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In-vitro Antioxidant and Alpha-Glucosidase Inhibitory Activities of Ethanolic Extracts of Aerial Part Extracts of *Gymnema sylvestre*

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ABSTRACT

Gymnema *sylvestre* is a medicinal woody climber that has been used in ethnomedicine for the treatment of stomach ailments, constipation, liver diseases. Similarly employed in the treatment of hypertension, tachycardia, diabetes and oxidative stress. This work investigated the antioxidant and α-glucosidase inhibitory activity of G. *sylvestre* aerial part extracts. Ethanol was used to macerate the powdered aerial portion, and the resulting crude extract was then separated into several fractions. Evaluation of the fractions for α-glucosidase inhibition and antioxidant activity was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and α-glucosidase by spectrophotometer. The ethanol and chloroform fractions demonstrate α-glucosidase inhibition with IC_{50} values of 618.7± 27µg/ml and 37.73± 0.38. µg/ml, respectively, and an IC_{50} value of 20.07 ± 19.75µg/ml and 91.65 ± 32.97µg/ml. In contrast, the aqueous and ethyl acetate fractions showed considerable α-glucosidase inhibition, with respective IC_{50} values of 116.47± 13.65 µg/ml and 97.46± 16.09 µg/ml. Based on the findings, it was proposed that the aerial portion of Gymnema sylvestre might contain substances with mild α-glucosidase inhibitory effects.

Keywords: *Gymnema sylvestre*, alpha-glucosidase inhibition, anti-oxidant, diabetes

INTRODUCTION

Gymnema *sylvestre* is a medicinal woody climber (Kanetkar, P *et al.,* 2007). In traditional medicine, it has been used to treat constipation, stomach problems, and liver illnesses. As an antioxidant and to lower serum cholesterol, it is also used to treat diabetes, hypertension, and tachycardia (Thakur *et al*., 2012). Locally, this plant has been utilized to treat cancer, diabetes mellitus, obesity, eye disorders, asthma, and antihyperlipidemia (Ibrahim *et al*., 2016; Chen and Guo, 2017). Several active phytochemicals have been found in the aerial part of G. sylvestre, which include Gymnemagenin, Gymnestrogenin, Hexadecanoic acid, 3-Allyl-2-methoxy phenol, 4-(1-Pentyl-heptyl) benzene sulfonic acid methyl ester, Gymnemic acid, and Gymnemanol (Anjum & Hasan, 2013, Laha & Paul, 2019, Tiwari *et al*., 2014). Cyclophenanthren and Gymnemoside E were also reported (Kumar & Jafar, 2017).

Gymnemic acid can block the absorption of glucose by attaching itself to intestinal epithelial receptors and the mouth's sweet taste buds (Phanjaroen *et al*., 2024). In 2019, hyperglycemia was ranked as the eighth most common cause of death and disability globally, and in 2021, it was projected that 529 million people would have diabetes. By 2050, there could be roughly 1.3 billion people with diabetes (Ong *et al*., 2023). Approximately 3% of people worldwide have diabetes, according to Tahseen *et al*. (2021), and by 2025, the prevalence is predicted to double, or reach 6.3%. According to Nganso *et al*. (2024), a diabetic dies every eight seconds worldwide.

A reduction in insulin excretion, action or both can lead to hyperglycemia, which is a defining characteristic of diabetes (Ghous *et*

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al., 2021). Long-term hyperglycemia damages and fails many organs, especially the heart, kidneys, blood vessels, eyes, and nerves. The onset and advancement of complications related to diabetes are caused by the body's heightened production of free radicals (oxidative stress) and weak antioxidant defense (Ghous *et al*., 2021).

Lipid peroxide buildup is a sign of elevated oxidative stress, which is linked to diabetes (Ibrahim *et al*., 2017). When used by diabetic patients, antioxidants can help maintain free radical level in the body and treat diabetic complications (Tahseen *et al*., 2021; Ghous *et al*., 2021). The inhibition of gastrointestinal tract carbohydrate-digesting enzymes, such as α-glucosidase and α-amylase, is one of the key strategies used to treat diabetes. The enzymes inhibition slow intestinal glucose absorption, which lowers postprandial blood glucose levels (Tahseen *et al*., 2021).

The α-glucosidase and α-amylase enzymes are targets for drug design in the treatment of diabetes (Tahseen *et al*., 2021). Miglitol and acarbose are common medications used to treat diabetes mellitus (Ibrahim *et al*., 2017). Because of its complexity, the side effects of the drugs that are available, and the strain on the healthcare system, managing diabetes has become difficult (Ong *et al*., 2023). This prompted the need of alternative effective medication with no side effect. In impoverished nations where the majority of people lack access to modern therapy, plants have traditionally been used to treat diabetes (Tahseen *et al*., 2021). This stems from the finding that certain medicinally effective plants, such as G. sylvestre, have demonstrated promise in treating diabetes Meletus (Zaharaddeen *et al*., 2022). Evidence from its significant phytochemical composition and traditional therapeutic applications. The phytochemical components of G. sylvestre leaf extracts, including flavonoids, steroids, quinones, phenols, triterpenoids, tannins, alkaloids, and saponins, may be thought to be responsible for the extract's possible effects (Tahseen *et al*., 2021).

Because plants are less harmful and safer than synthetic pharmaceuticals, herbal remedies have become more and more popular worldwide (Tahseen *et al*., 2021). Drug development heavily relies on natural products, which account for half of the new medications (Tahseen *et al*., 2021). Research on complementary and alternative therapies has demonstrated that natural medications can effectively treat hyperglycemia without causing negative side effects (Phanjaroen *et al*., 2024). The Phytochemical substances in functional foods scavenging free radicals in the body, preventing oxidation of biomolecules as such reducing oxidative stress (Phanjaroen *et al.,* 2024). The G. sylvestre leaves ethyl acetate extract afforded α-glucosidase and α-amylase inhibitory IC_{50} values of 130.77±3.15 mg/ml and170.45±13.61mg/ml respectively. The extract afforded DPPH, hydroxyl and hydrogen peroxide scavenging percentage of (53.87±17.12%), 28.10±14.35% and 34.26±20.12% respectively (Ibrahim *et al*., 2016). The n-hexane, ethyl acetate, and chloroform extracts from G. *sylvestre* leaves afforded an IC_{50} value of 170.2, 44.4, 131.6 µg/ml, respectively in α- glucosidase inhibition (Ghous *et al*., 2021). This research aim was to investigate the antioxidant and α-glucosidase inhibition property of G. *sylvestre* aerial part extracts.

MATERIALS AND METHODS

Plant Collection and Identification

The aerial part of G. *sylvestre* was collected from Zangon Danborno Sabon Gari Zaria, Kaduna State. Latitude: 11° 06'60.00" N Longitude: 7° 43'59.99" (8.567843°). The collected sample was authenticated by herbarium curator in the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria with voucher number 179.

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Extraction of Bioactive Compounds

Extraction was conducted according to the procedures of Mudi *et al*., (2010) and Thiantongin. (2014) with slight modification. The samples were washed with running tap water to remove soil debris. cut into smaller pieces and air-dried at room temperature. The 700g of air-dried G. sylvestre aerial parts samples were ground into a powder, and precisely 300g of that powder was macerated with 2.5 liters of ethanol. The filtrate was then concentrated using a rotary evaporator at 40 degrees Celsius under reduced pressure. The crude ethanol extract was then subjected to liquid-liquid partitioning using n-hexane, chloroform, and ethyl acetate in a 2:1 v/v ratio, each of which produced the different fractions.

Antioxidant Activity Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging activity

With minor modifications, the DPPH test technique of Ionita (2003), Mariko *et al*. (2016), and Jain *et al*. (2012) was used to measure the extract's antioxidant activity. Briefly, 195 µl of DPPH solution (0.1 mM) was added to the plate well in triplicate, after which 50 µl of each of the extract's concentrations—1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, and 10 µg/ml, respectively—were added. The absorbance at 517 nm was measured following a 30-minute dark incubation period at room temperature. Ethanol served as the blank and ascorbic acid as the standard.

The capability to scavenge the DPPH radicals was calculated using the following equation:

$$\text{DPPH free radical scavenging (\%)} = \frac{(AO-A1)}{AO} X\ 100 \text{ ..... (1)}$$

A0 = absorbance of blank sample

A1= absorbance of sample

In order to obtain IC_{50}, a graph of inhibition rate against the sample concentration was plotted.

Alpha-Glucosidase Inhibition Assay

With minor adjustments, the approach outlined by Fettach *et al*. (2022) and Kazeem *et al*. (2013) was used to determine the extract α-glucosidase enzyme inhibition. In summary, 50 µl of each extract concentration (30, 60, 120, and 240 µg/mL) was pre-incubated for 10 minutes in triplicate with precisely 100 µl of α-glucosidase from (Saccharomyces cerevisiae) (1.0 U/ml). Fifty microliters of 3.0 mM pNPG substrate were added to initiate the reaction. After 20 minutes of incubation at 37 ºC, the reaction was stopped by adding 2 milliliters of 0.1M Na_2CO_3. The yellow para-nitrophenol that was produced from pNPG at 405 nm was used to measure the α-glucosidase activity.

The extract ability to inhibited the enzymes was calculated using the following equation:

$$\% \text{ Inhibition } = \frac{(\text{Ac} - \text{Ae})}{\text{Ac}} X\ 100 \text{.....} (1)$$

where Ac and Ae are the absorbance of the control and extract, respectively. The (IC_{50}) was determined from a plot of inhibition rate against sample concentration using Microsoft Excel

RESULTS

Antioxidant activity

G. *sylvestre* aerial parts extracts antioxidant activity is shown in Fig. 1. and Table 1. The ethanol extract afforded the highest antioxidant activity of (20.07 ± 19.79 g/ml) followed sequentially by chloroform and ethyl acetate with 91.65± 32.97 and 163.26 ± 18.72 respectively. (44.59 ± 9.15 g/ml) then

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the aqueous extract afforded the lowest antioxidant (table1.). The extracts' antioxidant activity increased with an increase in concentrations of the extract's figure 1.

The crude extract and fractions were evaluated for their DPPH scavenging antioxidant activity.

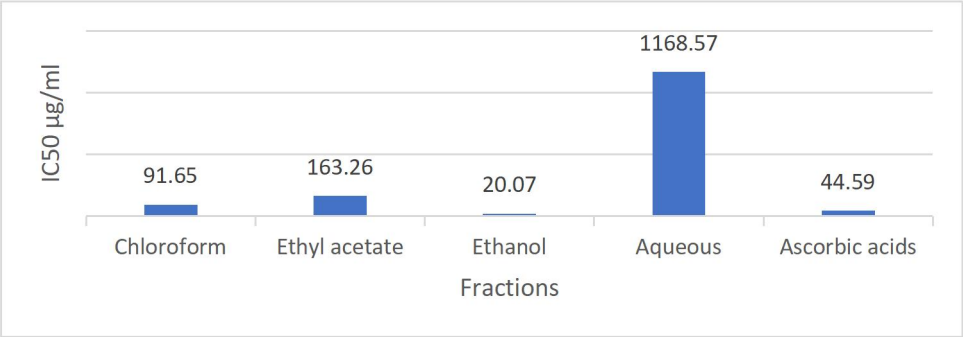

Figure 1: DPPH radical scavenging activity (IC_{50}) of aerial part extract of *G. sylvestre*.

Table 1: Antioxidant effect of the *G. sylvestre* extracts and fractions.

Extract	IC_{50} (µg/ml)
Chloroform	91.65 ± 32.97
Ethyl acetate	163.26 ± 18.72
Ethanol	20.07± 19.75
Aqueous	1168.57 ± 47
Ascorbic acid	44.59 ± 9.15

α- glucosidase inhibition activity

The ability of G. sylvestre aerial part extract to inhibit α- glucosidase activity in vitro was investigated and the result is presented in fig.2: and Table 2. The results revealed chloroform extract had the highest inhibition at 37.73 ± 0.38 µg/ml) fig.2 and the positive standard Acarbose with (64.13 ± 2.52 µg/ml) Table 2. The extracts' show the enzymes % inhibition in concentration dependent manner fig. 4.

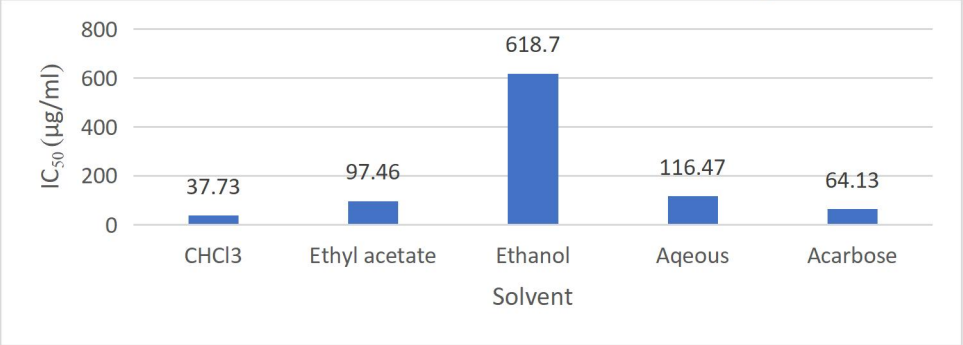

Figure 2: α-glucosidase inhibitory activity (IC_{50}) of stem bark extract and fraction of G. *sylvestre*

Table 2: α-glucosidase Inhibitory Activity of the G. sylvestre ethanol extract and fractions.

Extract	**IC_{50} (µg/ml)**
Chloroform	37.73 ± 0.38
Ethyl acetate	97.46 ± 16.09
Ethanol	618.7 ± 27
Aqueous	116.47 ± 13.65
Acarbose	64.13 ± 2.52

Percentage Inhibition of the DPPH free radical and α-Glucosidase enzyme

In order to examine the free radical scavenging, and α-glucosidase inhibitory activity of the extract and its fractions, their activity was measured at the different concentration.

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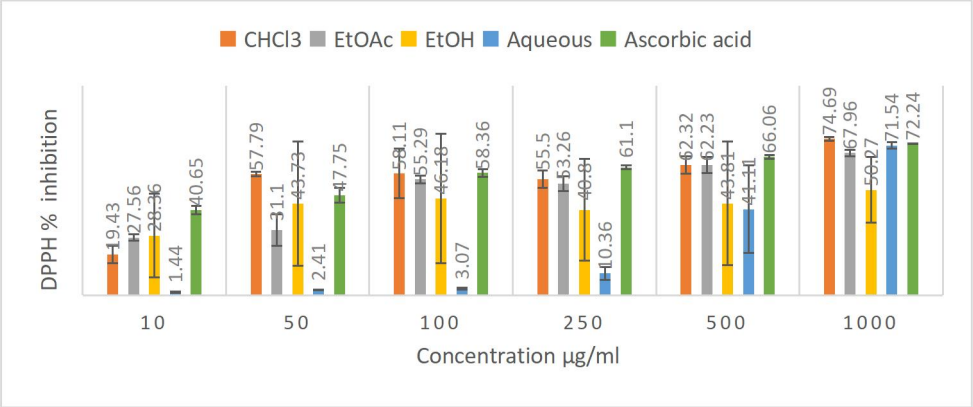

Figure 3: DPPH radical scavenging activity (%) of aerial part extracts of *G. sylvestre.*

The different solvent fractions DPPH radical scavenging activity (%) ranges from 1.44 µg/ml to 74.69 µg/ml fig 3. All the various solvents fractions showed a concentration dependent α-glucosidase % inhibition fig 4.

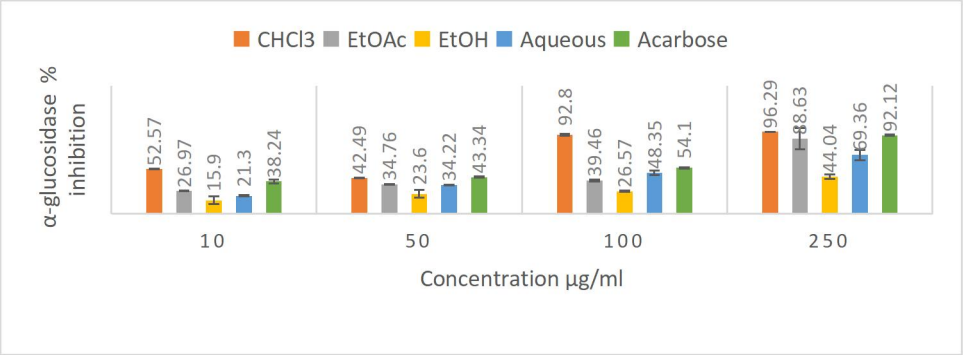

Figure 4: α-glucosidase inhibitory activity (%) of aerial part extracts of *G. sylvestre.*

The different solvent fractions α-glucosidase inhibitory activity (%) ranges from 15.9 µg/ml to 96.29 µg/ml fig 3. All the various solvents fractions showed a concentration dependent α-glucosidase % inhibition fig 4.

DISCUSSION

In this study, the *aerial part of G. sylvestre* was extracted with ethanol, the crude extract was fractionated using solvent-solvent partitioning with distilled water, petroleum ether, chloroform, and ethyl acetate. The fractions were screened for antioxidant and α-glucosidase inhibitory activity. Oxidative stress is significantly elevated in prolonged, unbroken hyperglycemia because of improved glycation, changes in polyol pathway activity, and an increase in free radical generation that precedes glucose auto-oxidation. Free radicals can cause damage to β-cells, and an imbalance in oxidation homeostasis leads to insulin resistance, which is a risk factor for type 2 diabetes. One strategy for preventing subsequent diabetic complications could be an antioxidant therapeutic approach that specifically targets the oxidative stress mechanisms brought on by diabetes (Abdul *et al*., 2021).

Findings of this work correlates well with those of earlier studies conducted using G. sylvestre aerial part extracts to scavenge DPPH radical and inhibited α-glucosidase enzymes. Phytochemical compounds have been shown to demonstrate significant antidiabetic properties. Gymnemic acid

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isolated from G. *sylvestre* inhibited glucose absorption by binding to the mouths sweet taste buds and intestinal epithelium receptors showing antihyperglycemic effect (Phanjaroen *et al*., 2024). Ghous *et al*., (2021) the methanol, acetone and aqueous extracts G. sylvestre leaf demonstrated maximal DPPH percentage scavenging activity of 83, 83, and 75%, respectively. According to Ibrahim *et al*. (2017), the ethyl acetate extract of G. *sylvestre* leaves demonstrated 53.87±17.12%, 34.26±20.12%, and 28.10±14.35% DPPH, H_2O_2, and OH radical scavenging ability, respectively. The extract also provided IC_{50} values of 130.77±3.15 and 170.45±13.61 mg/ml for α-glucosidase and α-amylase inhibition activity. Both the ethanol and aqueous extracts of G. sylvestre leaves had IC_{50} values of 65.2 ±1.9 µg/ml and 82.6 ± 2.1 µg/ml, respectively, which demonstrated effective inhibition of α-glucosidase (Venkata *et al*., 2023), demonstrating the plant extract's potential for anti-diabetic activity

Guilin and Mangana (2017) reported that extracts of G. sylvestre leaves had a significant inhibitory activity against α-glucosidase, with an IC_{50} of 68.70 ± 1.22 µg/ml compared to acarbose (positive control) at 59.03 ± 2.30 µg/mL.. The aqueous and ethanol extracts of G. sylvestre leaves inhibited α-glucosidase with 79.3±2.0 µg/mL and 63.2±1.6 µg/mL, respectively (Venkata *et al*., 223). This further demonstrated the plant extract's potential for anti-diabetic activity. n-hexane, ethyl acetate, and chloroform extracts of G. sylvestre leaves were reported by Ghous *et al*., (2021) to have α-glucosidase inhibitory capacity with IC_{50} values of 170.2, 44.4, and 131.6 µg/ml, respectively. Gymnemagenin a G. sylvestre isolated compound inhibited α-glucosidase and α-amylase enzymes' with IC_{50} values of 1.17±0.02 mg/ml and 2.04±0.17 mg/ml, respectively. This compound inhibited the enzymes in a concentration-dependent manner (Javed *et al*., 2023). The present research and various reported literature G. *sylvestre* extract α-glucosidase inhibition indicated the presence in G. *sylvestre,* compounds with varied α-glucosidase inhibition activity.

Rashmi *et al*, (2018) reported G. *sylvestre* leaves extracts -glucosidase inhibition % of 52.2 ± 0.50, 97.24 ± 0.24, 80.34 ± 0.63, 7.1 ± 0.05 and 75.24 ± 0.40 for methanol, ethanol, acetone, ethyl acetate and chloroform respectively. A triterpene glycoside fraction isolated and purified from ethanolic extract of Gymnema sylvestre exhibited effective inhibition of -glucosidase IC_{50} values 3.16 ± 0.05 g/mL Rashmi *et al*, (2018). The ethanol and chloroform fractions exhibited high antioxidant properties compared to other extracts. These extracts expressed their capacity to be used as target antioxidant therapy for diabetes-induced oxidative stress. The ethyl acetate with moderate antioxidant and non-excessive α-glucosidase inhibition. Therefore, natural antioxidant and glucosidase inhibitors from the medicinal plants can be used as an effective therapy for treating post prandial hyperglycemia, evident from the extracts DPPH radical scavenging and glucosidase inhibition potential range revealed by this study.

CONCLUSION

The study revealed the ethanol crude extract of the G. *sylvestre* containing components with a range of α-glucosidase inhibitory potential and antioxidant properties. The aqueous, chloroform and ethyl acetate fraction shows low, moderate and high capability in both α- glucosidase inhibition and DPPH scavenging activity respectively. The study also revealed the potentiality of the G. *sylvestre* aerial part antidiabetic compounds to have non-excessive α-glucosidase inhibition with no or fewer side effects. Further research is needed to isolate and characterize the bioactive compound responsible for the observed activities.

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