**Analysis of the Phytochemical Composition and Antidiabetic potential of *Anthocleista grandiflora* Ethanol Wood Bark extract in rats.**

**ABSTRACT**

Diabetes mellitus is one of the major global health issues characterized by hyperglycemia and insulin shortage or cellular insulin resistance. This study’s aim was to analyze the phytochemical composition and anti- diabetic potential of the ethanol bark extract of *A. grandiflora* in rats. The phytochemical analysis, as well as glucose concentrations were determined using standard methods. Twenty albino rats were randomly divided into five (5) groups of four rats each in which group 1 was the normal control, group 2 was induced with diabetes and not treated, group 3 was induced and treated with glibenclamide, group 4 and 5 were induced treated with the extract (100 and 200 mg/kg respectively) all for 12 days and blood glucose concentrations were measured on three days interval using a glucometer by cutting the tip of the tail. Results shows the presence of Alkaloids, steroids, carbohydrates, tannins and saponins while terpenes, anthraquinones, flavonoids and cardiac glycosides were absent. The detected phytochemicals were further quantified and the outcome showed a significantly (p<0.05) high amounts of alkaloids (496 ± 1.89), saponins (420.6 ± 2.5), steroids (408.2 ± 1.89) followed by phenols (396.7 ± 1.67), steroids (308.2 ± 1.89), Anthocyanosides (273.3 ± 1.53), Phlobatamins (251.5 ± 2.03) and tannins (228.9 ± 2.67 mg/ 100ml). Furthermore, There was significant (p < 0.05) reduction of serum glucose across the treated groups while (group 2) showed a sustained diabetic status in all rats, confirming the anti-diabetic properties of the ethanol extract.

**Keywords;** *Anthocleista grandiflora,*Diabetes, Hyperglycemia, Medicinal plants, Glucose

**1.0 INTRODUCTION**

The hallmark of diabetes mellitus, a chronic condition, is hyperglycemia caused by decreased insulin production or insulin resistance, which inhibits the body's ability to respond appropriately to insulin (Zhang et al., 2020).As a result, blood glucose levels rise, a condition known as hyperglycemia. Hyperglycemia is one of the major health problems that has been discovered globally, and its prevalence in adults is steadily increasing (6.4%) (Sheriff et al., 2019).According to Huang et al. (2020), 425 million individuals worldwide have diabetes mellitus in 2017, and 629 million are expected to have the condition by 2045.There is proof that diabetes mellitus is becoming more common over time. Africa is predicted to experience the greatest increase in the number of people with diabetes when compared to other continents to other continents. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. Between 2000 and 2019, there was a 3% increase in diabetes mortality rates by age. In 2019, diabetes and kidney disease due to diabetes caused an estimated 2 million deaths. Diabetes also causes hyperglycemia which has shown induced oxidative stress associated with many complications. The anti diabetic properties of A. grandiflora have been studied in a number of animal and cell- based studies (Abiola et al., 2017) Antioxidant therapy has been of great interest to combat this cause in the past few decades due to the potential of the presence of flavonoid, phenolic compounds to neutralize them.

Diabetes is a chronic illness with preventable but potentially fatal consequences. It is characterised by elevated blood glucose levels brought on by impairments in either the action or synthesis of insulin, or both. Type 2 diabetes affected 15.1 million persons globally in 2000. Between 1980 and 2014, the number of individuals with diabetes increased from 108 million to 422 million.Compared to highincome countries, prevalence has been increasing more quickly in low and middleincome countries.Globally, 36.6 million individuals are expected to have diabetes by 2030 (WHO, 2024).

Diabetes mellitus is a dangerous metabolic disease that is significantly aided by medicinal plants. According to reports, traditional plants have strong antidiabetic actions without any negative side effects.They are abundant in antidiabetic substances such flavonoids, alkaloids, phenolic, and tannins, which enhance the function of pancreatic tissues by either boosting insulin secretion or reducing intestinal glucose absorption.Glycolysis, gluconeogenesis, Krebs cycle, glycogen synthesis and its breakdown, insulin synthesis and release, cholesterol synthesis, carbohydrate metabolism, and absorption are among the metabolic pathways that have been demonstrated to be modulated by a number of medicinal plant extracts (Prabhakar and Doble 2008).The majority of the antidiabetic classes on the market are synthetic, expensive, and have a long list of adverse effects (Nasri et al., 2013). Nevertheless, the therapeutic potential of natural remedies should be explored in the development of novel classes of hypoglycemic drugs.

Plant medicine is the oldest and most widely used form of medicine that humans have ever encountered. Herbs are the main source of medicine for 85% of the world's population (WHO, 2013). Plants have long been used as a very important source of drugs to treat a wide range of diseases and conditions (Roja et al., 2000). Herbal medicine has expanded rapidly in recent years, and both developed and developing countries are increasingly using these drugs because of their natural origins and few adverse effects. Many of the traditional medicines that are currently in use come from medicinal plants, minerals, and organic components (Sajeth et al., 2011). The World Health Organization has compiled a list of 21,000 plants that are used medicinally worldwide (Maurya *et al.,* 2011). The antioxidant potential of medicinal plants is associated with the presence of phenolic, flavonoid, alkaloid, and terpenoid compounds that readily donate hydrogen atoms to the radicals to neutralise them. These plants are enriched with phytochemicals like tannins, saponins, flavonoids, essential oils, and alkaloids that appear to have therapeutic properties and are used in the traditional medical system to manage various ailments (Ashikaa et al., 2022).

In Nigerian traditional medicine, A. grandiflora, also known as the cabbage tree, forest big-leaf, or forest fever tree, is a common medicinal plant used to cure a range of ailments (Kalu et a., 2018).The dicot genus Anthocleista (Gentianaceae) contains over fifty species worldwide, with fourteen species found in tropical Africa, including Comoros, Madagascar, and Mascarene Island. The majority of these species are trees and shrubs with woody stems (Jimoh et al., 2017). Laxatives, syphilis, diabetes, stomachaches, typhoid fever, malaria fever, hypertension, purgatives, and wounds are just a few of the ailments they have historically been used to treat (Haruna et al., 2022).When alloxan is administered or injected into animals, it can result in alloxan-induced diabetes, a type of insulin-dependent diabetes mellitus (Dunn et al., 2010). Numerous animal species, including rabbits, mice, rats, monkeys, cats, and dogs, have been successfully induced to exhibit it.Several routes (intraperitoneal, intravenous, and subcutaneous) have been used to provide alloxan in single or multiple doses; the most common method appears to be intraperitoneal administration. Because of its strong attraction for water, the chemical molecule alloxan, which is based on a pyrimidine heterocyclic skeleton, can cause diabetes in experimental mice (Offor et al., 2017). Given the aforementioned, ongoing research is required to develop better medications for the management and treatment of diabetes; hence this research was aimed at assessing the anti-diabetic potential of *Anthocleista grandiflora* ethanol bark extract in Wistar rats.

Medicinal plants can create compounds that could be used to treat diabetes, according to Abbas et al. (2019). These compounds function as inhibitors of α-glucosidase, dipeptidyl peptidase-4, and SGLT2. In their review, Jacob and Narendhirakannan (2019) listed 81 medicinal plants that are thought to have anti-diabetic, anti-hyperglycemic, and hypoglycemic effects. Gupta (2018) listed active phytoconstituents that were extracted from 22 powerful anti-diabetic plants in another review. He also stated the plant sections that included the active compounds that might be helpful in the development of new drugs.

**2.0 MATERIALS AND METHODS**

**2.1 Materials**

**2.1.1 Plant material**

Fresh bark of *A. grandiflora* was gotten from a village in Kaena LGA, Nasarawa state, by the help of a local farmer. It was transported using a poly bag to the laboratory in the department of Biochemistry and molecular biology, Nasarawa state university, Keffi.

**2.1.2 Experimental animals**

The experimental animals used were Wister rats of both sexes weighing between 100-200g. They were obtained from the department of Zoology, university of Jos, Plateau state, Nigeria and transported in cages to the animal house in the department of Biochemistry and molecular biology, Nasarawa state university, keffi. They were acclimatized for seven (7) days before commencement of the experiment.

**2.1.3 Instrument/Equipment’s**

The instrument and equipment used in the study include:

Water bath (Dk-420, WOM), Spectrophotometer (752N, China), Rotatory Evaporator (RE300, China), AcuCheck Glucometer, Analytical balance (PA214, OHAUS corporation, USA), Weigh balance (G&G Electronic Scale, capacity 300g×0.01g).

**2.1.4 Chemicals/Reagents**

All the chemicals/reagents used were of analytical grade and products of Sigma Aldrich (USA) and BDH (UK).

**2.2 Methods**

**3.2.1 Processing of plant material**

The fresh bark of *A. grandiflora* was dried at room temperature in the laboratory. After which it was grounded to coarse powder with the aid of mortar and pestle, and also an electronic blender till a fine powdered sample was obtained.

**2.2.2 Extraction of the plant material**

The powdered sample (400g) was macerated in 2 liters of ethanol for 72hrs with occasional stirring to facilitate the extraction process. The mixture was then filtered using muslin cloth followed by what man no.4 filter paper and the filtrate concentrated using rotatory evaporator to get the crude extract at 65˚C and stored in containers at about 4˚C until use.

**2.2.3 Induction of diabetes with Alloxan**

The animals were given a single intraperitoneal injection of 80mg/kg of alloxan monohydrate in isotonic saline and allowed to stabilize for 3 days before the glucose levels were measured on three days interval for twelve (12) days. The glucose level were first measured to establish a baseline (Day 0) before induction and subsequent glucose measurements (Days 1, 4, 9 and 12).

**2.3 Qualitative phytochemical analysis**

The phytochemical analysis was carried out on the ethanol bark extracts of *A. grandiflora* using the method of Trease and Evans (2002). The presence or absence of a particular phytochemical compound involved the addition of appropriate standard chemicals/reagents in appropriate sequence to the sample. The following classes of phytochemicals were screened: Carbohydrates, tannins, Phlobatannins, saponins, flavonoids, alkaloids, anthraquinones, anthocyanosides, steroids, terpenoids, and glycosides.

***Test for carbohydrates***: 1ml Molisch reagent (a solution of α-naphthol in ethanol) was added to 2ml of the sample and few drops of concentrated sulfuric acid was slowly dripped and the resulted solution shaken carefully. The appearance of a violent ring at the interface of two liquids indicates the presence of carbohydrates.

***Test for tannins:***To 1 ml sample, 2ml of 5% ferric chloride was added and observed for a dark-blue or greenish – black colouration indicate the presence of tannins.

***Test for phlobatannins:*** Few drops of diluted HCl (1%) was added to 1ml sample and observed for a red precipitate.

***Test for saponins:*** 2ml of distilled water was added to 2ml of sample and shaken for 15 min in a graduated cylinder. Formation of 1 cm foam layer indicates the presence of saponins.

***Test for flavonoids:***1ml of 2N sodium hydroxide was added to 2ml of sample. Appearance of yellow colour indicates the presence of flavonoids.

***Test for alkaloids:*** 2ml of sample and 2ml Hagers reagent (a saturated aqueous solution of picric acid) are mixed together and a yellow precipitate indicates a positive test.

***Test for steroids:*** To 1 ml of sample, 10ml chloroform was added and then 10ml sulfuric acid is slowly dripped. A positive result indicates upper layer turns red and sulphuric acid layer turns yellow- green.

***Test for terpenoids:*** 2ml of chloroform was added to 1ml sample. Few drops of concentrated sulfuric acid is slowly added to solution and observed for a reddish brown precipitate.

***Test for glycosides:***2ml of sample reacts upon boiling with 2ml H2SO4. The solution is filtered, and equal volumes of chloroform was added and shaken vigorously, and two layers can be clearly observed. The organic layer is separated, and ammonia was added to form a pinkish – red colour as a sign of positive reaction.

**2.4 Quantitative phytochemical analysis**

This was done according to the method of M Madhu et al., (2016) as outlined thus;

*Estimation of Alkaloids***:** To 1ml of test sample, 5ml pH 4.7 phosphate Buffer was added and 5ml BCG solution and shake a mixture with 4ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust volume with chloroform. The concentration of the complex in chloroform was measured at 470nm.

*Quantitative estimation of flavonoids***:** 1ml of test sample and 4ml of water were added to a volumetric flask (10ml volume). After 5 min, 0.3ml of 5% sodium nitrite, 0.3 ml of 10% aluminum chloride was added. After 6 min incubation at room temperature, 2ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10ml with distilled water. The concentration of the reaction mixture was measured at 510nm spectrophotometrically.

*Quantitative estimation of Saponins:*Test sample was dissolved in 80% methanol. 2ml of vanillin in ethanol was added, mixed well and the 2ml of72% sulphuric acid solution was added, mixed well and heated on a water bath at 60°c for 10 min. Concentration was measured at 544nm against blank.

*Quantitative estimation of Steroids:*1ml of test extract of steroid solution was transferred into 10ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated in a water bath for 30 minutes with occasional shaking and diluted to the mark with distilled water. The concentration was measured at 780nm.

*Quantitative Estimation of Phenols***:** Thesample was mixed with 0.4ml follin-ciocalteu’s reagent (diluted 1:10 v/v). After 5 min, 4 ml of sodium carbonate solution was added. The final volume of the tubes were made up to 10ml with distilled water and allowed to stand for 90min at room temperature. Concentration of sample was measured at 750nm using a spectrophotometer.

**2.5 Experimental design for *in vivo* studies**

A total of twenty (20) rats were randomly grouped into 5 different groups and treated thus:

* Group 1: Control
* Group 2: induced but not treated
* Group 3: induced and treated with standard drug (5mg/kg-1 glibenclamide)
* Group 4: induced and treated with 100mg/kg-1 of the ethanol extract
* Group 5: induced and treated with 200mg/kg-1 of the ethanol extract

**2.5.1 Determination of blood glucose**

The blood sample was obtained from the tip of the rat tail and was collected on a reagent strip to determine the blood glucose level using a glucometer (Accu check inc. California, USA). The glucose levels were taken before the commencement of treatment and at 3 day intervals to determine the response to treatment. Glibenclamide (5mg/kg) was used as reference standard. Treatments were administered via oral route.

**2.6 Statistical analysis**

The data obtained were analyzed using analysis of variance (ANOVA) in SPSS version 23.0. The result was presented as mean ± standard deviations. The level of significance was further tested using LSD and Duncan. The acceptable level of significance was set at p < 0.05.

**3.0 RESULTS AND DISCUSSION**

**4.1 Results**

The qualitative phytochemical composition of *Anthocleistagrandiflora* ethanol extract are presented in table 1 and the results revealed the presence of alkaloids, steroids, saponins, carbohydrates and tannins while terpenes, anthroquinones, cardiac glycosides and flavonoids were absent.

**Table 1: Qualitative phytochemical composition of *A. grandiflora* ethanol bark extract.**

|  |  |
| --- | --- |
| Phytochemical | Inference |
| Alkaloids | + |
| Steroids | + |
| Saponins | + |
| Terpenes | - |
| Anthroquinones | - |
| Cardiac glycosides | - |
| Flavonoids | - |
| Carbohydrates | + |
| Tannins | + |

**Key:** The presence of phytochemical is denoted by + = Present - = absent.

**3.1.1 Quantitative phytochemical composition of *A. grandiflora* ethanol bark extract**

The results of quantitative phytochemical composition is presented in table 2. It revealed a significantly (p<0.05) high amounts of alkaloids (496 ± 1.89), saponins (420.6 ± 2.5), steroids (408.2 ± 1.89) followed by phenols (396.7 ± 1.67), steroids (308.2 ± 1.89), Anthocyanosides (273.3 ± 1.53), Phlobatamins (251.5 ± 2.03) and tannins (228.9 ± 2.67 mg/ 100ml)

**Table 2: Quantitative phytochemical composition of *A. grandiflora* ethanol bark extract**

|  |  |  |
| --- | --- | --- |
| Phytochemical |  | Composition (mg/ 100ml) |
| Alkaloids  Saponins  Steroids  Phenols  Anthocyanosides  Phlobatamins  Tannins  Steroids |  | 496 ± 1.89a  420.6 ± 2.5a  408.2 ± 1.89a  396.7 ± 1.67b  273.3 ± 1.53c  251.5 ± 2.03c  228.9 ± 2.67c  308.2 ± 1.89b |

Results are presented as Mean ± SEM; n = 3. Mean values with different letters as superscripts are considered to be statistically significant at p<0.05

**Blood Glucose Concentrations in Alloxan–Induced Diabetes treated with Ethanol Bark extract of *A. grandiflora***

As shown in table 3, there was a significant (p<0.05) reduction of serum glucose across the treated groups while (group 2) showed a sustained diabetic status in all the rats, confirming the anti-diabetic properties of the ethanol bark extract.

**Table 3**: Blood glucose concentration in Alloxan-induced diabetes treated with ethanol bark extract of *A. grandiflora*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **GROUPS** | **Day 0**  **(mg/dl)** | **Day1**  **(mg/dl)** | **Day4**  **(mg/dl)** | **Day8**  **(mg/dl)** | **Day12**  **(mg/dl)** |
| Group 1 | 66.5019.05a | 69.1915.31b | 99.53b | 97.805.51ab | 119.00a |
| Group 2 | 49.25 d | 239.7515.65d | 227.7512.2d | 210.503.7 d | 206.00d |
| Group 3 | c | 241.50b | 138.8325.93b | 134.33b | 160.25a |
| Group 4 | 91.5016.58 b | 277.0012.96a | 195.003.65a | 151.502.65a | 143.75b |
| Group 5 | 88.2510.935a | 209.753.78c | 174.256.99b | 136.504.04a | 116.50b |

The result are presented as Mean ± S.D (N=4) mean values with different letters as superscripts are considered to be statistically significant at P < 0.05. Group 1: Normal control, Group 2: positive control, Group 3: Induced and treated with standard drug (Glibenclamide), Group 4: Induced and treated with plant extract (100mg/kg-1), Group 5: Induced treated with plant extract (200mg/kg-1).

**4.2 Discussion**

Plants have always been an excellent source of drugs, and many of the drugs that are currently on the market are derived directly or indirectly from plants. Flavonoids are most commonly known for their antioxidant properties, which include inducing the body's biochemical reactions to carcinogenic chemicals, viruses, and allergy inducers. Plants have also received a lot of attention due to their bio-active substances with properties like antioxidants, hypoglycemic, and hypolipidemic factors. According to Ekam et al. (2007), several plants exhibit anticancer, anti-inflammatory, antibacterial, and antiallergic properties. They may also have therapeutic uses as antidiabetics (Jisika et al., 1992). Alkaloids are typically natural, organic substances that include nitrogen and have sedative and analgesic properties in addition to being physiologically active. They help lessen the symptoms of despair and stress. Because of their stimulatory properties, which cause excitation linked to cell and nerve diseases, alkaloids are typically toxic when consumed in large quantities (Obochi et al., 2006). Among secondary metabolites of plants, phenolic compounds are among the most prevalent molecules and are recognised for their inherent antioxidant properties (Jones et al., 1994).   
They also function as constitutive defences against invasive invaders and as flower pigments. Because of their ability to function as an adjuvant and enhance the immune response, saponins are widely used in animal vaccinations.

In the current study as shown in table 1, the result shows the following phytoconstituents; Alkaloids, steroids, carbohydrates, tannins, saponins were present while terpenes, anthraquinones, flavonoids and cardiac glycosides were absent.The detected phytohemicals were further quantified and the results showed a significantly (p<0.05) high amounts of alkaloids (496 ± 1.89), saponins (420.6 ± 2.5), steroids (408.2 ± 1.89) followed by phenols (396.7 ± 1.67), steroids (308.2 ± 1.89), Anthocyanosides (273.3 ± 1.53), Phlobatamins (251.5 ± 2.03) and tannins (228.9 ± 2.67 mg/ 100ml)

Table 3 is the results of glucose level and the diabetic rats treated with different doses of *A. grandiflora* ethanol bark extract and this study revealed that wistar rat’s serum glucose levels were significantly (p<0.05) lowered by *A. grandiflora’s* ethanol bark extract. Group 3 treated with standard drug compared with Groups 4 and 5 (treated with ethanol extract) shown a significant (p<0.05) decrease in glucose levels over time, but Group 2 (diabetic untreated) showed continuously high glucose levels throughout the study period. These results imply that the extract might possess potential anti diabetic qualities. The gradual decrease in blood glucose levels over the 12 days period support the hypothesis of the active components present. The occurrence of diabetes mellitus and its consequences, including micro and macro vascular illness have been linked to postprandial hyperglycemia which has been identified as an independent risk factor (Baron et al., 2000).

When rats were treated with ethanol bark extract of A. djalonensis, their diabetes condition improved. Extracts from certain plants have been shown to have a hypoglycemic impact by intensifying the insulin effect, either by releasing insulin from bound insulin or by boosting the pancreatic secretion of insulin from the islets of Langerhans cells (Qamar et al., 2011). One or more of the aforementioned modes of action may have been employed by the ethanol bark extract used in this investigation. Because of their bioactive compounds, such as antioxidants and hypoglycemic and hypolipidemic factors, plants have drawn a lot of attention and play a vital role in the identification of new beneficial therapeutic agents (Okigbo et al., 2008).

Alkaloids are typically natural, organic substances that include nitrogen and have sedative and analgesic properties in addition to being physiologically active. They help lessen the symptoms of despair and stress. Because of their stimulatory properties, which cause excitation linked to cell and nerve diseases, alkaloids are typically toxic when consumed in large quantities (Kala et al., 2005). Among secondary metabolites of plants, phenolic compounds are among the most prevalent molecules and are recognised for their inherent antioxidant properties (UNESCO, 2000). They also function as constitutive defences against invasive invaders and as flower pigments. Because of their ability to function as an adjuvant and enhance the immune response, saponins are widely used in animal vaccinations.

There are a number of disadvantages, despite the fact that the study on the anti-diabetic potential of A. grandiflora ethanol bark extract in wistar rats has provided valuable information on the plant's prospective medical applications. These include: The findings' generalisability is limited by the study's small sample size (n=20) and brief study period (4 weeks) (Kumar et al., 2017). Additionally, the oral delivery route and single dosage regimen might not be the best dosing methods (Mishra et al., 2019). Results may be impacted by strain-specific variations, and Wistar rats might not be an appropriate representation of human diabetes (Srinivasan et al., 2015). Unknown are possible medication interactions and chronic toxicity.

**4.3 Conclusion**

This study demonstrates the anti-diabetic potential of *A. grandiflora* ethanol bark extract. The extract’s ability to reduce blood glucose levels, improve insulin sensitivity and exhibit antioxidant properties suggest its potential as a natural remedy for diabetes management and its complications. The effects may be due to the presence of phytoconstituents in the plant extract. Further studies will be required for investigations of fractions of these plants to isolate potential lead for prophylaxis and therapeutic use for various diseases, especially diabetes.

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