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In vitro studies of magnesium oxide nano-particles of *Saccharum officinarum* root extract: Evaluation of the antidiabetic effect using alpha-glucosidase enzyme

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Abstract

Objective: Magnesium oxide nanoparticle green synthesis is said to have a wide range of biomedical uses. Inhibiting the activity of pancreatic enzymes is one method for treating diabetes. The goal of this study was to show in-vitro evidence that *Saccharum officinarum* magnesium oxide nanoparticles may inhibit the activity of the enzyme α-glucosidase. This study had a therapeutic focus as this could be emerging candidates of new drugs for controlling hyperglycemia.

Materials and Procedures: *Saccharum officinarum* root extract was used to create magnesium oxide nanoparticles, which were then examined for bioactive components and tested for α-glucosidase inhibition. When the enzyme substrate solution was incubated with various doses of the grass-root Magnesium oxide nanoparticles, the activity of the enzyme was monitored to identify the kind of inhibition.

Result: Magnesium oxide nanoparticles synthesized from *Saccharum officinarum* root extract showed concentration and time-dependent inhibition of α-glucosidase. An 85% inhibition was observed with 0.6 mg/ml of the synthesized nanoparticles.

Conclusion: The results showed that the ethanolic extract of MgO nanoparticles synthesized by *Saccharum officinarum* exhibited effective antidiabetic activity, supporting its use in the treatment and control of diabetes.

Keywords: α-glucosidase; Magnesium Oxide; Inhibition; Diabetes; Synthesis; Nanoparticles

1. Introduction

Nanotechnology, as a growing interdisciplinary research area in various fields, has the potential to enable groundbreaking applications (Lin *et al.*, 2018). Sugarcane has a rich source of bioactive phytochemicals, including flavonoids, polyphenols, and phytosterols, to name a few. Similarly, the roots and stems of sugarcane also have many medicinal applications that deserve further investigation (Sara *et al.,* 2021). Sugarcane root (*Saccharum officinarum*) is believed to be used in traditional medicine and disease treatment (Uchenna *et al*., 2015). Sugarcane is widely consumed by people in tropical and subtropical regions. The antioxidants present in concentrated sugarcane extracts extracted from the resins of sugar cane products are very stable and their antioxidant activity is not significantly reduced when heated or cooled over time (Fansheng, Shujuan, Feng and Xinlan., 2015). Diabetes is becoming a serious and major health threat in low- and middle-income countries. A patient is considered to have diabetes when their blood sugar is above 180 mg/dl (Mohammed *et al*., 2013). This is one of the greatest global health emergencies of the 21st century with increasing proportions. Approximately 463 million adults aged 20-79 are currently living with diabetes and by 2045 this number will increase to 700 million (Telephore *et al.*, 2020). Dipeptidyl peptidase-IV (DPP-IV), α-glucosidase and α-amylase play prominent roles in the regulation of postprandial blood glucose, which is considered a major target in the treatment of type 2 diabetes mellitus (DT2) (Ruotong *et al.,* 2023). MgO is well tolerated and absorbed by the

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body (Arslan *et al*., 2020). The synthesis of metal oxide nanoparticles by green method has recently become the focus of medicine because it is more environmentally friendly, low cost and low toxicity, long-term stability (Metz *et al.*, 2015). *Saccharum officinarum* plant has been used traditionally to treat and control a number of conditions including urinary tract infections, diabetes, constipation, tooth decay, and bad breath (Agbaje *et al.,* 2019). Despite the anti-diabetic claims of the locals, scientific evidence for this potential of *Saccharum officinarum* root has not been established. Magnesium oxide nanoparticles synthesized from *Saccharum officinarum* root extract screened by gas chromatography-mass spectrometry for present bioactive compounds and also evaluated anti-diabetic property using the enzyme α-glucosidase.

2. Material and methods

2.1. Chemical products

Starch, porcine pancreatic α-amylase (PPA) and 3,5-dinitrosalicylic acid (DNSA) were purchased from Sigma Aldrich, USA. The other chemicals used in this study were of the highest quality and are purchased from local suppliers.

2.2. Collect and prepare plant materials

Sugarcane roots were harvested on site from one of the main sugarcane farms in Papa Lantoro, Ogun State and brought to the laboratory. The cane body is cut, leaving only the root, stains and sand are quickly removed. The roots were air-dried at room temperature until constant mass was achieved. After drying, the sample was then pulverized with an electric mixer and packed in an airtight container for further analysis.

2.3. Prepare the raw extract

The 700 g powdered dried root of *Saccharum officinarum* was immersed in a 5 L round-bottom flask containing 0.5 L (500 ml) of distilled water in the shade for 72 h while stirring the extract for the intermediate time. The extract was filtered with a muslin cloth and the filtrate was cooled, while the residue was air-dried and weighed.

2.4. Nanoparticle synthesis process

The dried cane root extract was weighed and dissolved in sterile distilled water (5L). To prepare 1 mM MgO_2, 0.016 g MgO_2 was accurately weighed and made up to 100 ml with sterile distilled water. To prepare MgSO, 90 ml of 1 Mg MgO2 was added to 10 ml of root extract to form a final solution of 100 ml and centrifuged at 3000 rpm for 20 min.

Magnesium oxide formation was confirmed by color change and it was also verified by scanning electron microscopy (S.E.M.). The nanoparticles were washed with a mixture of acetone and methanol to remove any residual particles that were not hair styling agents. The suspension was dried and stored as a crystalline powder for further characterization (Amrulloh*., et al*., 2021). Analysis by gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis of the *Saccharum officinarum* extract was performed using GCMS-QP2010SE SHIMADZU, Japan, fused to a 5 ms (30 × 0.25 mm) Optima capillary column with a film thickness of 0.25 µm in the longitudinal direction. method described by Franklyn et al. (2019) with minor modifications. The gas chromatographic conditions are as follows:

Pure helium carrier gas (flow rate:1.56mL/min; linear speed: 37 cm/s), the initial temperature of the column furnace (60°C) was programmed to rise to 160°C at a rate of 10 °C/min and finally to 250°C with a holding time of 2 min/ increments, and an injection volume of 0.5 µL in undivided mode with a fractionation ratio of 1:

1 and the nozzle temperature is set to 200°C. The conditions of the mass spectrometer are as follows:

Ion source temperature (230°C), interface temperature (250°C), solvent hysteresis at 4.5 min and acquisition in the scanning range of 50 to 700 amu. The electron ionization mode and kernel voltage were set to 70 eV and 1859 V, respectively. Retention times, fragmentation patterns and mass spectrometry data of unknown components in the extract were compared with libraries of Wiley and the National Institute of Standards and Technology (NIST) for compound identification.

2.5. Measurement of enzyme activity

Pig pancreatic α-glucosidase (PPA) activity was determined by colorimetric method with soluble starch as substrate. Reducing sugars were measured by the dinitrosalicylic acid method described by (Miller 1959). In each reaction, 280 µl

of 1% (w/v) starch was dissolved in phosphate buffer, pH 7 containing 20 mM CaCl2; 20 µl of enzyme solution containing 20 µg of PPA was added and the samples were incubated at 37°C. The reaction was stopped by adding DNSA reagent.

2.6. Statistical analysis

Data were analyzed using SPSS version 25 (IBM Corp., New York, USA) and one-way analysis of variance (ANOVA) was performed using Duncan's multiple-range post-hoc test. The values reported are the mean ± standard deviation (SD) of three (3) replicates and are considered to be significantly different at $p < 0.05$.

3. Results

3.1. Sample identification

Taxonomic identification was performed at the Ibadan Forest Herbarium (FHI), identification number given as 112981.

3.2. MgO Nano Particle Synthesis

It was observed during the formation of MgO nanoparticles that the addition of a colorless solution of Magnesium Nitrate [Mg (NO_3)2] 5mM (drop by drop) to the Anona muricata extract caused a color change from light green to brown, this has been confirmed the synthesis of MgO nanoparticles (Vergheese & Vishal, 2018).

3.3. Gas chromatography-mass spectrometry of the synthesized MgONPs

- Total number of 27 bioactive compounds were identified within the first 2 minutes 1seconds, 20 compounds were identified, with the first been heptane with a molecular weight of 100.125 (amu)
- Within the first 2 minutes 2 seconds, 20 compounds were identified with the first been furan, tetrahydro-2,5-dimethyl with a molecular weight of 100.089(amu)
- Within the first 2 minutes 3 seconds, 20 compounds were identified with the first been cyclopentane, ethylidene, with a molecular weight of 96.094(amu)
- Within the first 2 minutes 7 seconds, 20 compounds were identified with the first been 3-furonol, tetrahydro 2,2,4,4 tetramethyl with the molecular weight of 144.115(amu)
- Within the 3 minutes 20 compounds were identified with the first been 3-aminopiperidin -2-one with a molecular weight of 114.079 (amu)
- Within the first 3 minutes 2 seconds, 20 compounds were identified with the first been [7a-isopropenyl-4,5-dimethyloctahydrinden-4-yl] methanol with a molecular weight of 222.198 (amu)
- Within the first 5 minutes 5 second 20 compounds were identified with the first been clopidol with a molecular weight of 190.99 (amu)
- Within the first 7 minutes 5 second, 20 compounds were identified with the first been benzene acetic acid, trimethylsilyl ester with a molecular weight of 208.092 (amu)
- Within the first 9 minutes, 20 compounds were identified with the first been disiloxane,1, 3 bis (1,1-dimethylethyl)-1,1,3,3-tetramethyl with the molecular weight of 246.184 (amu)
- Within the first 9 minutes 4 second 20 compounds were identified with the first been 2-phenyl-1, 2-bis (trimethylsilyloxy) propane with a molecular weight of 296.163 (amu)
- Within the first 9 minutes 6 second, 20 compounds were identified with the first been 3, 8-dioxa-2,9-disiladec-5-ene,2,2,9,9-tetra methyl-,(E)- with a molecular weight of 232.131 (amu)
- Within the first 11minutes 4 second, 20 compounds were identified with the first been ethanedioic acid, bis (trimethylsilyl)ester with a molecular weight of 234.074 (amu)
- Within the first 12 minutes 3 second, 20 compounds were identified with the first been disiloxane,1, 3-bis (1,1-dimethylethyl) -1,1,3,3-tetramethyl- with a molecular weight of 246.184(amu)
- Within the first 13 minutes 1second 20 compounds were identified with the first been tert-butyl pentamethyldisiloxane with a molecular weight of 204.137(amu)
- Within the first 14 minutes 2 second 20 compounds were identified with the first been 1, 2-Bis(trimethylsiloxy)ethane, with a molecular weight of 206.116 (amu)
- Within the first 14 minutes 5 second,20 compounds were identified with the first been 1,3-Bis(trimethylsilyloxyl) butane with a molecular weight of 234.146 (amu)
- Within the first 15 minutes 6 second, 20 compounds were identified with the first been 1, 2-Bis(trimethylsilyloxyl)ethane with a molecular weight of 206.116 (amu)

- Within the first 15 minutes 8 second, 20 compounds were identified, with the first been ethanedioic acid, bis(trimethylsilyl) ester with a molecular weight of 234.074 (amu)
- Within the first 16 minutes 9 second, 20 compounds were identified with the first been 1,2-Bis(trimethylsiloxy)ethane with a molecular weight of 206.116 (amu)
- Within the first 16 minutes 9 second ,20 compounds were identified with the first been 1,3Bis (trimethylsilyloxy) butane with the molecular weight of 234.147 (amu)
- Within the first 18 minutes 20 compounds were identified with the first been 1,2-Bis(trimethylsiloxy) ethane with the molecular weight of 206.116(amu)
- Within the first 18 minutes 5 second, 20 compounds were identified with the first been Trimethyl-[1-phenyl-2 (trimethylsilyloxy) ethoxy] silane with a molecular weight of 282.147 (amu)
- within the 19minutes 20 compounds were identified with the first been 1,2-Bis (trimethylsiloxy) ethane with a molecular weight of 206.116 (amu)
- Within the first 19 minutes 9 second, 20 compounds were identified with the first been tert-butyl pentanethyldisiloxane with molecular weight of 204.137 (amu)
- Within the first 20 minutes 8 second, 20 compounds were identified with the first been 1,2-Bis (trimethylsiloxy) ethane with a molecular weight of 206.116 (amu)
- Within the first 21 minutes 7 second, 20 compounds were identified with the first been 1,2-Bis (trimethylsiloxy) ethane with a molecular weight of 206.116 (amu)
- Within the first 22 minutes 7second, 20 active compounds were identified with the first been pentamethyldisilyloxytridecane with a molecular weight of 330.277 (amu).

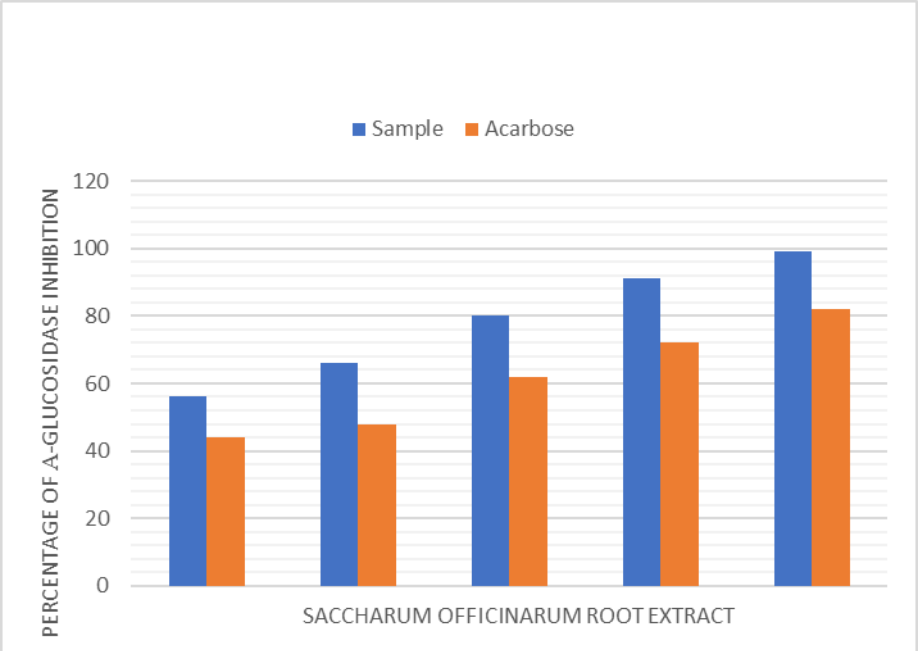

Figure 1 Percentage of α-glucosidase inhibition of *Saccharum officinarum*. Acarbose is taken as standard inhibition.

Therefore, the dose-dependent α-glucosidase inhibitory activity of *Saccharum officinarum* root extract was further studied and its IC_{50} value was calculated.

Table 1 α-glucosidase anti-diabetic activity of biosynthesized MgO nanoparticles *Saccharum officinarum*

	IC_{50} (mg/mL)	**Vmax (Mm/min)**	**Km (mM)**
SMgONps	0.44 ±0.06a	0.011	2.21
Acarbose	0.75 ±0.14b	_____	_____
Control	_____	0.020	0.47

Data are represented as mean ± SD ($n = 3$). Values with different superscripts down a column are significantly different at $p < 0.05$. IC_{50}: half maximal inhibitory concentration; *V*max: maximum velocity; *K*m: Michaelis constant.

MgONPs of *Saccharum officinarum* root extract is presented in the table above; where MgONPs elicited inhibitory effects that competed favorably with the standard drug (acarbose). It is significantly ($P<0.05$) higher than acarbose in its inhibitory effects with an IC_{50} of 0.44±0.06 and 0.75 ±0.14 mg/ml. MgONPs of *Saccharum officinarum* root extract displayed an inhibition of 0.011Mm/min and Km values of 2.21.

4. Discussions

4.1. *In vitro* antidiabetic potential of *Saccharum officinarum* root MgONPs

Vandna and Vipin, 2017 reported that the plant *Saccharum officinarum* produces several compounds including alkaloids, tannins, phenolics, flavonoids, proteins, reducing sugars, and resins. The components of the cane stalk have several medicinal properties and can be used therapeutically. different diseases from which they concluded that this herb can be used to treat various diseases without any side effects. The green synthesis of magnesium oxide nanoparticles leads to the formation of harmless nanoparticles due to the presence of many phytochemicals with pharmaceutical applications. Magnesium oxide (MgO) has been shown to be effective against diabetes mellitus (DM) (Arslan *et al*., 2022). Type II diabetes associated with postprandial hyperglycemia can be effectively controlled by α-amylase and α-glucosidase inhibitors (Badmus *et al.,* 2020). The extracts were also found to inhibit the enzymes alpha-glucosidase and alpha-amylase, suggesting a possible hyperglycemic effect. The enzyme α-glucosidase is one of the main enzymes that metabolize carbohydrates in the gastrointestinal tract, thereby influencing carbohydrate metabolism. Drugs attenuate its pharmacological effect by inhibiting this enzyme and these drugs are used as a therapeutic control in the management of diabetes through control of postprandial hyperglycemia. *Saccharum officinarum* root extract synthesized Magnesium oxide nanoparticles ($P<0.05$) significantly inhibited α-glucosidase with IC_{50} value of 0.44 ± 0.06 compared to standard acarbose with IC_{50} value of 0.75 ± 0.14mg/ml. Magnesium is considered beneficial in the treatment of insulin disorders, diabetes mellitus (Arslan *et al.,* 2022). The biosynthesized MgONPs synthesized from *Saccharum officinarum* root extract showed significantly stronger effects against α-glucosidase compared with standard acarbose. Several studies have reported the antidiabetic activity of MgoNPs evaluating α-glucosidase enzyme inhibitory activities. Intestinal α-Glucosidase hydrolyzes complex carbohydrates to glucose and other monosaccharides in the small intestine, the inhibition of this enzyme reduces the rate of complex carbohydrate digestion, thereby reducing the amount of glucose absorbed (Nair *et al.,* 2013) This result implies that the root extract of *Saccharum officinarum* MgONPs, as prepared in this study could be a potent antidiabetic pharmaceutical entity for the development of a safe drug. Furthermore, the inhibitory kinetics study of alpha-glucosidase showed that the particles inhibit the enzyme by noncompetitive inhibition. This implies that the particles inhibited the enzyme by binding to the enzyme site other than the active site and the enzyme-substrate complex. Furthermore, the particles have a strong affinity for the site of the enzyme other than the active site and do not interfere with substrate binding but prevent the catalytic step. The synthesis of environmentally friendly nanoparticles has become an effective and emerging treatment for diabetes mellitus and its associated sequelae and complications (Badmus *et al*., 2020). In summary, the inhibitory effect of sugarcane (*Saccharum officinarum*) root extract can be attributed to the synergistic effect of its rich content of functional and biologically active groups giving credence to its hypoglycemic nature. Timely recommendation of more in-depth work to be done in this area of research to substantiate and elucidate these findings is called for.

5. Conclusion

These arrays of unique bioactive potential could position *Saccharum officinarum* MgONPs as an agent for future biomedical application. Future in vivo studies and molecular studies will further establish its biomedical exploitation possibilities.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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