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Comparative Assessment of Antidiabetic Properties of Aqueous Extract and its Silver Nanoparticles from *Vernonia amygdalina* (ASTERACEAE)

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ABSTRACT

Background: Diabetes poses significant challenges globally, with modern drug costs and side effects often outweighing their benefits, leading to therapeutic failures. Natural sources, like Vernonia amygdalina (bitter-leaf), are being explored for their potential in diabetes management. This study used metformin as a benchmark to compare the anti-diabetic effects of aqueous extract and nano-particles from V. amygdalina.

Methods: The phytochemicals were analyzed quantitatively and qualitatively using standard methods. The anti-diabetic activities were assayed using glucose uptake by yeast method and the percentage of glucose uptakes by yeast was calculated.

Results: Phytochemical analysis identified saponins, tannins, alkaloids, phenols, and flavonoids in the plant, quantified as 0.156%, 0.013%, 0.022%, 0.003%, and 0.069%, respectively. Glucose uptake assays using yeast demonstrated enhanced uptake with silver nano-particles of the extract, particularly at 5mg/ml AgNPs, which exhibited 92.17% uptake compared to 53.66% for the aqueous extract and 80.79% for metformin, at a 100µg/ml glucose concentration. Statistical analysis (two-way ANOVA, $P<0.05$) confirmed the significance of results. The study indicated that silver nano-particles improved glucose utilization, potentially lowering blood glucose levels. Notably, nano-particles show superior anti-diabetic properties compared to the extract alone.

Conclusion: The research underscored the potential of green synthesis through nano-particle technology in enhancing the therapeutic efficacy of natural remedies for diabetes, offering a promising avenue for developing more effective and safer treatments.

Keywords: Diabetes mellitus, glucose uptake, nanoparticles, phytochemicals, yeast

1. INTRODUCTION

Diabetes mellitus (DM), a multifaceted metabolic disorder commonly referred to as diabetes, is characterized by hyperglycemia—an aberrant physiological state marked by persistently elevated blood glucose levels [1] Hyperglycemia stems from irregularities in either insulin secretion, insulin action, or both, presenting as chronic and heterogeneous disturbances in carbohydrate, fat, and protein metabolism. With profound implications for human health, diabetes exerts a debilitating impact worldwide. Recent data suggests an alarming escalation, with over 11.2 million reported cases in Nigeria, a figure projected to double by 2040 [2, 3]. The escalating prevalence of diabetes on a global scale has thrust it into the forefront of contemporary health challenges. This surge parallels rapid economic growth, urbanization, and the adoption of modern lifestyles across many regions. Estimates indicate a significant rise in global diabetes prevalence from 536.6 million people in 2021 to 783.2 million by 2045, emphasizing the urgency of addressing this burgeoning crisis [4]. In pursuit of viable solutions, ongoing research into natural remedies is underway, leveraging their perceived safety and freedom from the constraints associated with synthetic drugs. Traditional knowledge underscores the therapeutic potential of plants, which continues to inform modern medical practices. Several medicinal plants have demonstrated anti-diabetic properties, attributed to their rich content of phenolic compounds, flavonoids, terpenoids, alkaloids, and glycosides, which enhance insulin secretion and regulate blood glucose levels. Nigeria's favorable climatic conditions support the cultivation of numerous plants renowned for their anti-diabetic properties, including *Magnifera indica* L. (Mango), *Vernonia* amygdalina (Bitter leaf), *Aframomum melegueta* (Alligator pepper), *Syzygium aromaticum* (Cloves), *Azadirachta indica* (Neem), and *Momordica charantia* (Bitter melon). The advent of nanoparticle-based drug formulations presents promising avenues for addressing challenging diseases. Nanoparticles (NPs), typically ranging from 100

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to 500 nm, can be tailored to deliver drugs to specific tissues and offer controlled release therapy through manipulation of size, surface characteristics, and material composition. Their minute size and high surface area-to-

Figure 1; *Vernonia amygdalina* plant (live picture of plant from University of Uyo Pharmacy farm, 2023)

volume ratio confer enhanced solubility, bioavailability, and the ability to traverse physiological barriers such as the blood-brain barrier and pulmonary system, as well as endothelial tight junctions [5]. Studies conducted by [6] underscore the therapeutic potential of medicinal plants in Nigeria for treating diabetes, with a compilation of 132 plant species traditionally employed for this purpose. Notably, various plant parts—such as fruits, seeds, leaves, bulbs, and roots—have exhibited efficacy in managing the disease. Prominent among these are *Senna alata*, *amygdalina*, and *Allium* spp., showcasing their significant anti-diabetic properties. In recent years, the therapeutic landscape for diabetes has witnessed the introduction of novel glucose-lowering agents, including glucagon-like peptide-1 receptor (GLP-1R) agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors, and sodium/glucose cotransporter-2 (SGLT2) inhibitors, either as standalone treatments or in combination with established therapies like biguanides, sulfonylureas, and thiazolidinediones. While these agents offer potential benefits such as reduced risk of hypoglycemia and assistance with weight management, their long-term safety profiles remain under scrutiny. Metformin remains the cornerstone pharmacotherapy for type 2 diabetes mellitus (T2DM), with the utilization of other established agents such as sulfonylureas, meglitinides, pioglitazone, and α-glucosidase inhibitors varying across different regions [7]. *Vernonia amygdalina*, a member of the Asteraceae family, is a tropical African shrub recognized for its distinctive bitter taste, hence its colloquial name, bitter leaf. Despite its bitterness, which can be mitigated through boiling or soaking in multiple changes of water, this plant harbours a wealth of biologically active compounds. These include saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides, and sesquiterpenes, as documented by various studies [8, 9, 10]. Traditionally, the leaves of *Vernonia amygdalina* are utilized both as a culinary ingredient, incorporated into soups after maceration, and as aqueous extracts employed as tonics for medicinal purposes. Its applications in traditional medicine span a broad spectrum, including the treatment of emesis, nausea, diabetes, loss of appetite, dysentery, gastrointestinal tract issues, and even sexually transmitted diseases. These traditional uses find support in experimental research, providing scientific validation for many of the claimed health benefits. Phytochemicals such as saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes have been extracted and isolated from *Vernonia amygdalina. Vernonia amygdalina*, widely used in traditional African medicine, treats various ailments in humans and animals. Its leaves combat fevers, often replacing quinine in Nigeria (Masaba, 2000). Documented as a potent malaria remedy across Africa [11, 12, 13, 14], it also aids fertility [15] acts as a laxative, and treats parasitic infections like dysentery [16], helminthiasis [17] and typhoid fever [18]. It addresses diverse conditions including diabetes, inflammatory diseases, and cancer [19, 20, 21]. Its aqueous extract treats diabetes and gastrointestinal issues [22, 23] and possesses antimicrobial properties [24]. Moreover, it exhibits antioxidant, chemopreventive [25, 26], anthelmintic [27, 28]

and antibacterial [29] properties. This study therefore aimed to optimize the antidiabetic effects of Vernonia amygdalina through the utilization of aqueous extract nanoparticles as a novel drug delivery system.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Material: Leaves of *Vernonia amagdalina*

2.1.2 Equipment: Weighing balance (S. Mettler), UV-Visible spectrophotometer (Labon Med Inc.), Centrifuge, magnetic stirrer, 50ml beaker, glass rod, spatula, test tubes, conical flask, retort stand, 50ml burette, Virtis 2KBTXL-75 Bechtop SLC Freeze dryer.

2.1.3 Solvents, reagents and chemicals: Distilled water, 0.1N HCL, Folin-ciocalteu's reagent, 7% Na_2CO_3, 0.5M $NaNO_2$, 0.3M $AlCl_3.6H_2O$, 0.1M $FeCl_3$, 0.0088M $K_4Fe(CN)_6.3H_2O$, 0.1N NaOH, 30% Methanol, Baker's yeast, olive oil.

2.2 Methods

2.2.1 Collection, identification and sampling of the object of research, *Vernonia amygdalina*
The fresh and healthy leaves of the plant were collected from the Faculty of Pharmacy Garden, University of Uyo, Akwa Ibom state, Nigeria. The leaves were identified by Dr. Johnny Imoh of the Department of Pharmacognosy and Natural Medicines Herbarium, Faculty of Pharmacy, Faculty of Pharmacy, (Voucher No. 10(j)). They were washed with running tap water, rinsed with distilled water, cut into tiny pieces and dried under a shade at room temperature. The dried samples were stored in an air-tight container till further use.

2.2.2 Phytochemical screening
Chemical tests were carried out on the aqueous extract of the plant to identify the constituents using the standard procedures as described by [30].

2.2.2.1 Quantitative determination of saponins
Extract (0.5g) was dissolved in distilled water, vigorously shaken, and left to stand for one hour, during which the formation of stable foaming was observed. Subsequently, 1 mL of olive oil was introduced to 1 mL of the mixture and vigorously shaken until a cloudy appearance was achieved. The absorbance of the resulting solution was measured at 620 nm using a UV-Spectrophotometer. This procedure was performed in triplicate to ensure accuracy, and the total saponin content was quantified utilizing a standard graph for saponin concentration ($Y= 0.219X + 0.063$; $R^2=0.7460$).

2.2.2.2 Quantitative determination tannins
The process began with dissolving 0.5 g of the extract in distilled water, followed by filtration. Next, 1 mL of 0.1M $FeCl_3$ was introduced to 2.5 mL of the filtered solution, after which 0.3 mL of 0.0088M $K_4Fe(CN)_6.3H_2O$ was added. Subsequently, the absorbance was measured at 395 nm. The obtained result was then quantified in terms of tannic acid concentration (mg/ml) using a tannic acid graph ($Y = 0.917X + 0.398$; $R^2= 0.6195$).

2.2.2.3 Quantitative determination alkaloids
In a flask, 5 mL of 0.1N HCl was meticulously added to 5 mL of the supernatant, followed by thorough shaking for 3 minutes and subsequent settling. Subsequently, 5 mL of the lower layer was titrated with 0.1N NaOH until the color changed from red to yellow. The total alkaloid content was then determined, using the conversion factor: 1 mL of 0.1N HCl utilized in the titration = 0.0612 g of alkaloid.

2.2.2.4 Quantitative determination phenols
1 mL of Folin-ciocalteu's reagent was added to 1 mL of the plant extract (dissolved with distilled water) after which 2 mL of 7% Na_2CO_3 was added. 1 mL of distilled water was added after 5 minutes and mixed thoroughly. The mixture was incubated for 90 minutes at 25˚C. The absorbance was taken at 750 nm. The total phenol content was determined from standard curve of gallic acid solution ($Y = 0.213X + 0.6469$, $R^2 = 0.6469$) expressed as mg of Gallic Acid Equivalent (GAE) of dried sample. Folin-ciocalteu's reagent (1 mL) was added to 1 mL of the plant extract, previously dissolved in distilled water. Following this, 2 mL of a 7% Na2CO3 solution was introduced into the mixture. After a 5-minute incubation period, 1 mL of distilled water was added and thoroughly mixed. The

resulting solution was incubated for 90 minutes at 25°C. Absorbance readings were taken at 750 nm. The total phenol content was then determined using a standard curve derived from a gallic acid solution (Y = 0.213X + 0.6469, R^2 = 0.6469), expressed as milligrams of Gallic Acid Equivalent (GAE) of the dried sample.

2.2.2.5 Quantitative Determination of Flavonoids

Methanol (3.4 mL of 30%) was added to 0.3 mL of the extract followed by $NaNO_2$ (0.5M, 0.15%) and 0.5 mL of 0.3M $AlCl_3.6H_2O$ and mixed. 1 mL of 1M NaOH was added after 5 minutes and kept at room temperature for 30 minutes. The absorbance was taken at 586 nm using UV-Vis spectrophotometer. This procedure was repeated in triplicate and the total flavonoid was calculated using the standard graph of quercetin (Y = 0.296X + 0.053; R^2 = 0.6469). The result was expressed as quercetin equivalent (mg/ml).

2.2.3. Formulation of silver nanoparticles using the aqueous extract of *V. amygdalina*

The preparation process involved creating silver nitrate solutions at concentrations of 1 mg/mL, 2.5 mg/mL, and 5 mg/mL by dissolving the appropriate mass of silver nitrate in distilled water. Subsequently, 5 mL of each silver nitrate concentration was added to three separate beakers containing 5 mL, 3 mL, and 2 mL of the aqueous extract at a concentration of 100 mg/mL. The mixtures were continuously stirred using a magnetic stirrer assembly for 10 minutes to achieve dispersion of [Ag]+ ions. The resulting suspension of silver nanoparticles was then subjected to lyophilization using a Virtis 2KBTXL-75 Benchtop SLC Freeze Dryer [31].

2.2.4 Determination of glucose uptake by yeast [32]

2.2.4.1 Preparation of yeast solution

Baker's commercial yeast (20 g) was dissolved in 200 ml of distilled water and centrifuged (3,000 rpm for 5 minutes) until the supernatant was clear. A 10% v/v of the supernatant was prepared in distilled water.

2.2.4.2 Preparation of the aqueous extract of *Vernonia amygdalina* leaves

25.3 g of sliced *Vernonia amygdalina* leaves was soaked in 500 mL of distilled water and stirred for 30 minutes. The mixture was kept at room temperature for 72 hours followed by filtration through Whatman filter paper no.1.

2.2.4.4 Preparation of glucose

A stock solution of 1% w/v glucose solution was prepared by dissolving 1 g of glucose in 100 mL of distilled water.

2.2.4.5 Preparation of the standard (Metformin)

The drug, metformin (Brand name: Diabetmin by Hovid, Malaysia) was crushed in a mortar (1 tablet) and 1% w/v solution of the drug was prepared.

2.2.4.6 Estimation of yeast uptake

Various concentrations of the glucose solution (20, 40, 60, 80, and 100 µg/mL) were prepared from a one percent stock solution of glucose and distributed into different test tubes. Subsequently, 1 mL of each concentration was added to separate test tubes containing 4 mL of a 10%v/v suspension of yeast solution. After thorough vortexing, the mixtures were incubated in a dark cupboard at 37˚C for 60 minutes. Following this initial incubation, 1 mL of the extract was added to the test tubes containing the glucose and yeast solution mixture, and the contents were vortexed before further incubation at 37˚C for an additional 60 minutes. Similarly, 1 mL of the silver nanoparticles at different concentrations was added to separate test tubes containing the glucose and yeast solution mixture, followed by vortexing and incubation at 37˚C for 60 minutes. UV absorbance readings of the various solutions were then taken at 540 nm using a UV-visible spectrophotometer. These readings were compared to those obtained with a standard drug, metformin. The percentage increase in glucose uptake by yeast was calculated using a specific formula:

Increase in glucose uptake (%) = $\frac{ABSc-ABSs}{ABSc}$ x $\frac{100}{1}$

Where: ABSc = absorbance of the control reaction (containing all reagents except the test sample)
ABSs = absorbance of test sample

2.3 Statistical analysis

All results were expressed as mean ± SEM and were analyzed by two-way ANOVA using MS excel 2019. P <0.05 was taken as significant.

3.0 RESULTS

3.1 Phytochemical screening

The qualitative phytochemical analysis conducted on *Vernonia amygdalina* revealed saponins, tannins, alkaloids, phenols and flavonoids as major constituents.

Table 1: Quantitative estimates Phytochemicals present in aqueous extract of *V. amygdalina*

Phytochemical	Concentration (mg/g)	% Composition
Saponin	1.557 ± 0.01	0.156
Tannins	0.129 ± 0.03	0.013
Alkaloids	0.220 ± 0.02	0.022
Phenols	0.033 ± 0.02	0.003
Flavonoids	0.069 ± 0.01	0.069

3.2 Glucose uptake by yeast

Table 2: % increase of glucose uptake by yeast cells due to effect of the various samples

Conc. µg/mL	% Aqueous extract	1 mg/mL AgNPs	2.5 mg/mL AgNPs	5 mg/mL AgNPs	STANDARD (Metformin)
20	53.93	64.42	64.42	45.08	75.26
40	56.79	63.76	75.55	67.75	79.57
60	54.17	67.03	74.55	68.52	78.82
80	53.28	67.99	77.29	82.07	80.03
100	53.66	80.88	81.10	92.17	80.79

The absorbance of the negative control at the wavelength (540 nm) was very negligible and was ignored.

4. DISCUSSION

In yeast, glucose transport occurs via facilitated diffusion [33]. Table 2 illustrates the percentage increase in glucose uptake by yeast cells at various glucose concentrations in the presence of the plant's aqueous extract and different concentrations of its silver nanoparticles compared to the standard drug, metformin. Notably, a higher percentage increase in glucose uptake was observed in the presence of the silver nanoparticles compared to the aqueous extract alone, attributable to enhanced absorption of the silver nanoparticles by yeast cells. Consequently, there was a proportional rise in glucose absorption, akin to human cells. Statistical analysis via two-way ANOVA, with a significance level set at $P<0.05$, confirmed the significance of the findings. This study underscores a notable difference in the anti-diabetic activity between the aqueous leaf extract and nanoparticles of *V. amygdalina*, with the latter exhibiting superior anti-diabetic properties. The phytochemical screening of the crude aqueous extract of *V. amygdalina* revealed the presence of saponins, tannins, alkaloids, phenols, and flavonoids with respective compositions as shown in Table 1. Saponins were found to be the most abundant compound. The antidiabetic potential of medicinal plants and vitamins stems from their rich composition of phytochemicals such as flavonoids, polyphenols and alkaloids. These constituents work synergistically to reduce blood glucose levels effectively [34]. Scientific studies, including those by Malode *et al*. [35] and Ansari *et al*. [36], have underscored the ability of properly administered medicinal plants and vitamins to significantly lower fasting blood sugar levels in individuals with diabetes. Moreover, they aid in enhancing blood circulation, fostering wound healing, and mitigating diabetes-related complications. It is imperative to amplify awareness regarding the invaluable health benefits offered by medicinal plants and vitamins in both preventing and treating diabetes mellitus (DM), particularly in resource-constrained regions where access to modern medical interventions is limited due to financial constraints. By advocating for the integration of these cost-effective alternatives into healthcare practices, we can effectively address the needs of diabetic patients worldwide. Research findings have also demonstrated the remarkable efficacy of hypoglycemic herbs in regulating blood sugar levels through multifaceted mechanisms. These natural remedies have been shown to bolster insulin secretion, facilitating the transportation of glucose into adipose and muscle tissues, where it is efficiently utilized for energy production. Additionally, they exert inhibitory effects on glucose

absorption within the intestine, thereby attenuating postprandial spikes in blood glucose levels [37]. Furthermore, hypoglycemic herbs exhibit the unique ability to curb glucose production in the liver, thus contributing to the maintenance of stable blood sugar levels. The studies conducted by Turdu *et al*. [38] and Olawale *et al*. [39] corroborate these findings, providing valuable insights into the intricate pathways through which hypoglycemic herbs exert their therapeutic effects. Such holistic approaches to glycemic management offer promising avenues for individuals seeking natural and sustainable strategies to support their metabolic health. As awareness grows regarding the potential benefits of herbal interventions, further exploration and integration of these botanical agents into conventional diabetes management protocols hold significant promise for improving overall patient outcomes and quality of life.

Significance of the Research

This research has provided compelling evidence that the formulation of medicinal plant extracts into nanoparticles significantly enhances their pharmacological activities. This advancement holds immense promise in the realm of drug manufacturing and drug delivery systems. By leveraging nanotechnology to encapsulate bioactive compounds extracted from plants, scientists can amplify their therapeutic potential, leading to more effective treatments for various ailments. Furthermore, the utilization of nanoparticle-based delivery systems facilitates targeted and controlled release of these potent compounds, enhancing their bioavailability and minimizing potential side effects. Thus, this innovative approach not only optimizes the efficacy of traditional herbal remedies but also paves the way for the development of novel therapeutics with improved safety and efficacy profiles.

5. CONCLUSION

This study yielded compelling findings indicating that the silver nanoparticles derived from the aqueous extract of *V. amygdalina* demonstrate superior anti-diabetic properties compared to the plant's plain aqueous extract. Furthermore, it elucidated that both the aqueous extract of *V. amygdalina* and its silver nanoparticles exhibit notable anti-diabetic activity. However, the enhanced efficacy observed with the silver nanoparticles underscores their potential as a more potent therapeutic agent for managing diabetes. These results not only validate the traditional medicinal use of *V. amygdalina* but also highlight the significant advancement achieved through nanotechnology in augmenting the plant's therapeutic effects. Such insights offer valuable contributions to the development of innovative treatments for diabetes and underscore the potential of nanoparticle-based formulations in enhancing the efficacy of herbal remedies for various health conditions.

Author Contributions
Conceptualization, ECJ. Methodology, CGE, software, ECJ.;. Formal analysis, ECJ and CGE. Investigation, CGE, resources, ECJ.; writing—original draft preparation, ECJ.; writing—review and editing, ECJ and CGE. supervision, ECJ; project administration, ECJ.

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