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Antidiabetic Activity of Phytosynthesized Ag/CuO Nanocomposites Using *Murraya koenigii* and *Zingiber officinale* Extracts

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Abstract

In this paper, we report the phytosynthesis of Ag/CuO nanocomposites (NCs) using *Murraya koenigii* and *Zingiber officinale* green extracts containing unique metabolites. The silver and copper oxide nanoparticles, and Ag/CuO NCs were also synthesized by chemical methods for the purpose of comparison with phytosynthesized Ag/CuO NCs. All the synthesized nanomaterials were characterized by diverse spectro-analytical methods. The phytochemical analysis confirmed the presence of active phytoconstituents in the *Murraya koenigii* and *Zingiber officinale* extracts. The major biomolecules present in the plant extracts act as reducing and capping agents in the green synthesis process, as identified using FT IR spectroscopy. The maximum absorbance at ~ 290 and ~ 460 nm corresponding to CuO and Ag evidenced the formation of Ag/CuO NCs. Further, the XRD patterns also confirmed the formation of nanocomposites by exhibiting the fcc crystalline and monoclinic nature of Ag and CuO nanoparticles, respectively. The SEM images displayed the spherical shape of the synthesized NCs, and the EDX spectra confirmed the presence of Ag, Cu and O elements in the synthesized NCs. The TEM images revealed the relatively uniform size of the synthesized

nanomaterials ranging between 18 and 22 nm. The *in vitro* antidiabetic activity with α -amylase, α -glucosidase and glucose-6-phosphatase enzymes, and glucose uptake assay showed that the Ag/CuO NCs synthesized using *Zingiber officinale* extract displayed highest activity than the rest of the synthesized nanomaterials.

Keywords: *Murraya koenigii* extract • *Zingiber officinale* extract • Antidiabetic activity • Glucose-6-phosphatase activity • Glucose uptake assay

1 Introduction

In recent years, nanotechnology has gained wide-ranging interest due to its versatile properties and potential applications in modern material science. The wide scopes of research in various scientific disciplines are due to the unique properties of nanomaterials when compared with the bulk materials. The synthesis of nanomaterials through biological routes have attracted much attention in modern nanotechnology due to their unique advantages such as simple, scalable and non-toxic process, and improved biomedical applications [1, 2]. In this connection, the metal and metal oxide nanoparticles are picking up more consideration because of their surface plasmonic properties and potential technological application in biomedical fields, sensors and photocatalysis [3]. The development of nanomaterials in biological and medicinal research leads to be strong strategies to control medical issues coupled with several diseases and infections.

Nowadays, distinctive combination of nanomaterials has attracted researchers due to their stability and versatile behaviour in which the synthesis and application of metal/metal oxide nanocomposites have gained much importance as the combination of two nanomaterials provide a distinctive model for the production of nanomaterials with specified properties [4, 5]. Copper oxide (CuO), a p-type semiconductor with a narrow bandgap of 1.2–

1.9 eV and monoclinic crystal structure is well-known for diverse practical applications in heterogeneous catalysis, sensors, lithium ion electrode materials, field-emission sources, semiconductors *etc.*, The combination of CuO with noble metal is an energizing area of research due to their enhanced biomedical, electronic, photocatalytic and other applications. For example, in the case of CuO decorated Ag nanoparticles, the charge transfer occurred from the CuO to the Ag nanoparticles, suppressing the recombination of charge carriers, and thus exhibiting the efficient photocatalytic properties [6–9]. Additionally, the CuO nanoparticles are stable and have longer shelf-life due to which it can be utilized for many therapeutic applications [10].

Among the several green synthesis sources, the plant extracts have attracted much attention because of their advantages such as abundant availability, safe to handle, and healing as well as curing of human diseases due to the presence of various metabolites. The active biomolecules present in the plant extracts also played a crucial role in the reduction process during the synthesis of nanomaterials. *Murraya koenigii* from Rutaceae family, generally called curry leaves having a slight pungent taste and used as flavouring agent in food items. It is also used in Siddha medicine as tonic for stomach ache, as stimulant and carminative, hypoglycemic agent, antifungal agent, and also exhibit anticancer activity against colon cancer [11, 12]. *Zingiber officinale* is a flowering plant belongs to Zingiberaceae family, whose rhizome, ginger root is widely used for remedial purposes over 2000 years in folk medicine [13].

The present study was designed to synthesize Ag/CuO NCs using *Murraya koenigii* and *Zingiber officinale* extracts. The preliminary qualitative and quantitative phytochemical analysis was carried out for *Murraya koenigii* and *Zingiber officinale* extracts in order to identify and quantify their phytochemical constituents. The antidiabetic potential of the synthesized Ag/CuO NCs were assessed against α -amylase, α -glucosidase and glucose-6-

phosphatase enzymes. The gulucose uptake activity of the synthesized Ag/CuO NCs were also analysed using 3T3-L1 preadipocyte cells.

2 Experimental methods

2.1 Materials and methods

Analytical grade silver nitrate was obtained from SD Fine Chemicals Limited (SDFCL), India. Copper acetate tetrahydrate, ethanol and sodium hydroxide were purchased from Fisher Scientific, India. 3T3-L1 preadipocyte cells were obtained from Microbial Type Culture Collection Center (MTCC), Chandigarh, India. *Murraya koenigii* and *Zingiber officinale* were acquired from nearby market, Royapettah, Chennai. The detailed procedure for the synthesis of silver and copper oxide nanoparticles are given in our earlier publications [14, 15]. The Ag/CuO NCs were successfully synthesized using the plant extracts and chemical method by adopting the reported procedures with slight modifications [16, 17].

2.2 Synthesis of Ag/CuO nanocomposites (Ag/CuO NCs)

2.2.1 Synthesis of Ag/CuO NC by chemical method

Copper acetate tetrahydrate (0.2 mol) and equimolar sodium hydroxide were dissolved slowly in deionized water, and stirred for 1 h at 80 °C during which a black coloured precipitate was formed, indicating the formation of CuO nanoparticles. Then, silver nitrate (0.42 g, 0.025 mol) was added, and the mixture was stirred for 2 h at 80 °C. The final product was collected by centrifugation at 500 rpm, washed with ethanol followed by deionized water, and dried in a hot air oven for 2 h at 120 °C.

2.2.2 Synthesis of Ag/CuO NCs using *Murraya koenigii* and *Zingiber officinale* extracts

Ag/CuO NCs were synthesized by green method using *Murraya koenigii* and *Zingiber officinale* extracts. *Murraya koenigii* leaves were thoroughly washed with deionized water and air dried at room temperature. 10 g of air dried leaves were gauged and boiled at 100 °C with 250 mL of deionized water for 15 min. The greenish extract was separated from the

leaves using Whatman No.1 filter paper, and stored in refrigerator. *Zingiber officinale* was washed multiple times with deionized water to remove surface contaminations. The green extract was prepared by boiling 6 g of *Zingiber officinale* in 50 mL of deionized water and kept at normal room temperature for 24 h. The resulting solution was decanted to remove solid pieces, filtered through Whatman No. 1 filter paper and stored in refrigerator.

Copper acetate tetrahydrate (0.2 M) was dissolved in deionized water (15 mL) and silver nitrate (0.42 g, 0.025 mol) was slowly added to the solution and stirred for 10 min. Finally, 20 mL of leaf extract (*Murraya koenigii*/*Zingiber officinale*) was added to the above mixture under constant stirring using magnetic stirrer for 10 min. After complete dissolution of the mixture, the solution was boiled at 80 °C for 10 min. The immediate colour change after adding the leaf extract indicates the formation of Ag/CuO NCs. The final product was washed with ethanol followed by deionized water and then it was allowed to dry under room temperature to get the Ag/CuO nanocomposites.

The synthesized Ag and CuO nanoparticles are denoted as S1 and C1, respectively. Ag/CuO NCs synthesized through chemical method is denoted as SC1, while that obtained from *Murraya koenigii* and *Zingiber officinale* extracts are denoted as SC2 and SC3, respectively.

2.3 Characterization

FT IR spectra of synthesized nanomaterials were recorded at room temperature on Perkin–Elmer spectrophotometer in the range 4000–400 cm^{-1} . Diffuse reflectance spectra were recorded using UV140404B spectrophotometer in the wavelength range 200–800 nm, and numerical data were plotted in the 'Origin 8' software. Photoluminescence spectra were recorded using FLUOROLOG-FL3-11 fluorescence spectrometer. Powder X-ray diffraction data were collected between 10–90° (2 θ) at 0.5° min^{-1} from a highly stabilized and automated ZESSI X-ray generator (PW 1830) operated at 40 kV and 20 mA, with Cu K α radiation and λ

value is 1.5406 Å. SEM and EDX analysis were performed through JEOL 6500F instrument. TEM measurements were performed on a JEOL model 1200EX operated at an accelerating voltage of 120 kV.

2.4 Phytochemical studies

The aqueous *Murraya koenigii* and *Zingiber officinale* were subjected to qualitative and quantitative analysis for the identification of their active phytoconstituents [18–20]. The detailed procedures are reported in supplementary material (S1 and S2).

2.5 Antidiabetic activity

The *in vitro* antidiabetic activities against α -amylase, α -glucosidase and glucose-6-phosphatase enzymes, glucose uptake activity were evaluated according to the reported procedures with some modification [21–23].

2.5.1 α -Amylase inhibitory activity

α -Amylase (0.05 g of α -amylase in 100 mL of ice-cold distilled water) was pre-mixed with the synthesized nanomaterials at different concentrations (100–1.52 μ g/mL) and sonicated at room temperature for 30 min. Starch as a substrate was added as a 0.5% starch solution in phosphate buffer to start the reaction. The assay mixture was incubated at 37 °C for 15 min and terminated by adding 2 mL of DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The reaction combination was then heated for 15 min at 100 °C in a boiling water. The α -amylase inhibitory activity was calculated as percentage inhibition using the following formula;

$$I_{\alpha\text{-Amylase}} (\%) = \frac{A_{540 \text{ control}} - A_{540 \text{ sample}}}{A_{540 \text{ control}}} \times 100$$

where $A_{540 \text{ control}}$ = absorbance without nanomaterials, and $A_{540 \text{ sample}}$ = absorbance with nanomaterials.

2.5.2 α -Glucosidase inhibitory activity

α -Glucosidase (0.05 g of α -glucosidase in 100 mL of ice-cold distilled water) was pre-mixed with the synthesized nanomaterials at different concentration (100–1.52 μ g/mL) and sonicated at room temperature for 30 min. In order to start the reaction, *p*-nitrophenyl- α -D-glucopyranoside (3 mM) as a substrate in potassium phosphate buffer was added to the mixture. The reaction was incubated at 37 °C for 20 min and terminated by the addition of Na₂CO₃ (2 mL, 0.1 M). A tube containing α -glucosidase without nanomaterials served as the control with 100% enzyme activity. α -Glucosidase inhibitory activity was determined by measuring the release of *p*-nitrophenyl- α -D-glucopyranoside as percentage inhibition using the following formula;

$$I_{\alpha\text{-Glucosidase}} (\%) = \frac{A_{405 \text{ control}} - A_{405 \text{ sample}}}{A_{405 \text{ control}}} \times 100$$

where $A_{405 \text{ control}}$ = absorbance without nanomaterials, and $A_{405 \text{ sample}}$ = absorbance with nanomaterials.

2.5.3 Glucose-6-phosphatase inhibition activity

The glucose-6-phosphatase activity was carried out using glucose-6-phosphatase from a rabbit liver (Sigma, G5758), and was determined by measuring the release of glucose-6-phosphate at 660 nm. Inhibition rates were calculated by using the following formula;

$$(\%) \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

IC₅₀ values were determined from dose-response curve of percentage inhibition *versus* nanomaterials concentration and compared with the IC₅₀ value of the synthetic inhibitor of glucose-6-phosphatase (metformin), under similar conditions.

2.6 Glucose uptake determination

The 3T3-L1 preadipocyte cells were cultured and plated in 24-well plate, and incubated for 24 h in DMEM growth medium containing glucose (5 mM). Initially (1st day), the growth medium was replaced by DMEM supplemented with 10% fetal calf serum (FCS), 10 µg/mL insulin, dexamethasone (DEX; 10⁻⁸ M) and 3-isobutyl-1-methylxanthine (IBMX; 0.1 mM). After 70 h (4th day), this medium was replaced with growth medium. After 48 h incubation (6th day), the cells were analyzed by removing 10 µL of the media and placing in the 96 well plates to which GOD-POD reagent (200 µL) was added and incubated for 15 min at 37 °C. The amount of glucose content (mg/dL) in each well was determined by using the following formula;

$$\text{Unknown sample} = \frac{\text{Concentration of sample}}{\text{OD}_{\text{standard}} - \text{OD}_{\text{blank}}} \times (\text{OD}_{\text{of unknown sample}} - \text{OD}_{\text{of blank}})$$

Finally, the glucose uptake over control was calculated as the difference between initial and final glucose content in the incubated medium.

3 Results and discussion

Ag/CuO nanocomposites (NCs) were synthesized by green route using aqueous extracts of *Murraya koenigii* and *Zingiber officinale*. The use of these extracts leads to the reduction of metal ion to zero valent metal nanoparticles by avoiding the usage of common reducing agents such as sodium borohydride. In order to carry out comparison study, we have also synthesized the same nanocomposite by chemical methodology. The use of optimum plant extracts, which contain phytoconstituents of therapeutic values for the synthesis of NCs have induced us to investigate the *in vitro* antidiabetic activities of the synthesized NCs.

3.1 Phytochemical screening of green extracts

The biomolecules present in plant extracts are safe and owns remedial merits as compared to the artificial drugs. Interestingly, *Murraya koenigii* and *Zingiber officinale* possesses a wealthy phytochemistry with reputable medicinal uses in traditional medicine, which originates from Rutaceae and Zingiberaceae families, respectively. *Zingiber officinale* has been used widely in Chinese, Indian and Unani medicines because of its potential therapeutic activity [24–26]. The green extracts contain several biomolecules such as amino acids, proteins, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins and tannins (**Table S1**). Gums and mucilages, and terpenoids are missing in both the extracts. Rest of the compounds including alkaloids and phytosterols are available only in *Zingiber officinale* extract. In this regard, we have also quantitatively determined the essential phytoconstituents present in both the extracts (**Table S2**). The biomolecules such as flavonoids and phenolic compounds may have the potential to bind metal ions and cause reduction to metal nanoparticles. The results support the role of flavonoids and phenolic compounds in the bioreduction.

3.2 Structural, optical and morphological characterization

3.2.1 FT IR analysis

The FT IR spectra revealed the bioreduction role of major phytoconstituents present in the extracts and the effective incorporation of green extracts on the synthesized nanomaterials (**Fig. 1**). The band observed at $\sim 3450\text{ cm}^{-1}$ for all the synthesized NCs attributed to the stretching vibration of the moisture content and the band in the region $1650\text{--}1670\text{ cm}^{-1}$ corresponding to the H–O–H bending vibration of water molecule [27]. The photosynthesized NCs exhibit absorption band at 1038 cm^{-1} ascribed to C–O stretching characteristics of polysaccharides. The bands at ~ 2920 and $\sim 2860\text{ cm}^{-1}$ are due to C–H vibration modes and that observed at ~ 1720 and $\sim 1610\text{ cm}^{-1}$ attributed to stretching vibration of carboxyl group (C=O) of the extracts [28]. The band observed at $\sim 1100\text{ cm}^{-1}$ is due to C–C vibration (skeletal). The characteristic absorption peaks of Cu–O vibrations are indexed around 530 cm^{-1} [29–31]. The FT IR spectra of Ag/CuO NCs show all the characteristics vibrations of functional groups of silver and CuO NPs. The characteristic peaks of functional groups of the extracts indicate the role of secondary metabolites in the synthesis of nanocomposites.

(A)

(B)

Fig. 1. FT IR spectra of green extracts (A): *Murraya koenigii* extract (a) and *Zingiber officinale* extract (b), and nanomaterials (B): C1 (c), SC2 (d) and SC3 (e).

3.2.2 UV-Vis DRS spectral studies

The UV-Vis DRS spectroscopy is used to understand the formation of Ag/CuO NCs (**Fig. 2**). The optical characteristics of the metal nanoparticles in composite are considerably differing from individual metal nanoparticles. The maximum absorption at ~ 290 and ~ 470 nm for the Ag/ZnO NCs corresponds to CuO and AgNPs, respectively. The successful deposition of AgNPs into CuO NPs were confirmed by the stronger absorption surface plasmon resonance peaks observed for nanocomposites compared to the original nanomaterials [2, 28]. The diffuse reflectance spectroscopy is used to analyse the width of the energy gap using the Kubelka-Munk (K-M) model [32]. A graph plotted between $[F(R)/h\nu]^2$ and $h\nu$, in which the intercept value is the bandgap energy, and the bandgap values is found to be in the range between 1.61 and 1.77 eV for Ag/CuO NCs, and 1.39 eV for CuO nanoparticles, which authenticate the deposition of Ag into CuO nanoparticles (**Fig. 3** and **Table 1**). The bandgaps of Ag/CuO NCs are higher than that of CuO NPs. The larger bandgaps are generally linked with smaller size that leads to exhibit higher level of biological activity [33].

Fig. 2 UV-Vis DRS spectra of C1 (a), SC1 (b), SC2 (c) and SC3 (d).

Fig. 3 Estimated bandgap of C1 (a), SC1 (b), SC2 (c) and SC3 (d).

3.2.3 Powder XRD analysis

The XRD analysis was performed to ascertain the crystalline nature and phase purity of the synthesized nanomaterials (**Fig. 4**). The diffractogram of CuO NPs show diffraction peaks at 2θ values 32.3, 35.6, 38.2, 48.7, 53.6, 58.2, 61.5, 66.2, 68.2 and 72.3° , which can be indexed to (110), (-111), (111), (-202), (020), (202), (-113), (-311), (220), and (311) planes with monoclinic CuO phase (JCPDS card No. 05-0661). There are two mixed sets of diffraction peaks appeared for Ag/CuO NCs. The diffraction peaks at 2θ values 38.1, 44.3 and 64.4° can be indexed to (111), (200) and (220) planes assigned to crystalline face-centered cubic Ag structure (JCPDS card No. 65-2841) [34, 35]. The XRD patterns confirmed the presence of both CuO nanocrystals and Ag nanoparticles in the synthesized Ag/CuO NCs with no other peaks due to any impurities. The crystalline sizes of the synthesized nanomaterials are calculated by using the Debye-Scherrer's formula;

$$D = \frac{K\lambda}{\beta \cos \theta}$$

where D is the average crystallite size, K is a grain sharp factor (0.94), λ is the incident X-ray wavelength ($1.5418 \text{ \AA}$), β is the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg's angle of peak. The average crystalline size of the synthesized nanomaterials is shown in **Table 1**.

Fig. 4. X-Ray diffraction patterns of S1 (a), C1 (b), SC1 (c), SC2 (d) and SC3 (e).

Table 1 Average crystallite size and bandgap values of nanomaterials determined by XRD, TEM and UV-Vis DRS techniques.

Nanomaterials	Crystalline size (nm) (XRD)	Particle size (nm) (TEM)	Bandgap (eV) (UV-Vis DRS)
S1	18.54	18.12	—
C1	19.71	19.64	1.38
SC1	21.59	21.78	1.62
SC2	22.10	21.83	1.61
SC3	22.43	20.14	1.63

3.2.4 SEM and EDX analysis

The SEM images of CuO and Ag/CuO NCs revealed the spherical shapes of the synthesized nanomaterials [31, 36]. It could be seen that the AgNPs are deposited on the surface of the CuO nanostructures, and having roughened surfaces. The elemental composition of the nanocomposites was demonstrated by EDX analysis, which shows the presence of Ag, Cu and O elements in the synthesized nanocomposites. The presence of strong Ag signal authenticate the deposition of Ag into CuO. In addition to the strong signals due to Ag and Cu, the NCs synthesized using green extracts also exhibit weak signals from C, S, Cl, O and P due to the phytoconstituents of the extracts [37]. The nanomaterials synthesized through chemical method shows no other extra signal, which confirm the purity of the samples (**Figs. 5 & 6**).

3.2.5 TEM analysis

The TEM images of the synthesized Ag/CuO NCs exhibit the uniform dispersion by contrast and colour differences with an average size between 18 and 22 nm (**Fig. 7**). The size of the nanomaterials calculated from XRD analysis is closely matched with TEM results. The AgNPs appeared to be a black in colour and CuO was light due to high mass thickness of Ag [36, 37].

Fig. 5 SEM images of nanomaterials: S1 (a), C1 (b), SC1 (c) and SC2 (d).

Fig. 6 Energy dispersive spectra of nanomaterials: S1 (a), C1 (b), SC1 (c) and SC2 (d).

3.3 Antidiabetic activity

Diabetes is a tricky disease that refers to a metabolic disorder distinguished by higher range of blood glucose level, and classified into Type 1, Type 2 and gestational type diabetes. The Type 2 diabetes received considerable attention as it influences a large proportion of populates worldwide by increasing the blood glucose level to insulin resistance in adipose tissue, muscle and liver [38]. The inhibition of carbohydrate enzymes prevent the increase of glucose level in blood. Sulfonylureas, biguanides, genistein, astilbin and hesperidin are generally recommended antidiabetic drugs for the purpose of controlling the blood glucose level [39, 40]. The carbohydrate digesting enzymes such as α -amylase and α -glucosidase avoid the sudden raise of glucose level in blood. Acarbose, miglitol and voglibose are commonly utilized as standards in α -amylase and α -glucosidase inhibiton activity [41].

Fig. 7. TEM images of nanomaterials: C1 (a), SC1 (b), SC2 (c) and SC3 (d).

3.3.1 α -Amylase and α -glucosidase inhibition activity

The *in vitro* α -amylase and α -glucosidase inhibition activity of the synthesized nanomaterials were carried out with respect to the standard drug acarbose (**Table 2** and **Fig. 8**). The inhibitory reports revealed that the nanomaterials synthesized utilizing green extracts exhibit higher antidiabetic activity than that synthesized by chemical method, and Ag and CuO nanoparticles. The Ag/CuO NC synthesized using *Zingiber officinale* extract (SC3) displayed higher α -amylase and α -glucosidase inhibition activity when compared to that synthesized using *Murraya koenigii* extract and other nanomaterials. The presence of higher content of phenolic (137.41 ± 3.161 mg GAE/g dw) and flavonoid (93.82 ± 1.201 mg QE/g dw) compounds in *Zingiber officinale* extract when compared to *Murraya koenigii* ($127.68 \pm$

2.142 mg GAE/g dw and 61.27 ± 2.124 mg QE/g dw, respectively) may be the reason for the higher antidiabetic activity exhibited by SC3 compared to the rest of the nanomaterials. Based on the obtained results, the phytosynthesized Ag/CuO NCs could be suggested for further antidiabetic studies in order to explore the medicinal applications.

Table 2 Inhibitory effect of nanomaterials on α -amylase and α -glucosidase activity.

Nanomaterials/ Standard	*IC ₅₀ (μ g/mL)	
	α -Amylase	α -Glucosidase
S1	33.46 ± 1.825	13.74 ± 1.236
C1	38.91 ± 1.741	17.41 ± 1.241
SC1	36.38 ± 1.297	19.62 ± 1.864
SC2	24.32 ± 1.448	11.42 ± 0.964
SC3	22.37 ± 1.268	10.33 ± 0.984
Acarbose	12.426 ± 1.246	4.281 ± 0.164

Data are expressed as mean $\pm$ SEM; *Average of three independent determinations.

Fig. 8 Percentage of α -amylase and α -glucosidase inhibitory activity of nanomaterials.

3.3.2 Glucose-6-phosphatase inhibitory activity

Glucose-6-phosphatase is an enzyme made up of proteins, phosphate group and glucose. Glucose exported from cell *via* glucose transporter membrane proteins. This catalysis is used in gluconeogenesis and plays a key role in the homeostatic regulation of blood glucose level [23]. Generally, glucose-6-phosphatase targets insulin action by inhibiting hyperglycaemia state and it prevent the production of glucose. In type II diabetes, liver becomes resistant to insulin and overexpression of glucose-6-phosphatase leads to uncontrolled gluconeogenesis [42, 43]. The inhibition activity of the synthesized nanomaterials was carried out against glucose-6-phosphatase with respect to the standard drug, metformin under identical conditions (**Table 3** and **Fig. 9**). The obtained results revealed that Ag and CuO NPs, and the nanocomposite synthesized by chemical method showed weak activity when compared to the nanocomposites synthesized by green methods. The nanocomposite synthesized using *Zingiber officinale* extract (SC3) showed higher activity among the nanomaterials with respect to their IC₅₀ values because of the rich amount of phytochemicals present in the green extract, which leads to reduce the glucose formation.

Table 3. Inhibitory effect of nanomaterials on glucose-6-phosphatase enzyme.

Nanomaterials/ Standard	IC ₅₀ (μg/mL)*
	Glucose-6-Phosphatase
S1	68.53 ± 3.258
C1	65.47 ± 4.202
SC1	62.49 ± 2.986
SC2	49.02 ± 2.613
SC3	42.13 ± 3.151
Metformin	28.61 ± 1.823

Data are expressed as mean ± SEM; *Average of three independent determinations.

Fig. 9. Bar diagram showing the inhibition of glucose-6-phosphatase enzyme by nanomaterials.

3.3.3 Glucose uptake assay

Glucose uptake assay is a simple, non-radioactive assay, which helps in measuring the uptake of glucose inside the cells. The Type 2 diabetes is associated with lack of insulin-stimulated glucose uptake, while high glucose uptake is a sign of high glycolytic rate associated with cancer. Glucose uptake assay was carried out against 3T3-L1 adipocyte cells at a concentration of 100 $\mu\text{g/mL}$ in the presence of insulin and metformin, and the synthesized nanomaterials, both separately and in combination to each other (**Table 4** and **Fig. 10**). The results revealed that all the nanomaterials exhibit considerable activity against the 3T3-L1 cells. Addition of insulin and metformin to the nanomaterials further increased the uptake of glucose percentage. It could be noticed that the NCs synthesized using green extracts (SC2 and SC3) showed considerable glucose uptake when combined with insulin and metformin. Further, among the nanocomposites, the Ag/CuO NCs synthesized using *Zingiber officinale* extract (SC3) shows higher activity compared to the other nanomaterials.

Table 4 Effect of insulin, metformin and nanomaterials on glucose uptake %.

Test/Standard	Glucose uptake (%)
Insulin	98.41 ± 4.326
Metformin	95.22 ± 3.249
Insulin + Metformin	99.36 ± 2.947
S1	76.42 ± 2.265
S1 + Insulin	82.43 ± 2.861
S1 + Insulin + Metformin	91.47 ± 3.752
C1	71.94 ± 2.287
C1 + Insulin	85.38 ± 3.912
C1 + Insulin + Metformin	90.38 ± 3.123
SC1	82.32 ± 2.642
SC1 + Insulin	91.47 ± 2.281
SC1 + Insulin + Metformin	96.86 ± 3.112
SC2	92.37 ± 4.381
SC2 + Insulin	96.78 ± 3.227
SC2 + Insulin + Metformin	98.42 ± 2.608
SC3	92.64 ± 3.123
SC3 + Insulin	96.81 ± 3.476
SC3 + Insulin + Metformin	99.12 ± 2.822

Data are expressed as mean ± SEM; *Average of three independent determinations.

Fig. 10. Effect of insulin, metformin and nanomaterials on 3T3-L1 adipocyte cells showing glucose uptake %.

4 Conclusion

In this chapter, we have reported eco-friendly and simple biosynthesis of Ag/CuO NCs using *Murraya koiengii* and *Zingiber officinale* extracts. The biomolecules present in the green extracts assist the reduction process and contribute interesting methodology towards the fabrication of nanomaterials. The functional groups present in the green synthesized NCs were confirmed by FT IR spectroscopy. The UV-Vis DRS spectra revealed the formation of nanocomposites by exhibiting two absorption bands at ~ 290 nm and ~ 460 nm corresponding to CuO and AgNPs, respectively. The morphological analysis confirmed the spherical shape of the synthesized nanomaterials, whereas the elemental composition authenticated the presence of Ag, O, S, C, P and Cu elements in the phytosynthesized nanomaterials. The TEM results confirmed the formation of nanocomposites with an average particle size of 18–22 nm. The nanocomposites synthesized using *Zingiber officinale* extract showed higher *in vitro* inhibition activity against α -amylase, α -glucosidase and glucose-6-phosphatase enzymes. Further, the glucose uptake assay in 3T3-L1 adipocyte cells revealed the higher uptake of glucose for the nanocomposite synthesized using *Zingiber officinale* extract on addition of insulin and metformin. The presence of higher amount of phytochemicals such as phenolic (137.41 ± 3.161 mg GAE/g dw) and flavonoid (93.82 ± 1.201 mg QE/g dw) compounds in the *Zingiber officinale* extract may be the prime reason for the higher antidiabetic activity exhibited by the Ag/ZnO NC synthesized using *Zingiber officinale* extract (SC3). The Ag/CuO NCs synthesized through green extracts may be utilized as therapeutic agents in future.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interest.

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Figures

(A)

(B)

Figure 1

FT IR spectra of green extracts (A): *Murraya koenigii* extract (a) and *Zingiber officinale* extract (b), and nanomaterials (B): C1 (c), SC2 (d) and SC3 (e).

Figure 2

UV-Vis DRS spectra of C1 (a), SC1 (b), SC2 (c) and SC3 (d).

Figure 3

Estimated bandgap of C1 (a), SC1 (b), SC2 (c) and SC3 (d).

Figure 4

X-Ray diffraction patterns of S1 (a), C1 (b), SC1 (c), SC2 (d) and SC3 (e).

Figure 5

SEM images of nanomaterials: S1 (a), C1 (b), SC1 (c) and SC2 (d).

Figure 6

Energy dispersive spectra of nanomaterials: S1 (a), C1 (b), SC1 (c) and SC2 (d).

Figure 7

TEM images of nanomaterials: C1 (a), SC1 (b), SC2 (c) and SC3 (d).

Figure 8

Percentage of α -amylase and α -glucosidase inhibitory activity of nanomaterials.

Figure 9

Bar diagram showing the inhibition of glucose-6-phosphatase enzyme by nanomaterials.

Figure 10

Effect of insulin, metformin and nanomaterials on 3T3-L1 adipocyte cells showing glucose uptake %.

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