27}H_{42}O_3$	5.89×10^{07}	414
19	31.214	Psi.,.psi-Carotene, 3,4-Didehydro-1,1′, 2,2′- Tetrahydro-1′-Hydroxy-1-Methoxy	$C_{41}H_{60}O_2$	2.10×10^{08}	584
20	35.136	1-Monolinoleoylglyceroltrimethylsily Ether	$C_{27}H_{54}O_4Si_2$	5.45×10^{08}	498

ester (4.515%), 13-octadecanoic acid methyl ester (1.230%), 9,12,15-octadecatrienoic acid 2,3-bis [(trimethylsilyl)oxy] propyl ester (1.503%), diosgenin (8.360%), psi, psi-carotene, 3,4-didehydro-1,1′, 2,2′- tetrahydro-1′-hydroxy-1-methoxy (2.365%) and 1-monolinoleoylglycerolteimethylsily ether (13.922%). The retention and molecular formula of the above-mentioned compounds are described in Table 2.

3.4. UPLC-MS Analysis of the Seed Extracts of *B.aegyptiaca*. The UPLC-MS analysis led to detection of approximately 55 compounds mainly categoriesed into fatty acid and derivatives, organic acids, steroidal saponins, sugar derivatives, sugar alcohols, amino acids, flavonoids, coumarins, etc., explained in Table 3. Fatty acids and its derivatives include 3-Oxotetradecanoic acid, 7-(3-Pyridinyl)heptanoic acid, (2E)-2-Ethyl-2-hexenoate, 1,6,10,14-Hexadecatetraen-3-ol, 9-Octadecenedioic acid (oleic acid) and octadecyl octadecenoate. Several flavonoids are also detected like (+)-Gallocatechin gallate, formylphenyl 4-n-dodecyloxycinnamate, Luteolin-7-O-neohesperidoside, Dihydro-resveratrol, Epimestrol, 7,8-dimethoxyflavone, Deazaflavin, flavan myristinin A, 4-n-decyloxycinnamic acid etc. Sugar and its derivative including steroidal glycosides, Methyl 4-S-beta-D-mannopyranosyl-4-thio-beta-D-glucopyranoside, 1(F)-alpha-D-galactosylraffinose, 1,2-O-Isopropylidene-3-O-methyl-D-xylofuranose, Tetra-O-acetyl-L-rhamnopyranose, (5S)-1,2-O-Isopropylidene-5-C-phenyl-alpha-D-xylofuranose, 2-Aminoethyl alpha-D-glucopyranosyl-(1 → 4)-6-deoxy-alpha-L-mannopyranosyl-(1 → 3)-2-acetamido-2-deoxy-beta-D-glucopyranoside, Astaxanthin b-D-glucopyranoside, 1,4-D-xylobiose, 1-O-(4-coumaroyl)-beta-D-glucose, (1R)-2,2,4-Trimethyl-3-(3-oxobutyl)-3-cyclohexen-1-yl beta-D-glucopyranoside etc. Coumarins includes 3-tolylisocoumarin, licocoumarone, 6,7-di-2-ethylhexyloxy-4-methylcoumarin etc. Organic compounds including pyridine and imidazole derivatives and amino acids are 2′-Deoxy-5-[(2-methylphenyl)-ethynyl]uridine, 4-(5-Methoxy-1H-indol-3-yl)-6-methyl-3(2H)-pyridazinone. 1-(4-Cyanobenzyl)-L-proline, N-Acetyl-L-histidine, Glycyl-L-phenylalanine, saccharose acetate isobutyrate, dipyridophenazine, D-Aspartatic acid, (E)-1-[5-(Benzyloxy)-4-methoxy-2-nitrophenyl]-N-(4-methylphenyl)-methanimine, diquinolylmethyl-lysine, 3,4-Dihydroxy-2-pyrrolidinone, 5-Chloro-6-(2-imino-1-pyrrolidinyl)-2,4(1H,3H)-pyrimidinedione hydrochloride (1:1), 1-[2-Ethoxy-2-(3-pyridinyl)ethyl]-4-(2-methoxyphenyl)piperazine and 6-Methyl-3-(2-pyridinyl)-2H-1,2-thiazine 1,1-dioxide.

3.5. MTT Assay. The cytotoxicity assessment using the MTT assay involved exposing C2C12 and MIN6 cell lines to seed extract and their AgNPs for 24 h. The plant extracts were not toxic at any tested concentration in either cell lines. The cell viability of C2C12 cells increased significantly in a dose-dependent manner when exposed to plant extracts (6.25−100 μg/mL) compared to untreated cells. The cell viability ranged from 101.85% to 154.13% at tested concentrations, with the maximum increase observed at 100 μg/mL (54.13% greater than untreated cells, $p \leq 0.0001$). The positive control, hydrogen peroxide, reduced C2C12 cell viability by 25.96% (74.04% of untreated cells, $p \leq 0.001$), while 0.1% DMSO served as a control. The AgNPs synthesized from seed extract of *B.aegyptiaca* were tested at concentrations ranging from 6.25 to 100 μg/mL, and they were not found to be toxic at any concentration. The cell viability of C2C12 cells exposed to AgNPs ranged from 101.62% to 93.3%. The calculated IC50 for AgNPs was 206.0 μg/mL. Unlike plant extracts, AgNPs did not significantly increase cell viability compared to plant extracts. The toxicity of AgNPs was found to be 7.7% on C2C12 cells at 100 μg/mL. None of the tested concentrations of plant extracts and AgNPs showed toxicity, suggesting their suitability for further assays on C2C12 cells.

Similar to C2C12 cells, all tested concentrations (6.25−100 μg/mL) of seed extract and their synthesized AgNPs were not found to be toxic on MIN6 cells. Similarly, plant extract increased cell viability significantly at certain tested concentrations (25−100 μg/mL), although the increase was not significant at 6.25 and 12.5 μg/mL. In MIN6 cells, the cell viability at all tested concentrations of plant extract ranged from 109.44% to 116.88%, as illustrated in Figure 3. The maximum cell viability was observed at a concentration of 100 μg/mL of

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Table 3. Details of the Chemical Compounds Obtained after UPLC-MS/MS Analysis of Seed Extract of *B. aegyptiaca*

Sr. No.	Chemical formula	Mass error (ppm)	Compound Name	*m/z* ratio	Retention time (min)
1	$C_{14}H_{26}O_3$	3.8931	3-Oxotetradecanoic acid	265.1784	2.00
2	$C_6H_6O_8$	0.2829	2-Oxosuccinic acid - oxoacetic acid (1:1)	248.0402	2.08
3	$C_{18}H_{18}N_2O_5$	−5.5147	2′-Deoxy-5-[(2-methylphenyl)ethynyl]uridine	183.0581	2.18
4	$C_{13}H_{24}O_{10}S$	−0.9212	Methyl 4-S-beta-D-mannopyranosyl-4-thio-beta-D-glucopyranoside	395.0979	2.29
5	$C_{22}H_{18}O_{10}$	5.5031	(+)-Gallocatechin gallate	484.1263	2.46
6	$C_{14}H_{14}O_3$	2.5050	Dihydro-resveratrol	231.1022	2.49
7	$C_{14}H_{13}N_3O_2$	3.5399	4-(5-Methoxy-1H-indol-3-yl)-6-methyl-3(2H)-pyridazinone	220.0878	2.63
8	$C_{13}H_{14}N_2O_2$	4.6268	1-(4-Cyanobenzyl)-L-proline	195.0927	2.71
9	$C_{60}H_{39}N_7$	4.3121	4-[4-(4-Pyridinyl)-2-quinolinyl]-N,N-bis{4 -[4-(4-pyridinyl)-2-quinolinyl]phenyl}aniline	822.3166	2.80
10	$C_{18}H_{32}O_{16}$	4.1695	Melezitose	527.1604	2.94
11	$C_{25}H_{28}N_6O_7S_3$	1.0642	Cefditoren pivoxil	659.0820	3.02
12	$C_4H_{10}NOSi$	4.1093	Aminosiloxane	82.04762	3.50
13	$C_{27}H_{30}O_{15}$	−5.4941	Luteolin-7-O-neohesperidoside	617.1444	3.53
14	$C_{11}H_7N_3O_2$	−2.2992	Deazaflavin	236.0426	3.56
15	$C_{24}H_{42}O_{21}$	4.9929	1(F)-alpha-D-galactosylraffinose	689.2144	3.67
16	$C_7H_8N_2O$	3.2203	2-Acetamidopyridine	175.0273	3.95
17	$C_{16}H_{12}O_6$	4.3817	Tectorigenin	265.0508	4.29
18	$C_{11}H_{16}N_6$	−4.0970	2-[2-(1-Pyrrolidinyl)ethyl]-2H-pyrazolo[3,4-d]pyrimidin-4-amine	271.1059	4.60
19	$C_9H_{16}O_5$	0.0882	1,2-O-Isopropylidene-3-O-methyl-D-xylofuranose	227.0890	5.50
20	$C_{14}H_{20}O_9$	1.0149	Tetra-O-acetyl-L-rhamnopyranose	333.1183	6.77
21	$C_{11}H_{14}N_2O_3$	4.5463	Glycyl-L-phenylalanine	187.0876	7.88
22	$C_{17}H_{14}O_4$	5.0069	7,8-Dimethoxyflavone	305.0798	7.90
23	$C_{10}H_{11}N_5O_3$	−2.0415	1H-Imidazol-2-ylcarbonyl histidinate	214.0718	8.22
24	$C_{22}H_{40}N_2O_{15}$	0.5832	2-Aminoethyl alpha-D-glucopyranosyl-(1→4)-6-deoxy-alpha-L-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-beta-D-glucopyranoside	573.2498	10.11
25	$C_{17}H_{22}O_{10}$	3.4215	Sinapoylglucose	428.1565	10.30
26	$C_{18}H_{30}O_{13}$	−0.2180	Saccharose acetate isobutyrate	419.1547	10.87
27	$C_{15}H_{18}O_8$	3.1254	1-O-(4-coumaroyl)-beta-D-glucose	327.1085	11.57
28	$C_{18}H_{10}N_4$	2.9796	Dipyridophenazine	305.0806	11.91
29	$C_{16}H_{12}O_2$	3.5470	3-Tolylisocoumarin	201.0707	11.94
30	$C_{19}H_{32}O_7$	3.2701	(1R)-2,2,4-Trimethyl-3-(3-oxobutyl)-3-cyclohexen-1-yl beta-D-glucopyranoside	395.2053	12.57
31	$C_{20}H_{20}O_5$	1.9486	Licocoumarone	341.139	13.01
32	$C_{19}H_{26}O_3$	2.5110	Epimestrol	303.1962	13.33
33	$C_{12}H_{17}NO_2$	−2.8937	7-(3-Pyridinyl)heptanoic acid	249.1592	13.36
34	$C_{16}H_{26}O$	4.3044	1,6,10,14-Hexadecatetraen-3-ol	257.1886	13.75
35	$C_{22}H_{20}N_2O_4$	4.6417	(E)-1-[5-(Benzyloxy)-4-methoxy-2-nitrophenyl]-N-(4-methylphenyl)methanimine	341.1267	13.92
36	$C_{48}H_{54}O_8$	3.1097	Flavan myristinin A	797.3474	14.46
37	$C_{19}H_{28}O_3$	3.8309	4-n-Decyloxycinnamic acid	269.1912	14.91
38	$C_{20}H_{28}O_3$	−1.4500	6,7-Di-2-ethylhexyloxy-4-methylcoumarin	317.2107	14.99
39	$C_{10}H_{18}O_9$	4.9127	1,4-D-Xylobiose	283.1038	15.78
40	$C_4H_7NO_4$	5.4354	D-Aspartatic acid	134.0455	16.29
41	$C_{19}H_{33}N_3O$	−0.5812	3,5-Diamino-N-dodecylbenzamide	361.296	16.60
42	$C_{46}H_{62}O_9$	−3.6841	Astaxanthin b-D-glucopyranoside	723.4227	18.86
43	$C_{18}H_{32}O_4$	4.0763	9-Octadecenedioic acid	277.2175	20.86
44	$C_8H_{13}O_2$	−2.4044	(2E)-2-Ethyl-2-hexenoate	106.0779	22.67
45	$C_{16}H_{32}O_2$	−2.9864	Palmitic Acid	279.2287	26.31
46	$C_{73}H_{75}O_4P$	0.9161	5-Diphenylphosphino-11,17,23-tri(p-tolyl)-25,26,27,28-tetrapropoxycalix[4] arene	1047.547	28.75
47	$C_{28}H_{36}O_4$	−2.5864	Formylphenyl 4-n-dodecyloxycinnamate	478.3307	30.34
48	$C_{30}H_{38}O_4$	−0.2694	3,20-Dioxopregn-4-en-21-yl hydratropate	504.3453	31.17
49	$C_4H_7NO_3$	−5.9630	3,4-Dihydroxy-2-pyrrolidinone	156.0051	33.03
50	$C_8H_9C_{l2}N_4O_2$	−4.2431	5-Chloro-6-(2-imino-1-pyrrolidinyl)-2,4(1H,3H)-pyrimidinedione hydrochloride (1:1)	265.0242	33.59
51	$C_9H_{10}O_5$	−3.39254	Syringic acid	199.0594	34.38
52	$C_{46}H_{29}N_5O_6$	−2.4252	4,4′,4”-[5-(3-Pyridinyl)-2,3,21(23H)-porphinetriyl]tribenzoic acid	789.2438	36.08
53	$C_{10}H_{10}N_2O_2S$	−0.0096	6-Methyl-3-(2-pyridinyl)-2H-1,2-thiazine 1,1-dioxide	187.0324	37.10
54	$C_{20}H_{27}N_3O_2$	−5.5039	1-[2-Ethoxy-2-(3-pyridinyl)ethyl]-4-(2-methoxyphenyl)piperazine	364.1977	37.66
55	$C_5H_7BN_2$	−3.2416	(2E)-4-Methyl-2-(methylborylene)-2H-imidazole	71.0560	37.75

plant extracts. The positive control, hydrogen peroxide, reduced MIN6 cell viability by 34.55% (65.45% of untreated cells, $p \leq 0.001$).

Similarly, the AgNPs seeds were not toxic at the same tested concentrations as the plant extracts. The cell viability ranged from 100.72% to 81.08%, as shown in the Figure 3. The

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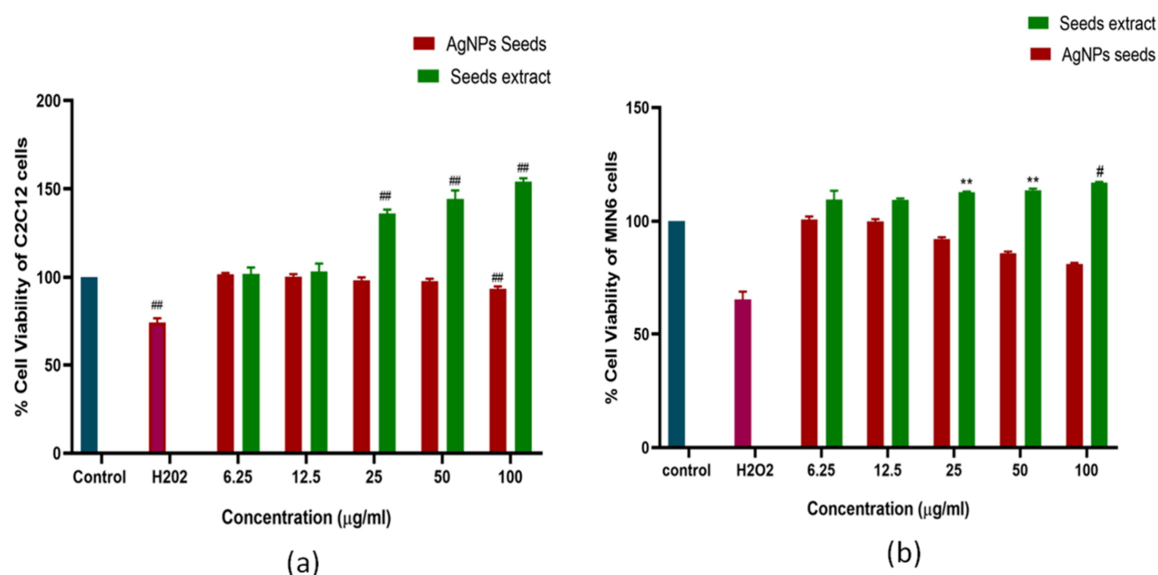

Figure 3. Histogram showing the mean viability of C2C12 cells (a) and MIN6 cells (b) exposed with the different concentration of seed extracts and AgNPs seeds of *B. aegyptiaca*. Results as mean ± standard error; ** Significance level with control ($p \leq 0.001$), # Significance level with control ($p \leq 0.001$), and ## Significance level with control ($p \leq 0.0001$).

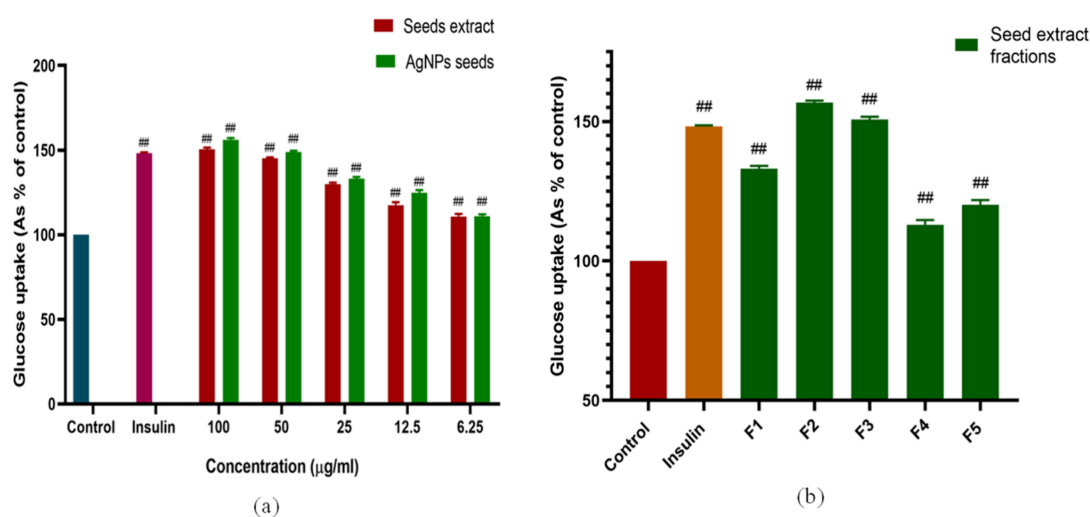

Figure 4. Histogram showing the percent glucose uptake in C2C12 cells (as of control), exposed with the different concentration of seeds extract, AgNPs seeds (a) and fractions (b) obtained from the seed extract of *B. aegyptiaca* Results are expressed as mean ± SE of three independent experiments performed with three replicates. ## Represents that the results are significantly different from the control with a significance level of ($p \leq 0.0001$).

calculated IC_{50} for AgNPs seeds on MIN6 cells was 140.44 μg/mL. The maximum concentration of AgNPs seeds decreased the cell viability by 18.92%, reaching 81.08%. All tested concentrations were deemed to be suitable for further analysis on MIN6 cells.

3.6. Glucose Uptake Assay. The glucose uptake assay was conducted to assess the concentration of glucose absorbed by C2C12 cells following treatment with the seeds extract, AgNPs seeds, and corresponding fractions of the seed extract. Seed extracts, AgNPs, and extract fractions significantly augmented glucose uptake in C2C12 cells compared with untreated cells. All tested concentrations of plant extracts (6.25−100 μg/mL) dose-dependently increased glucose uptake within the range of 110.82−150.458% ($p \leq 0.0001$) of the control, as illustrated in the Figure 4. The highest glucose uptake occurred at a plant extract concentration of 100 μg/mL, surpassing the control by 50.48% ($p \leq 0.0001$). Insulin, used as a positive control, enhanced glucose uptake to 147.31% ($p \leq 0.0001$) of the control value at a concentration of 100 nM.

Glucose uptake was also elevated with AgNPs treatment from the seed extract. All tested concentrations of AgNPs (6.25−100 μg/mL) increased glucose uptake in C2C12 cells within the range of 111.08−156.007% of the control ($p \leq 0.0001$). Similar to seed extracts, the maximum glucose uptake was observed at 100 μg/mL, surpassing the control by 56.00%. AgNPs increased glucose uptake by 5.55% more than seed extract at 100 μg/mL. All fractions (F1−F5) also significantly enhanced glucose uptake in C2C12 cells at 100 μg/mL. Among the fractions, F2 exhibited the highest glucose uptake (156.81% of control) at the same concentration ($p \leq 0.0001$), surpassing plant extracts by 6.36% and AgNPs by 0.81%.

3.7. Insulin Secretion Assay. All tested concentrations of seed extract (6.25−100 μg/mL) significantly increased insulin concentration in a dose-dependent manner compared to the

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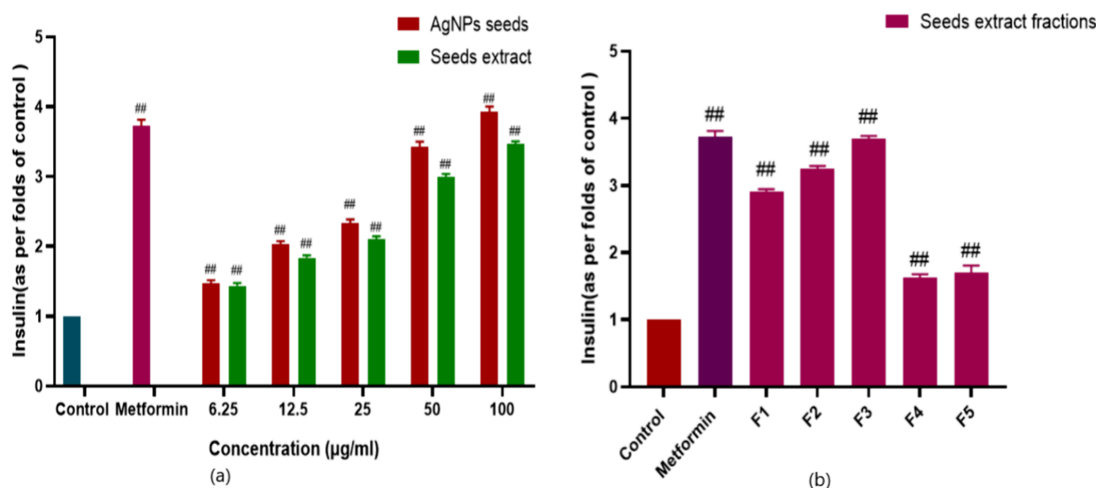

Figure 5. Histogram showing the increase in folds of insulin secretion from MIN6 (as of control), exposed with the seed extract, AgNPs seeds (a) and fraction (b) obtained from the seed extract of *B. aegyptiaca*. Results are expressed as mean ± SE of three independent experiments performed with three replicates. ## Represents that the results are significantly different from the control with a significance level of ($p \leq 0.0001$).

Table 4. Details of the Chemical Compounds Obtained after GC-MS Analysis of Fraction F2 Obtained from the Seed Extract of *B. aegyptiaca*

Sr. No.	Retention time	Compound name	Molecular Formula	Area	Molecular weight
1	3.252	Propanamide, N-[4-(4-chlorophenyl)-2 thiozole]-3-pyrrolidinyl)-	$C_{16}H_{18}ClN_3OS$	7.09×10^{08}	335
2	4.531	5-Pyrimidinecarboxyldehyde, 1,2,3,4-tetrahydro-2, 4-dioxo	$C_5H_3N_2O_3$	1.59×10^{09}	140
3	4.687	2-Cyclopenten-1-one, 2-hydroxy-3-methyl	$C_6H_8O_2$	2.04×10^{09}	112
4	5.605	Phenol, 2-methoxy-	$C_7H_8O_2$	1.36×10^{09}	105
5	5.714	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	3.20×10^{08}	280
6	6.115	Maltol	$C_6H_6O_3$	3.09×10^{09}	126
7	6.285	4-Hexen-3-ol, 2,5-dimethyl-	$C_8H_{16}O$	6.16×10^{08}	128
8	7.046	Fumaric acid, di(cis-non-3-enyl) ester	$C_{22}H_{36}O_4$	8.23×10^{08}	364
9	7.375	Benzene, 1-chloro-4-methoxy-	C_7H_7ClO	2.62×10^{08}	142
10	7.750	Succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester	$C_{24}H_{42}O_4$	8.07×10^{08}	394
11	7.937	Catechol	$C_6H_6O_2$	1.58×10^{09}	110
12	8.615	Malonic acid, di(3-methylpent-2-yl) ester	$C_{15}H_{28}O_4$	7.15×10^{08}	272
13	9.450	1,2-Benznediol, 3-methoxy	$C_7H_8O_3$	1.49×10^{09}	140
14	10.533	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	3.96×10^{08}	150
15	11.029	1,4-Benzendiol, 2-methoxy	$C_7H_8O_3$	2.37×10^{09}	140
16	12.510	Myo-inosital, 4-C-methyl-	$C_7H_{14}O_6$	4.94×10^{08}	194
17	15.863	4,4′-Dimethyl-2,2′-dimethylenebicyclohexyl-3,3′-diene	$C_{16}H_{22}$	2.11×10^{08}	214
18	17.696	Benzene, (triethoxymethyl)-	$C_{16}H_{16}O_3$	2.99×10^{08}	256
19	18.520	Androst-5-en-17-one, 3,16-bis[(trimethlysilyl)oxy]-(3, beta, 16 alpha)	$C_{25}H_{44}O_3SiO_2$	3.52×10^{09}	448
20	19.751	2-(2′,4′,4′,6′,6′,8′,8′-Heptamethyltetrasiloxan-2′-yloxy)	$C_{16}H_{48}O_{10}Si_9$	3.46×10^{08}	652
21	24.199	Pregna-14-en-3-one, (5, beta)	$C_{21}H_{32}O$	6.70×10^{08}	300
22	24.722	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	2.75×10^{08}	330
23	25.871	Pseudosarsasapogenin-5,20-dien diacetate	$C_{31}H_{46}O_5$	2.80×10^{08}	498
24	27.369	Butyl 4,7,10,13,16,19-docosahexaenoate	$C_{26}H_{40}O_2$	4.55×10^{08}	384
25	28.536	3, Beta, 17 beta-dihydroxyestr-4-ene	$C_{18}H_{28}O_2$	5.31×10^{08}	276
26	29.834	1(3H)-isobenzofuranone, 3a,4,5,7a-tetrahydro-4-hydroxy-3a, 7a-dimethyl-	$C_{10}H_{14}O_3$	3.72×10^{08}	182
27	30.620	Tigogenin tosylate	$C_{34}H_{50}O_5S$	4.08×10^{08}	570
28	30.778	Diosgenin	$C_{27}H_{42}O_3$	2.75×10^{08}	414
29	34.348	(+)-Lariciresinol	$C_{20}H_{24}O_6$	1.77×10^{09}	360

control, ranging from 1.43- to 3.47-fold the control value ($p \leq 0.0001$). The highest insulin concentration (3.47-fold of control) was observed in the cell supernatant treated with seed extract at a concentration of 100 μg/mL ($p \leq 0.0001$) depicted in Figure 5. Metformin hydrochloride, used as a positive control, enhanced insulin concentration to 3.72-fold of the control value at a concentration of 100 μg/mL.

Similarly, treatment with AgNPs seeds at different concentrations (6.25−100 μg/mL) also significantly increased insulin secretion ($p \leq 0.0001$). Comparable to seed extracts, the maximum increase in insulin secretion was 3.92-fold that of the control at a concentration of 100 μg/mL. The enhancement in insulin secretion with AgNPs seeds was 0.45-fold of the control more than that observed with plant extracts at 100 μg/mL, and

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Table 5. Details of the Chemical Compounds Obtained after UPLC-MS/MS Analysis of Fraction F2 Obtained from the Seed Extract of *B. aegyptiaca*

Sr. No.	Chemical formula	Mass error (ppm)	Compound Name	*m/z* ratio	Retention time (min)
1	$C_{12}H_{12}N_6O$	3.6580	1-(1H-Imidazo[2,1-i]purin-7-ylmethyl)-2-pyrrolidinone	279.0974	0.67
2	$C_{11}H_{11}NO_{5\text{-}2}$	−5.1378	Salicylaspartate	279.0974	0.68
3	$C_{11}H_{11}NO_5$	−0.5097	N-Benzoyl-D-aspartic acid	279.0974	0.93
4	$C_{14}H_{10}N_6O$	−5.1693	4-[(6-Ethynyl-9H-purin-2-yl)amino]benzamide	279.0975	1.24
5	$C_{12}H_{12}N_6O$	3.4449	7-((4-Methylbenzyl)oxy)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-5-amine	279.0974	1.55
6	$C_6H_8FeO_7$	0.7153	Iron(iii) citric acid	270.9497	1.94
7	$C_2H_2O_3S$	1.0233	Monothiooxalic acid	144.9357	1.97
8	$C_{15}H_{18}I_2N_2O_6$	1.3605	N-Acetyl-N, alpha-diiodo-L-tyrosyl-D-threonine	614.8894	2.11
9	$C_{20}H_{28}O_6$	−5.2076	Gibberellin A44 diacid	365.194	2.34
10	$C_{12}H_{22}O_{11}$	0.4827	Maltose	183.0564	2.39
11	$C_6H_{11}O_6$-	1.2007	Glucopyranoside	144.0425	2.45
12	$C_6H_7ClN_2O_2$	5.2642	4-Chloro-1,5-dimethyl-1H-pyrazole-3-carboxylic acid	175.0278	2.60
13	$C_7H_{10}O_2$	−1.0245	(2E,5E)-2,5-Heptadienoic acid	168.1018	2.74
14	$C_{28}H_{26}N_4O_5$	1.4758	Tryptophan -2-[2-amino-3-(1H-indol-3-yl)propanoyl]-1,4-benzoquinone (1:1)	463.1772	2.80
15	$C_{16}H_{18}O_9$	−5.5406	Chlorogenic acid	377.0824	3.98
16	$C_{12}H_{12}N_6$	4.7575	*N*′-[(Z)-Imino(2-pyridinyl)methyl]-2-pyridinecarboximidohydrazide	205.0996	10.78
17	$C_7H_{14}O_5$	−4.9395	6-Deoxy-2-O-methyl-beta-D-mannopyranose	201.0725	12.02
18	$C_{37}H_{41}NO_7$	−5.1654	Methyl 3-{(2R,3R,4R,5S)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]-1-hydroxy-2-pyrrolidinyl}-3-(4-methoxyphenyl)propanoate	612.2924	13.10
19	$C_{28}H_{32}O_6$	−4.4876	Methyl 2,3,4-tri-O-benzyl-alpha-D-glucopyranoside	503.181	13.19
20	$C_{19}H_{26}O_3$	3.4054	16-î^2-Hydroxyandrostenedione	303.1888	13.78
21	$C_{20}H_{25}N_7O_6$	4.3432	N-(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)-4-methyl-L-glutamic acid	498.1518	13.98
22	$C_{16}H_{15}NO_4$	0.8290	Coumarin 343	327.1342	14.46
23	$C_8H_{10}O_2$	1.8769	1,4-Cyclohexadien-1-ylacetic acid	103.0545	15.50
24	$C_{46}H_{62}O_9$	4.8807	Astaxanthin b-D-glucopyranoside	723.4292	16.60
25	$C_{27}H_{42}O_3$	−1.3971	Diosgenin	415.3201	16.63
26	$C_{11}H_{13}N_3O_5$	2.5816	1-(2-Deoxy-D-erythro-pentofuranosyl)-1H-pyrimido[4,5-c]^{1,2}oxazin-7(3H)-one	309.12	17.05
27	$C_{10}H_{15}ClN$	2.0138	(+)-Metamfetamine hydrochloride	186.1048	17.70
28	$C_4H_{10}ClN$	−5.9956	Pyrrolidine hydrochloride	108.0568	18.44
29	$C_{12}H_{24}N_2O_2$	−5.6637	2-Methyl-2-propanyl 3-isopropyl-5-methyl-1-imidazolidinecarboxylate	229.1898	18.78
30	$C_{21}H_{32}O_3$	−3.2442	4-n-Dodecyloxycinnamic acid	297.2202	19.87
31	$C_{40}H_{52}O_4$	3.5312	Astaxanthin, (13z)-	619.3779	20.36
32	$C_{43}H_{60}N_8O_{12}S$	2.1058	N-Acetyl-L-alpha-aspartyl-D-alpha-glutamyl-L-phenylalanyl-*N*-methyl-L-phenylalanylglycyl-L-leucyl-L-methioninamide	877.3932	20.58
33	$C_8H_{25}H^1{}_5O_2$	−5.9208	Methyl 1,2,3,4,5-pentadeuteriobenzoate	185.1324	21.43
34	$C_{10}H_{18}O_3$	5.9479	3-Oxodecanoic acid	151.1128	21.88
35	$C_{27}H_{35}N_3$	2.4664	Tris(t-butyl)terpyridine	424.2738	22.41
36	$C_{16}H_{24}N_2O$	2.1612	7-(2-Pyridinyl)-1-(1-pyrrolidinyl)-1-heptanone	225.1756	23.30
37	$C_{48}H_{70}O_6$	−1.7340	(2’S)-Deoxymyxol 2′-(2,4-di-O-methyl-alpha-L-fucoside)	781.4791	23.37
38	$C_{18}H_{32}O_4$	−2.0320	9-Hydroperoxy-11,12-octadecadienoic acid	354.2633	23.40
39	$C_9H_{13}N_3$	2.8233	Pyridinylpiperazine	186.1006	23.91
40	$C_{18}H_{32}O_2$	3.2915	Linoleic acid	322.275	24.14
41	$C_{27}H_{44}O_3$	1.7393	Sarsasapogenin	381.3159	24.37
42	$C_{18}H_{22}N_6$	3.8987	4-(2-Methyl-3-pyridinyl)-1-[2-(1-piperidinyl)ethyl]-1H-pyrazolo[3,4-d]pyrimidine	323.1991	25.13
43	$C_{12}H_{16}N_2O_3$	−2.98998	Phenylalanylalanine	237.1227	25.44
44	$C_{18}H_{32}O_3$	−1.16929	(2E,4E)-9-Hydroxy-2,4-octadecadienoic acid	261.2209	25.64
45	$C_{55}H_{60}O_{11}$	−5.8564	Methyl 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-beta-D-glucopyranosyl)-alpha-D-glucopyranoside	471.1934	25.69
46	$C_{57}H_{50}N_2$	−5.0213	2′,7′-Bis(2-methyl-2-propanyl)-N,N,*N*′,*N*′-tetraphenyl-9,9′-spirobi[fluorene]-2,7-diamine	785.3828	26.34
47	$C_6H_{13}N_3O_3$	1.4180	D-Citrulline	198.0852	26.82
48	$C_6H_{10}O_7$	2.6456	alpha-D-Glucopyranuronic acid	159.0293	28.58
49	$C_6H_{10}O_7$	1.5062	Glucuronic acid	159.0291	28.89
50	$C_{14}H_{14}N_2O_2$	−3.4022	2-Methoxy-5-[1-(4-methoxyphenyl)vinyl]pyrimidine	281.0679	30.30
51	$C_{14}H_{28}O_6$	4.4642	Octyl beta-D-glucopyranoside	331.2258	30.72
52	$C_{21}H_{39}N_2$+	5.4383	3-Hexadecyl-1-vinyl-1H-imidazol-3-ium	284.2987	33.90
53	$C_{34}H_{43}N_5$	−3.0580	*N*-Methyl-*N*′-(1,2,3,4-tetrahydro-9-acridinyl)-N-[3-(1,2,3,4-tetrahydro-9-acridinylamino)propyl]-1,4-butanediamine	522.3576	31.14
54	$C_{31}H_{52}O_2$	−2.2720	Beta-Sitosterol acetate	251.1871	33.03
55	$C_9H_{10}O_5$	−3.3925	Syringic acid	199.0594	34.39

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Table 5. continued

Sr. No.	Chemical formula	Mass error (ppm)	Compound Name	m/z ratio	Retention time (min)
56	$C_{23}H_{23}ClN_4O_2$	−1.3840	2-Chloro-N-[1-(2-ethylphenyl)-3-(5-methoxy-1-methyl-1H-indol-3-yl)-1H-pyrazol-5-yl] acetamide	445.1396	37.13
57	$C_{42}H_{82}NO_8P$	4.8405	2-[(9E)-9-Octadecenoyloxy]-3-(palmitoyloxy)propyl 2-(trimethylammonio)ethyl phosphate	760.5888	37.72

Table 6. Details of the Chemical Compounds Obtained after GC-MS Analysis of Fraction F3 Obtained from the Seed Extract of *B. aegyptiaca*

Sr. No	Retention time	Compound name	Molecular Formula	Area	Molecular weight
1	3.232	Propanamide, n-[4-(4-chlorophenyl)-2 thiozole]-3-pyrrolidinyl)-	$C_{16}H_{18}ClN_3OS$	3.80×10^{08}	335
2	3.935	Phosphonic acid, (p-hydroxyphenyl)-	$C_6H_7O_4P$	3.70×10^{08}	174
3	4.499	5-Pyrimidinecarboxyldehyde, 1,2,3,4-tetrahydro-2, 4-dioxo	$C_5H_3N_2O_3$	5.37×10^{08}	140
4	4.645	2-Cyclopenten-1-one, 2-hydroxy-3-methyl	$C_6H_8O_2$	5.06×10^{08}	112
5	5.602	Phenol, 2-methoxy-	$C_7H_8O_2$	4.67×10^{08}	105
6	6.081	Cyanogen chloride	CClN	8.59×10^{08}	61
7	7.034	Benzene-1-chloro-4-methoxy	C_7H_7ClO	6.42×10^{08}	142
8	7.693	Succinic acid, di (3,7-dimethyloct-6-en-1-yl) ester	$C_{24}H_{42}O_4$	3.07×10^{08}	394
9	7.868	1,4,3,6-Dianhydro- alpha-D-glucopyranose	$C_6H_8O_4$	6.19×10^{08}	144
10	8.073	2-Azindinone, 1-*tert*-butyl-3-(1-methylcyclohexyl)-	$C_{13}H_{23}NO$	2.57×10^{08}	209
11	8.588	1,2-Benznediol, 3-methoxy	$C_7H_8O_3$	1.93×10^{08}	140
12	9.04	Benzenemethanol, alpha-2-cyclohexen-1-yl	$C_{13}H_{16}O$	3.64×10^{08}	188
13	9.15	4-Chlorophenyl benzoate	$C_{13}H_9ClO_2$	5.94×10^{08}	232
14	9.445	Propane,2-(9-borabicyclo[3,3,1]non-9-yloxy)-3-(9-borabicyclo[3.3.1]non-9-yl	$C_{25}H_{38}B_2O_2S$	6.55×10^{08}	424
15	10.498	Benzene, 1,2,3,4-tetrachloro-	$C_6H_2C_{l4}$	3.32×10^{08}	214
16	10.752	Chloroxylenol	C_8H_9ClO	2.87×10^{08}	156
17	10.974	Vanilline lactoside	$C_{20}H_{28}O_{13}$	7.81×10^{08}	476
18	11.814	3-Isopropaxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxyl)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	6.08×10^{08}	576
19	12.559	Myo-inosital, 4-c-methyl-	$C_7H_{14}O_6$	2.43×10^{08}	194
20	12.884	Pregna-5,9(11)-diene-20-ol-3-one ethylene ketal	$C_{23}H_{34}O_3$	1.03×10^{09}	358
21	13.45	5,5′-Isopropylidenebis[2-(benzoyloxy)toluene	$C_{31}H_{28}O_4$	2.36×10^{08}	464
22	31.246	Diosgenin	$C_{27}H_{42}O_3$	5.52×10^{08}	414
23	38.112	Pregnenoloneacetate	$C_{23}H_{33}O_3$	7.06×10^{08}	358

this enhancement was consistent across all treated concentrations.

All fractions (F1−F5) also demonstrated a significant ($p \leq 0.0001$) increase in insulin concentration secreted by MIN6 cells at 100 μg/mL. Fraction F3 exhibited the maximum increase in insulin secretion, reaching 3.700-fold that of the control at 100 μg/mL ($p \leq 0.0001$), which was higher than both plant extracts and AgNPs. Fraction F3 increased insulin secretion to 3.24-fold of the control ($p \leq 0.0001$). Among all three treatments, AgNPs seeds showed the maximum enhancement (3.92-fold of control) in insulin secretion, surpassing plant extracts and F3 fraction by 0.45- and 0.55-fold of the control, respectively.

Out of the above results, this can be concluded that fraction F2 increased the glucose uptake in C2C12 cells higher than all the fractions and fraction F3 stimulated the secretion of insulin from MIN6 cells more than other fractions. So, further F2 and F3 were analyzed for GC-MS and UPLC-MS to identify the active antidiabetic components presents in the seed extract of *B. aegyptiaca*

3.8. GC-MS Analysis of Fraction F2 Obtained from the Seed Extract of *B. aegyptiaca*. The GC-chromatogram of fraction F2 reveals about 29 compounds greater than the compounds present in other fractions. The identified compounds are, propanamide, N-[4-(4-chlorophenyl)-2 thiozole]-3-pyrrolidinyl)- (3.143%), 5-Pyrimidinecarboxyldehyde, 1,2,3,4-tetrahydro-2, 4-dioxo (2.102%), 2-cyclopenten-1-one, 2-hydroxy-3-methyl (0.494%), Phenol, 2-methoxy-(4.767%), (0.949%), Maltol (1.269), 4-Hexen-3-ol, 2,5-dimethyl-(0.403%), Fumaric acid, di(cis-non-3-enyl) ester (1.244%), Benzene, 1-chloro-4-methoxy- (2.430%), Succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester (1.103%), Catechol (2.298%), Malonic acid, di(3-methylpent-2-yl) ester (0.610%), 1,2-benznediol, 3-methoxy (3.647%), 2-methoxy-4-vinylphenol (0.762%), 1,4-Benzendiol, 2-methoxy (0.325%), 1,4-Benzendiol, 2-methoxy(0.461%), Myo-inosital, 4-C-methyl- (5.441%), 4,4′-dimethyl-2,2′-dimethylenebicyclohexyl-3,3′-diene (0.534%), benzene, (triethoxymethyl)-(1.033%), Androst-5-en-17-one, 3,16-bis[(trimethlysilyl)oxy]-(3, beta, 16 alpha) (0.424%), 2-(2′,4′,4′,6′,6′,8′,8′-Heptamethyltetrasiloxan-2′-yloxy) (0.432), pregna-14-en-3-one, (5, beta) (0.701%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.819%), Pseudosarsasapogenin-5,20-dien diacetate (0.573%), Butyl 4,7,10,13,16,19-docosahexaenoate (2.431%), 3, beta, 17 beta-dihydroxyestr-4-ene (2.733), 1(3H)-isobenzofuranone, 3a,4,5,7a-tetrahydro-4-hydroxy-3a, 7a-dimethyl-(0.630%), Diosgenin (37.857%), Tigogenin tosylate (9.735%), (+)-Lariciresinol (7.260). The retention time and molecular formula of the following compounds are explained in Table 4.

3.9. UPLC-MS Analysis of the Fraction F2 Obtained from the Seed Extract of *B.aegyptiaca*. The UPLC-MS/MS analysis of fraction F2 led to detection of approximately 57 chemical compounds mainly categoriesed into fatty acid and derivatives, organic acids, steroidal saponins, sugar derivatives, sugar alcohols, amino acids, flavonoids, coumarins, and phenolic

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Table 7. Details of the Chemical Compounds Obtained after UPLC-MS/MS Analysis of Fraction F3 Obtained from the Seed Extract of *B. aegyptiaca*

Sr. No.	Chemical Formula	Mass error (ppm)	Compound name	*m/z* ratio	Retention time (min)
1	$C_{18}H_{33}N_4O_{24}P_3$	−5.4398	Diuridine triphosphate	821.0633	2.22
2	$C_{13}H_6BrN_3S_2$	−3.8313	4-(1-Benzothiophen-2-yl)-7-bromo[1,2,5]thiadiazolo[3,4-c]pyridine	385.8805	1.97
3	$C_8H_{15}NO_6$	−5.6594	N-acetyl D-glucosamine	244.0779	2.11
4	$C_{13}H_{24}O_{11}$	3.4717	2,3,4,5-Tetrahydroxy-6-methoxycyclohexyl hexopyranoside	379.1223	2.35
5	$C_8H_{11}N_3O_3$	−1.6337	N-Acetyl-L-histidine	239.1136	2.46
6	$C_7H_8N_2O$	5.5917	2-Acetamidopyridine	175.0276	2.60
7	$C_{24}H_{20}F_2N_2$	1.5709	6-(Difluoromethyl)-1-(3-methylphenyl)-2,4-diphenyl-1,2-dihydropyrimidine	375.1673	2.80
8	$C_{18}H_{32}O_{16}$	0.5953	Melezitose	505.1766	2.85
9	$C_{25}H_{27}N_3O_6$	−5.1361	Ethyl 3-(4-pyridinyl)propyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate	466.1949	3.36
10	$C_{42}H_{44}O_{17}S$	−3.4403	Methyl 2,3,6-tri-O-benzoyl-4-S-(2,3,4,6-tetra-O-acetyl-beta-D-mannopyranosyl)-4-thio-beta-D-glucopyranoside	853.234	3.53
11	$C_{11}H_7N_4O_3$-	1.6385	4,6-Dioxo-1-phenyl-1,4,6,7-tetrahydro-5H-pyrazolo[3,4-d]pyrimidin-5-olate	285.0866	3.95
12	$C_{16}H_{23}N_5O_6$	−1.7893	9-(Alpha-D-glucosyl)-trans-zeatin	404.1534	7.57
13	$C_{19}H_{28}O_{11}$	−1.6153	Benzyl 4-O-beta-D-glucopyranosyl-beta-D-glucopyranoside	433.1697	9.85
14	$C_7H_{12}O_3S$	2.7632	(3R,4E)-3-Hydroxy-7-mercapto-4-heptenoic acid	177.0585	11.15
15	$C_{19}H_{30}O_5$	2.4954	Dodecyl gallate	303.1963	13.40
16	$C_{11}H_{13}NO_2$	−2.5448	3-Hydroxy-1-methyl-5-phenyl-2-pyrrolidinone	233.128	13.74
17	$C_{23}H_{29}N_3O_7$	−4.7306	2-Azidoethyl 4-O-benzyl-3-O-(4-methoxybenzyl)-alpha-D-mannopyranoside	498.1615	13.98
18	$C_{12}H_{24}O_8$	−1.5250	Acetone - alpha-D-glucopyranose (2:1)	297.1539	14.29
19	$C_{11}H_{12}N_2O_2$	−1.1361	DL-Tryptophan	227.0789	15.30
20	$C_{16}H_{25}N_5O_6$	−2.1039	Dihydrozeatin-O-glucoside	348.1658	15.67
21	$C_{37}H_{62}O_{12}$	0.7924	16-Deethyl-3-O-demethyl-16-methyl-3-O-(1-oxopropyl)monensin	737.4072	16.06
22	$C_{16}H_{28}O_2$	2.6424	9,12-Hexadecadienoic acid	275.1988	16.09
23	$C_{19}H_{28}O_3$	4.1314	4-n-Decyloxycinnamic acid	269.1913	16.20
24	$C_{27}H_{42}O_3$	4.2263	4-Octadecyloxylcoumarin	415.3224	16.82
25	$C_{17}H_{17}NO_3$	−0.6215	Coumarin 334	306.1099	17.47
26	$C_{16}H_{28}O_2$	3.4275	9,12-Hexadecadienoic acid	275.199	17.92
27	$C_{18}H_{32}O_4$	−2.5017	9-Octadecenedioic acid	277.2154	18.69
28	$C_{58}H_{85}ClN_{12}O_{12}S$	1.2991	N-[2-(4-Chlorophenyl)ethyl]glycyl-N-cyclopentylglycyl-N-(tetrahydro-2-furanylmethyl)glycyl-N-(2-furylmethyl)glycyl-N-allylglycyl-N-(2-furylmethyl)glycyl-N-(4-aminobutyl)glycyl-N-(4-aminobutyl)glycyl-L -cysteinamide	1231.573	18.72
29	$C_8H_{14}N_4O_6$	3.9167	2-[(Azidoacetyl)amino]-2-deoxy-D-mannopyranose	263.1093	19.23
30	$C_{70}H_{68}N_6O_4$	4.4466	2,2′-(2,2′-Bipyridine-6,6′-diyl)bis[6-{6′-[2-hydroxy-5-(2-methyl-2-propanyl)phenyl]-2,2′-bipyridin-6-yl}-4-(2-methyl-2-propanyl)phenol]	1057.542	22.41
31	$C_6H_{10}O_7$	−5.3516	beta-D-Glucopyranuronic acid	159.0278	25.83
32	$C_{16}H_{32}O_2$	−2.9864	Palmitic Acid	279.2287	26.31
33	$C_{12}H_{16}O_6$	−3.5989	Phenyl D-mannopyranoside	257.1011	27.19
35	$C_{46}H_{62}O_9$	−5.5301	Astaxanthin b-D-glucopyranoside	723.4213	27.24
36	$C_{32}H_{58}O_{17}$	1.0851	Octyl 6-deoxy-alpha-L-galactopyranosyl-(1→4)-6-deoxy-alpha-L-galactopyranosyl-(1→4)-6-deoxy-alpha-L-galactopyranosyl-(1→4)-6-deoxy-beta-L-galactopyranoside	756.402	27.61
37	$C_{18}H_{30}O_2$	−0.8216	α-Linolenic Acid	279.2316	27.95
38	$C_{12}H_{16}N_2O_3$	5.5327	L-Phenylalanylalanine	237.1247	29.05
39	$C_{18}H_{34}O_4$	0.2699	2,3-Dihydroxy-2-octadecenoic acid	279.2319	30.04
40	$C_{30}H_{31}N_3O_4$	−0.0303	Methyl N-(3-phenylpropanoyl)-L-tryptophylphenylalaninate	539.2653	30.44
41	$C_{11}H_{17}NO_3$	0.7845	3-[2-(1,3-Dioxolan-2-yl)ethyl]-1-vinyl-2-pyrrolidinone	253.1548	33.06
42	$C_{23}H_{34}O_4$	3.3393	Digitoxigenin	375.2542	33.37
43	$C_{20}H_{28}$	−5.1638	1,3-di*tert*-butyl-5-phenyl-1,3-cyclohexadiene	307.182	33.93
44	$C_{17}H_{32}O_3Si$	1.0072	3a-({[Dimethyl(2-methyl-2-propanyl)silyl]oxy}methyl)-2-hydroxy-6-methyloctahydro-4H-inden-4-one	335.2016	34.78
45	$C_{26}H_{50}O_4Si$	4.8945	(3*S*,6*S*)-3-Hydroxy-1-pentadecen-6-yl (3R)-3-{[dimethyl(2-methyl-2-propanyl)silyl]oxy}-4-pentenoate	496.3839	37.13
46	$C_{18}H_{18}Br_3N$	−2.4584	3,6-Dibromo-9-(6-bromohexyl)-9H-carbazole	449.8839	37.72

compounds explained in Table 5. The description and retention times of the compounds are shown in Table 5. Fatty acid and its derivatives include (2E,5E)-2,5-Heptadienoic acid, 16-î²-hydroxyandrostenedione, 3-Oxodecanoic acid, 16-î²-hydroxyandrostenedione, 9-Hydroperoxy-11,12-octadecadienoic acid, (2E,4E)-9-Hydroxy-2,4-octadecadienoic acid, 5,12-Dihydroxy-6,8,10,14,17-icosapentaenoic acid, beta-Sitosterol acetate, methyl 1,2,3,4,5-pentadeuteriobenzoate, and linoleic acid. Syringic acid and chlorogenic acid are polyphenolic compounds that are detected in fraction F2. Organic compounds mainly includes amino acids, pyridine and imidazole derivatives and organic acids. These are 1-(1H-Imidazo[2,1-i]purin-7-ylmethyl)-2-

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pyrrolidinone, salicylaspartate, N-Benzoyl-D-aspartic acid, 4-[(6-Ethynyl-9H-purin-2-yl)amino]benzamide, 7-((4-methylbenzyl)oxy)-3H-[1,2,3]triazolo[4,5-*d*]pyrimidin-5-amine, iron(iii) citric acid, monothiooxalic acid, N-Acetyl-N, alpha-diiodo-L-tyrosyl-D-threonine, Gibberellin A44 diacid, pyridine pyrrole, Tryptophan -2-[2-amino-3-(1H-indol-3-yl)-propanoyl]-1,4-benzoquinone (1:1), *N*′-[(Z)-Imino(2-pyridinyl)methyl]-2-pyridinecarboximidohydrazide, Methyl 3-{(2R,3R,4R,5S)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]-1-hydroxy-2-pyrrolidinyl}-3-(4-methoxyphenyl)propanoate, N-(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)-methyl]amino}benzoyl)-4-methyl-L-glutamic acid, 1,4-Cyclohexadien-1-ylacetic acid, 1-(2-Deoxy-D-erythro-pentofuranosyl)-1H-pyrimido[4,5-*c*][1,2] oxazin-7(3H)-one, N-Acetyl-L-alpha-aspartyl-D-alpha-glutamyl-L-phenylalanyl-*N*-methyl-L-phenylalanylglycyl-L-leucyl-L-methioninamide, 7-(2-Pyridinyl)-1-(1-pyrrolidinyl)-1-heptanone, Phenylalanylalanine, D-Citrulline, Pyridinylpiperazine, 2-Chloro-N-[1-(2-ethylphenyl)-3-(5-methoxy-1-methyl-1H-indol-3-yl)-1H-pyrazol-5-yl]acetamide.

3.10. GC-MS Analysis of Fraction F3 Obtained from the Seed Extract of *B. aegyptiaca*. The GC-chromatogram of fraction F3 revealed the presence of 23 compounds. The compounds are propanamide, n-[4-(4-chlorophenyl)-2 thiozole]-3-pyrrolidinyl)- (2.316%), phosphonic acid, (p-hydroxyphenyl)- (2.251%), 5-pyrimidinecarboxyldehyde, 1,2,3,4-tetrahydro-2, 4-dioxo (3.270%), 2-cyclopenten-1-one, 2-hydroxy-3-methyl (3.079%), phenol, 2-methoxy-(2.844%), cyanogen chloride (0.861%), (5.226%), succinic acid, di (3,7-dimethyloct-6-en-1-yl) ester (3.909%), 1,4,3,6-dianhydro- alpha-D-glucopyranose (3.867%), 2-azindinone, 1-*tert*-butyl-3-(1-methylcyclohexyl)- (1.563%), 1,2-benznediol, 3-methoxy (1.175%), benzenemethanol, alpha-2-cyclohexen-1-yl (2.218%), 4-chlorophenyl benzoate (3.616%), propane,2-(9-borabicyclo[3,3,1]-non-9-yloxy)-3-(9-borabicyclo[3.3.1]non-9-yl (3.988%), benzene, 1,2,3,4-tetrachloro- (2.018%), chloroxylenol (1.745%), myo-inosital, 4-c-methyl- (4.755%), pregna-5,9(11)-diene-20-ol-3-one ethylene ketal (6.238%), 5,5′-isopropylidenebis[2-(benzoyloxy)toluene (3.362%), diosgenin (25.082%) and pregnenoloneacetate (4.300%). The chemical formula and retention time of the identified compounds are described in Table 6.

3.11. UPLC-MS Analysis of the Fraction F3 Obtained from the Seed Extract of *B.aegyptiaca*. The UPLC-MS/MS analysis of fraction F3 led to detection of approximately 46 chemical compounds mainly categories into fatty acid and derivatives, organic acids, steroidal saponins, sugar derivatives, sugar alcohols, amino acids, flavonoids, coumarins, and phenolic compounds. The description and retention time of the compounds are shown in Table 7. Fatty acids and derivatives include (3R,4E)-3-hydroxy-7-mercapto-4-heptenoic acid, Propyl 2-octadecenoate, methyl stearic acid, 9,12-Hexadecadienoic acid, 9,12-Hexadecadienoic acid, Palmitic Acid, Isopropyl palmitate, 2,3-Dihydroxy-2-octadecenoic acid, and 9-Octadecenedioic acid. Organic compounds mainly include amino acids, imidazole and pyridine derivative, and organic acids. These are 4-(1-Benzothiophen-2-yl)-7-bromo[1,2,5]thiadiazolo[3,4-*c*]-pyridine, N-Acetyl-L-histidine, 2-Acetamidopyridine, Tetrakis-(4-hydroxy-3-methyl-1-buten-1-yl) diphosphate, 6-(Difluoromethyl)-1-(3-methylphenyl)-2,4-diphenyl-1,2-dihydropyrimidine, Ethyl 3-(4-pyridinyl)propyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate, 4,6-Dioxo-1-phenyl-1,4,6,7-tetrahydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-olate, 3-Hydroxy-1-methyl-5-phenyl-2-pyrrolidinone, DL-Tryptophan, n-Glycyl-DL-leucine, 2,6-Di(1H-tetrazol-5-yl)pyridine, L-Phenylalanylalanine, 3a-({[Dimethyl(2-methyl-2-propanyl)-silyl]oxy}methyl)-2-hydroxy-6-methyloctahydro-4H-inden-4-one, 3,6-Dibromo-9-(6-bromohexyl)-9H-carbazole.

4. DISCUSSION

Nanotechnology has become a prominent technology with applications spanning various sectors including agriculture, food, pharmaceuticals, and biomedical engineering. Nanoparticles, known for their small size, versatility, and compatibility with various substances such as optical, textile, magnetic, electronic, mechanical, and chemical materials, offer innovative possibilities in therapeutic applications. These applications encompass a wide range, including antimicrobial, antioxidant, antidiabetic, and anticancer treatments. The exploration of nanoparticles extends across the fields of physical, chemical, and biological sciences.[43]

Recent successful reports have emphasized the production of nanomaterials derived from natural sources, such as plants and microbes. The biosynthesis of nanoparticles has gained significant attention in biomedical applications due to their biocompatibility and multifunctional capabilities.[44] Contemporary oral antidiabetic medications often face challenges in providing sustained glycemic control. To address this limitation, various extracts from medicinal plants are recognized for their efficacy in reducing blood glucose levels and are utilized as antidiabetic agents.[45]

Based on the aforementioned analysis, it can be inferred that diosgenin plays a pivotal role as the primary compound contributing to the antidiabetic properties found in the seeds of *B. aegyptiaca*. Additionally, other compounds, such as linolenic acid, oleic acid, linoleic acid, chlorogenic acid, palmitic acid and its esters, and myo-inositol, which are recognized for their antidiabetic effects, contribute to the overall antidiabetic potential. Notably, the F2 fraction exhibits the highest concentration of diosgenin among all fractions, likely accounting for its superior antidiabetic activity, while other beneficial compounds are notably present in the F3 fraction.

Linoleic acid falls under the category of essential fatty acids and exhibits a hypocholesterolemic effect, as reported by Attia et al.[46] Furthermore, it plays a role in regulating blood glucose levels by enhancing insulin secretion from pancreatic cells, as indicated by Zhang et al.,[47] and by increasing glucose uptake in C2C12 muscle cells, as demonstrated by Sawada et al.[48] Chlorogenic acid detected in the seeds, leaves and fruits of *Viscum album* also reported to have antidiabetic potential in terms of the inhibition of α- amylase and α-glucosidase enzyme.[49] Oleic acid, another fatty acid, has been shown to stimulate insulin secretion from glucose-sensitive INS-1 cells, even in the presence of the inflammatory cytokine TNF-γ, according to Vassiliou et al.[50] Oleic acid and lenolenic acid present in the seeds of *Vitis vinifera* showed antidiabetic activity through α-glucosidase inhibition.[51] The influence on glucose uptake and the expression of GLUT-4 transporter is notably affected by polyunsaturated fatty acids, leading to increased expression of GLUT-4 and GLUT-1, as highlighted by Nugent et al.[52] and Manco et al.[53] Natural flavonoids are also recognized for their ability to reduce blood glucose levels, thereby enhancing insulin sensitivity, as reported by Parsath et al.[54] Additionally, various polyphenols exhibit antidiabetic activity, with chlorogenic acid, for instance, known for reducing blood glucose levels and improving insulin sensitivity, as documented by Meng et al.[55] Other compounds like flavonone, syringic acids,

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and naringin demonstrate antihyperglycemic activity by enhancing glycemic control and reducing glucose absorption[56−58] respectively.

Diosgenin's antidiabetic properties have been documented in various studies involving animal models and cell lines. According to Skett,[59] it was observed to reduce glucose absorption in rat hepatocytes and inhibit glycogen decomposition in rats. In HepG2 cells, as noted by Fang et al.,[60] Diosgenin demonstrated an ability to improve insulin resistance. Additionally, Uemura et al.[61] reported that Diosgenin stimulated adipocyte differentiation, enhanced glucose uptake, and increased intracellular lipid accumulation in 3T3-L1 cells. In studies involving male Wistar rats, Sato et al.[62] found that Diosgenin led to a decrease in blood glucose levels. Wang et al.[63] observed in male 57 mice that Diosgenin not only lowered blood glucose levels but also increased glucose and insulin intolerance, while simultaneously suppressing lipid accumulation.

The study's results indicate that *B. aegyptiaca* seed extracts were nontoxic at all concentrations tested. Instead, they elevated cell viability to 154.13% in C2C12 cells and 116.88% in MIN6 cells at a concentration of 100 μg/mL. The calculated IC50 for AgNPs seeds on C2C12 and MIN6 cells was 206.0 μg/mL and 140.44 μg/mL, respectively. A similar observation was documented by Kamga-Simo III et al.[64] when assessing the toxicity impact of *Cassia abbreviata* seeds, leaves, and bark extracts on C2C12 cells. The study revealed an increase in cell viability by 236%, 192%, and 127% compared to the control after treatment with seeds, leaves, and bark extracts, respectively, at a concentration of 5 mg/mL. The extract of *Geranium graveolens*, *Sarcopoterium spinosum* and *Varthemia iphionoides* increased the cell viability of MIN6 cells to 120.6 ± 1.5%, 109.4 ± 3.0% and 127.6 ± 14.6% of control at 100 μg/mL concentration.[65] Naveed et al.[66] reported the calculated IC50 values of AgNPs synthesized from leaves extracts of *Brachychiton populneus* on U87 and HEK293 cells as 64.85 μg/mL and 150.38 μg/mL, respectively, using the MTT assay. The extract of *Achillea santolina*, *Eryngium creticum* and *Pistacia atlantica* was also not found toxic at 100 μg/mL on MIN6 cells and maintain the cell viability at 95.9 ± 2.1%, 91.4 ± 7.2% and 93.1 ± 3.1% respectively.[67] All these studies support the validation of the present study.

The study's results indicate a significant increase in glucose uptake in C2C12 cells following treatment with plant extracts, AgNPs seeds, and the F2 fraction. Similar results were shown by study reported by Kamga-Simo III et al.,[64] the seeds (500 μg/mL), leaves (500 μg/mL), and bark extracts (100 μg/mL) of *Cassia abbreviata* demonstrated a substantial increase in glucose uptake, reaching 142%, 133%, and 123% of the control, respectively. Similarly, Safarzad et al.[68] reported that the leaf extract of *Rubus anotolicus* significantly increased glucose uptake in HepG2 cells ($p \leq 0.0001$) at 100 and 200 μg/mL, C2C12 cells ($p \leq 0.0001$) at 100 and 200 μg/mL, and CRI-D2 cells ($p \leq 0.0001$) at 50 and 100 μg/mL. Additionally, in the study by Han et al.,[69] the seeds (100 μg/mL) and fruits (500 μg/mL) extracts of *Synsepalum dulsificum* exhibited notable increases in glucose uptake in C2C12 cells, reaching up to 33.18% and 56.6%, respectively. The AgNPs synthesized from the leaves extracts of *Cantella asiatica* are reported to increase the glucose uptake of 63.27 ± 0.57% in yeast cells at 200 μg/mL.[70] Similarly, the extract derived from *Achillea santolina, Eryngium creticum*, and *Pistacia atlantica* exhibited a statistically significant increase in insulin secretion from MIN6 cells, with significance levels at $p \leq 0.05$, $p \leq 0.001$, and $p \leq 0.001$, respectively, as reported by Kasabri et al. in.[65] *Rubus anotolicus* was reported to increase the insulin secretion from CRI-D2 significantly ($p \leq 0.001$) at 200, 50, and 100 μg/mL.[68]

5. CONCLUSION

While numerous studies have highlighted diverse medicinal properties associated with different parts of the *B. aegyptiaca* plant, our current findings present the inaugural pharmacological insights into the *in vitro* antidiabetic potential of seed extracts and silver nanoparticles (AgNPs). This study also aimed to explore the presence of various phytochemicals responsible for this observed activity. Notably, the seed extracts and AgNPs exhibited no toxicity at tested concentration on both treated cells and demonstrated significant antidiabetic effects, as evidenced by enhanced glucose uptake and insulin secretion. Phytochemical analysis revealed the presence of compounds such as diosgenin, coumarins, oleic acid, palmitic acid, and its esters, linoleic acid, and syringic acid, among others, which may contribute to these medicinal properties. From the fractions analysis, it was evaluated that diosgenin contributes majorly to the antidiabetic activity. The findings from this research could serve as valuable insights for future investigations into utilizing traditional medicinal plants in the development of nutraceuticals and pharmaceuticals.

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M.B., P.Y., and M.Y. completed experimental design and writing of the article. S.K and S.D. contributed for review and editing of the research article.

Notes

The authors declare no competing financial interest.

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