

Hypoglycemic Properties of Ethanolic and Aqueous Leaf Extract of *Acanthus Montanus* (*Acanthaceae*)

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ABSTRACT

This study evaluated the hypoglycemic properties of ethanolic and aqueous leaf extract of *acanthus montanus*. The leaves were subjected to different extraction methods (ethanolic extract and aqueous extract) and evaluated the phytochemical and hypoglycemic potential of the aqueous and ethanolic extract in alloxan-induced diabetic rats. Flavonoid content varied widely, ranging from 12.03% in milled samples to 54.00% in ethanolic extracts with the latter being significantly higher ($p < 0.05$). Alkaloid levels followed a similar trend, spanning 2.06 % (milled) to 15.02 % (ethanolic extract) with significant differences among all samples ($p < 0.05$). Saponin content ranged from 5.00 % (ethanolic extract) to 19.00 % (aqueous extract), while tannins ranged from 1.03 % (aqueous extract) to 2.50 % (ethanolic extract); both showed significant variations ($p < 0.05$). In the animal study, Group A (normal control) maintained glucose levels within the normal range (3.8–7.8 mMol/L). Group B (alloxan-induced) exhibited an initial drop 24 h post-induction followed by persistent hyperglycemia (>7.8 mMol/L). Group C (standard drug) returned glucose to normoglycemia, while Group D (treated with ethanolic extract) showed a gradual decline from 9.8 mMol/L to normoglycemic levels (8.0 mMol/L) over two weeks. Alloxan selectively destroyed pancreatic β -cells, confirming its use as a diabetic inducer. These findings demonstrate that ethanolic extraction maximizes flavonoids and alkaloids, and its extract possesses notable hypoglycemic activity, suggesting potential for developing anti-diabetic phytomedicine.

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Introduction

Medicinal plants have been used in folk medicine for generations in most of the cultures throughout the world and are still one of the primary sources of treatment in many areas today. In Nigeria and most developing countries of the world, plant based traditional medicine system play essential role in health care with about 80% of the population relying on it due to its availability and cheap source [1]. These medicinal plants have always been among the common sources of medicine either processed as traditional preparations or used to extract bioactive compounds that might serve as lead for the development of novel drugs [2].

Acanthus montanus is a member of the Acanthaceae family. The genus *Acanthus* has about 30 species of flowering plants. The generic name is derived from the Greek word *Acanthus* (*acanthos*), meaning thorny [3]. It is known and called by different names including “Ahon Ekon” (Yoruba), “Agameebu” or “Agamsoso” (Igbo), “Gautar Fadama” (Hausa), “Elele-nyijuo” (Igede), “Idumngbe” (Etulo) and “Shishaikyo”, “Ityoukibua” or “Pevkyekye” (Tiv) in Nigeria and across the world [4]. The common name of this thorny herbaceous plant which grows in grasslands, woods, scrubs and rocky hills in different parts of the world include “Bear’s breech”, “Mountain thistle”, “False thistle”, “Alligator plant” and “Thorny pigweed” [5].

Different portions of the *A. montanus* plant are used in different parts of the world in the treatment of various ailments in humans including furunculosis, cough, pneumonia, fever, gastrointestinal upsets, heart troubles, urinary tract infections and other inflammatory processes, menstrual irregularities, ‘morning sickness’ in pregnant women, abortion, rheumatism, yaws, as well as in ceremonies of purification and exorcism [6-8]. Recent research has demonstrated that leaf extracts of this plant have an antiparasitic effect against gastrointestinal nematodes [4]. Secondary metabolites such as cardiac glycosides, unsaturated steroids and sterols, saponins, tannins, anthracenes, triterpenes, flavonoids and alkaloids were previously detected in *A. montanus* [4], as well as a new phenylethanoid di-glycoside named ‘Acanmontanoside’ has been isolated [9]. In South Eastern Nigeria, the leaves of *A. montanus* are used in traditional herbal practices for the treatment of gonorrhoea, syphilis, deep and dangerous wounds and boils among other uses. Farmers in Ubakala, Umuahia South Area of South Eastern Nigeria, use the fresh leaves of *A. montanus* to ward off rodents from their produce probably because of the thorns associated with the leaves [10].

Traditionally, the stem and leaves are used locally to treat various illnesses. In Traditional Medicine and Pharmacopoeia of Cameroon, *A. montanus* is used to treat pain, cough, epilepsy, dysmenorrhoea, miscarriages and false labour [11]. Soups made from it are also used for abdominal pains caused by indigestion. Asongalem et al. reported that aqueous extracts of the plant

showed anti-inflammatory, lack of central analgesia and antipyretic properties [12]. They demonstrated that the same plant extracts exhibited a dose dependent inhibition of writhing and did not affect the second phase of formalin test, thus, showing its poor centrally acting activity. Previous studies have showed that *Acanthus montanus* inhibited uterine contractions induced by histamine, serotonin and prostaglandin [13]. This plant is devoid of toxic effects in rats at the doses often used by the population to treat inflammatory and analgesic ailments, but its MeOH/CH₂Cl₂ extract was not teratogenic compared to its aqueous extract and possesses anti-fertility activities in female rats [14].

This study therefore, investigated the phytochemical constituents present in *Acanthus montanus* and the possible hypoglycemic effects of the aqueous and ethanolic leaf extracts in alloxan diabetic rats which in turn may be used in the management and treatment of diabetes mellitus.

Materials and Methods

Sources of Raw Materials

Fresh leaves of *Acanthus montanus* used for this research was obtained in Ahaba Oloko in Ikwuano L.G.A of Abia state, Nigeria following both stipulated local and national guidelines.



Figure 1: *Acanthus Montanus* Plant

Sample Preparation

The leaves was sorted to separate the healthy ones from the bad ones and washed with clean running water to remove debris. The sample was oven dried at 55°C in an oven (model: KZ 760 4SS China) to a final moisture content of 5% and milled into powder using a mechanical grinder {model: BLG-595(MK2) China} according to the method of Enyi et al. and stored in air tight container until needed for analysis [15].

Preparation of Aqueous Extract of the Sample

Twenty {20 g} of the sample was soaked in 400 ml of hot (60°C) distilled water with constant shaking for about 7 hr. The mixture was filtered and the filtrate concentrated.

Preparation of Ethanolic Extract of the Sample

Ten {10 g} of the sample was wrapped with Whatman No 1 filter paper. This was subjected to extraction using 95% ethanol as the solvent through soxhlet extraction method for a, period of 2 hr. The extract was concentrated by evaporation and stored in air tight container until needed for analysis.

Determination of Phytochemical Properties

Alkaloid was determined and calculated using the method of Herborne [16]. One {1 mg} of the sample was dispersed in 10 mL of 10 % acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4hours at 28 °C. It was later filtered with a Whatman No 42 grade filter paper. The filtrate was concentrated to one quarter of its original

volume by evaporation and treated with drop wise addition of concentrated aqueous NH₄OH until the alkaloid was precipitated. The alkaloid precipitated was filtered, received in a weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°C for 30 minutes.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

W = weight of sample, W₁ = weight of empty filter paper, W₂ = weight of paper + alkaloid precipitate

Flavonoid was determined and calculated using the method of Meda and Lamien [17]. Five {5 g} weight of each sample was boiled in 50 ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered using Whatman No 42 filter paper. A known volume {5 mL} of the extract was treated drop wise with equal volume of ethyl acetate. The flavonoid precipitated was recovered by filtration using weighed filter paper. The weight difference gave the weight of flavonoid in the sample.

$$\% \text{ Flavonoids} = \frac{W_2 - W_1}{W} \times \frac{100}{1}$$

W = weight of sample, W₁ = weight of empty crucible, W₂ = weight of paper + flavonoid precipitate

Tannins were determined and calculated using the method of Pearson [18]. Five {5 g} weight of each sample was dispersed in 50 mL of distilled water, shaken and allowed to stand for 30 minutes at 28 °C before it was filtered through whatman No.42 grade filter paper. Two {2 mL} volume of each sample extract was dispensed into a 50 mL volumetric flask and mixed with 2 mL standard reagent and 2.5 mL of saturated Na₂CO₃ solution. After mixing, the content of each flask was made up to 50 mL with distilled water and allowed to incubate at 28 °C for 90 minutes. The respective absorbance was measured in a spectrophotometer at wave length of 260 nm. The reagent blank was used to calibrate the instrument while the absorbance values of the samples was plotted to determine tannin content against the weight of the sample. Tannin content was calculated as:

$$\% \text{ Tannin} = \frac{100}{w} \times AU \times \frac{C}{AS} \times C \times \frac{Vf}{1000} \times D/Va$$

W = weight of sample analyzed, AU = Absorbance of the test sample, AS = Absorbance of the standard solution in mg/ml, C = Concentration of standard solution in mg/ml, Vf = Total volume of extract, Va= Volume of extract analyzed, D = Dilution factor

Saponin content of the samples was determined by the double solvent extraction gravimetric method using the method of Herborne [16]. Five {5 g} of the powdered sample was weighed and mixed with 50 mL of 20% aqueous ethanol solution. The mixture was heated with periodic agitation on a water bath for 90 minutes at 55°C. It was filtered through Whatman No. 42 filter paper and the residue re-extracted with 50 mL of 20% ethanol, both extracts were combined together. The combined extracts were reduced to 40 mL over a water bath at 90°C and the concentration transferred into 250 mL separating funnel and 40 mL of diethyl ether added and shaken vigorously. Separation was by partition during which the aqueous layer recovered and the ether layer discarded. Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour. The saponins was extracted with 60 mL of normal butanol and the combined n-butanol extract washed with 5% aqueous NaCl (sodium chloride)

solution and evaporated to dryness in a pre-weighed evaporating dish. It was further dried at 60°C in the oven and weighed. The saponin content was determined and expressed as percentage of the weight analyzed using the formula:

$$\% \text{ Flavonoids} = \frac{W2 - W1}{W} \times \frac{100}{1}$$

W = weight of sample, W1 = weight of empty evaporating dish, W2 = weight of dish + saponin extract

Animals Experiment

Twenty (20) albino rats of the Wistar strain 24-day old male weighing between 53 – 58 g at the beginning of experiment were obtained from the Department of Zoology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The experimental house and cages were cleaned and disinfected before the arrival of the experimental rats. The rats were weighed and divided into four groups (A-D) of five rats each per group and housed at room temperature (29°C). They were fed on commercial diet for a period of 7 days for proper acclimatization to the environment and re-weighed before commencement of the experiments.

Group A served as normal control (given 0.9% saline).

Group B served as diabetic control (alloxan diabetic but was not given any treatment).

Group C was alloxan diabetic treated with the aqueous extract of *A. montanus* (200 mg/kg body weight).

Group D was alloxan diabetic treated with the ethanolic extract of *A. montanus* (200 mg/kg body weight).

The glucose level was determined by allowing the animals to fast overnight but allowed free access to water. At the end of the fasting period, blood was collected from the tail of each rat and the glucose level was determined by the glucose oxidase method. After checking the normal glucose levels of all the rats, diabetes was induced by intraperitoneal injection of 5% alloxan monohydrate at 90 mg/kg body weight in groups B-D. Once the rats were confirmed diabetic, treatments with the extracts were immediately given and this was done for two weeks. The glucose levels were monitored until there was a notable drop in the glucose level to the normal range.

Statistical Analysis

All experiments were conducted in triplicate, with results reported as mean values ± standard deviation (SD). Statistical significance between groups was evaluated using a one way analysis of variance (ANOVA), followed by Duncan's Multiple range test. Differences were considered statistically significant at p-values ≤ 0.05. These analyses were performed using statistical software (SPSS version 22).

Results and Discussion

Phytochemical Properties of the Leaves of *A. Montanus*

The phytochemical properties of the leaves of *A. montanus* are shown in Tables 1. The phytochemical report of the leaves showed the presence of flavonoids, alkaloids, tannins and saponin. The flavonoid contents of the samples ranged from 12.03 to 54.00%. Milled sample had the least value (12.03%) while ethanolic extract had the highest value (54.00%). The ethanolic extract

differ significantly ($p < 0.05$) from other samples. The variation in the flavonoid content of the samples may be due to the method of analysis, method of processing and variation in solvent for extraction. This is in line with the findings of Onyeka and Nwambekwe who reported that method of processing affect the phytochemical properties of vegetables [19]. The samples were higher than the value (1.6%) reported by Nwachukwu et al. for phytochemical content of the leaf of *Acanthus montanus* and 0.36% and 0.18% for chloroform and methanolic extract of *A. montanus* respectively reported by Parker for phytochemical composition of chloroform and methanol leaf extract of *Acanthus montanus* [20,21]. Flavonoids are hydroxylated polyphenolic compounds and have been reported to possess significant pharmacological properties which include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepato-toxins, viruses and tumor [22]. Flavonoids also exhibit anti-fungal, anti-viral and anti-cancer properties [23]. Flavonoids also have significant activities when ingested and there is great interest in their potential health benefits, particularly for compounds such as iso flavonoids, which have been linked to the anticancer benefits of soy-based foods and the stilbenes in red wine that are believed to contribute to reduced heart disease. It has been known for several years that plant polyphenols such as steroids, terpenoids, flavonoids etc. are antioxidants in vitro [24]. Therefore, flavonoids protect against oxidative cell breakdown and damage has anti-inflammatory properties. This may be the reason *A. montanus* is used in the treatment of furuncles [25]. Flavonoid also lowers the risk of heart diseases and cancer. Quercetin which is used to treat atherosclerosis, CVDs and prevent cancer is a flavonoid.

The alkaloid contents of the samples ranged from 2.06 to 15.02%. Milled sample had the least value (2.06%) while ethanolic extract had the highest value (15.02%). There were significant ($p < 0.05$) differences among the samples. The variation in the alkaloid content of the samples may be due to the method of analysis, method of processing and variation in solvent for extraction. This is in line with the findings of Onyeka and Nwambek we who reported that method of processing affect the phytochemical properties of vegetables [19]. The samples vary from the value (5.6%) reported by Nwachukwu et al. for phytochemical content of the leaf of *Acanthus montanus* and, 3.16 - 4.04% reported by Igwe and Eleazu for phytochemical composition of raw and processed *Acanthus montanus* leaves [20,26]. Alkaloids are very important in phytomedicine and constitute most of the valuable drugs, for example, anti malarial. Alkaloids are well known for their anti-microbial activity. Some studies have been reported on the anti microbial activity of alkaloids from several medicinal plants such as *Jatropha curcas*, *Carica papaya*, *Mangifera indica* and *Psidium guajava* [27]. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents all over the world for their analgesic, antispasmodic and bactericidal effects. These are used for the help of mankind and found beneficial for certain life-threatening disease [28]. Morphine, Codeine and berberine are among the isoquinoline alkaloids that have been used in the treatments of neurodegenerative diseases (NDDs) such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Epilepsy. Also, caffeine is a stimulant for the Central Nervous System (CNS) promoting CNS coordination and fighting NDDs [29]. Thus the presence of alkaloids alludes to the ability of the fraction of *A. montanus* to exhibit numerous pharmacological activities.

The saponin contents of the samples ranged from 5.00 to 19.00%. ethanolic extract had the least value (5.00%) while aqueous extract

had the highest value (19.00%). There were significant ($p < 0.05$) differences among the samples. The variation in the saponin content of the samples may be due to the method of analysis, method of processing and variation in solvent for extraction. This is in line with the findings of Onyeka and Nwambek we who reported that method of processing affect the phytochemical properties of vegetables [19]. The samples vary from the values 3.22 - 5.35% reported by Igwe and Eleazu for phytochemical composition of raw and processed *Acanthus montanus* leaves and (6.45%) reported by Nwachukwu et al. for phytochemical content of the leaf of *Acanthus montanus* [20,26]. Roa et al. have shown that saponins possess antioxidant, antitumor, and anti-mutagenic activities and may also reduce the incidence of human cancers by inhibiting the growth of cancer cells [30].

The tannin contents of the samples ranged from 1.03 to 2.50%. Aqueous extract had the least value (1.03%) while ethanolic extract had the highest value (2.50%). The aqueous extract differ significantly ($p < 0.05$) from other samples. The variation in the tannin content of the samples may be due to the method of analysis, method of processing and variation in solvent for extraction. This is in line with the findings of Onyeka and Nwambek we

who reported that method of processing affect the phytochemical properties of vegetables [19]. The samples were lower than the value (6.68%) reported by Nwachukwu et al. for phytochemical content of the leaf of *Acanthus montanus* and 11.78% and 12.05% for chloroform and methanolic extract of *A. montanus* respectively reported by Parker for phytochemical composition of chloroform and methanol leaf extract of *Acanthus montanus* but vary slightly from the values 0.76 - 1.56% reported by Igwe and Eleazu for phytochemical composition of raw and processed *Acanthus montanus* leaves [20,21,26]. Tannins have high potency for intestinal disorders such as diarrhoea and dysentery [31]. Tannins bind to produce rich proteins in the body and interfere with protein synthesis. They are known to exert anti-microbial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells [32]. Presence of tannins also shows antimicrobial and antifungal activities by coagulating the protoplasm of microorganisms. Tannins can also be effective in protecting the kidneys and as anti-inflammatory, antiseptic; antioxidant and haemostatic pharmaceuticals [33]. Thus the presence of tannins in *A. montanus* fractions could suggest its activity against microbial related diseases.

Table 1: Phytochemical Properties of the Leaves of *A. montanus*

Samples	Flvonoids (%)	Alkaloids (%)	Saponin (%)	Tannins (%)
Milled sample	12.03 ^b ±0.03	2.06 ^c ±0.03	9.01 ^b ±0.02	1.91 ^a ±0.02
Aqueous extract	13.01 ^b ±0.01	12.00 ^b ±0.02	19.00 ^a ±0.01	1.03 ^b ±0.03
Ethanolic extract	54.00 ^a ±0.01	15.02 ^a ±0.02	5.00 ^c ±0.02	2.50 ^a ±0.02

Values shows the mean of triplicate analysis and ± standard deviation. Figures with different superscript down the column are significantly different ($p < 0.05$).

Phytochemical Properties of the Leaves of *A. montanus*

The results on the blood glucose concentrations of the saline control, diabetic control, aqueous and ethanolic extract of *A. montanus* treated groups for 14 days are shown in Tables 2. For group A, the variations in the glucose levels were observed to fall within the normal 3.8mMol/L-7.8mMol/L. The glucose level for group B animals were seen to have a drop, 24 hrs after inducement with alloxan monohydrate and then picked up and rose continually 48 hrs after inducement until it reach the hyperglycemic phase (>7.8mMol/L) and persisted. This corresponds to the mechanism of action of alloxan as documented by Lenzen [34]. With group C animals, there was a sharp decline in the blood glucose level from 13.1mMol/L - 8.8mMol/L. This continued until it returned to the normal range 3.8mMol/L-7.8mMol/L. There was a gradual drop in the glucose level from 9.8mMol/L - 8.0mMol/L normal range for about 2 weeks in group D animals following the treatment with the ethanolic extract. Alloxan is a specific toxic substance that destroys the β-cells provoking a state of primary deficiency of insulin without affecting other islet types. Hence alloxan was selected to induce diabetes in the present study.

Table 2: The Blood Glucose Concentrations of Alloxan-Diabetic Wistar Rat Treated with Aqueous and Ethanolic Leaf Extract of *A. Montanus*

Day	Normal Mmol/L	Diabetic Mmol/L	Aqueous Mmol/L	Ethanolic Mmol/L
0	3.27±0.31	6.10±0.10	5.80±0.62	6.67±0.76
1	3.67±0.42	5.32±0.46	3.67±0.15	3.83±0.25
2	4.10±0.10	11.20±0.44	13.10±2.00	9.86±1.50
3	5.50±0.76	10.10±0.39	8.77±0.70	8.07±1.39

4	4.97±0.15	9.15±0.53	7.17±0.95	7.77±1.82
5	5.23±0.60	8.80±0.79	7.17±0.32	7.57±1.15
6	4.73±1.36	8.40±0.47	6.67±0.67	7.43±2.03
7	3.97±1.00	8.40±0.86	5.67±0.65	7.10±1.35
8	4.13±1.21	9.00±0.1.13	5.67±0.67	6.37±1.12
9	4.04±0.86	9.50±0.86	5.40±0.36	6.17±0.91
10	4.10±0.60	9.51±0.48	5.13±0.32	5.10±1.51
11	3.80±0.62	10.00±0.53	4.90±0.26	5.60±0.53
12	4.33±0.21	9.61±1.10	4.67±0.42	4.50±0.79
13	4.66±0.95	9.80±0.0.80	4.24±0.0.61	3.73±0.85
14	4.10±0.40	9.90±0.97	4.33±0.31	3.53±0.50

Values shows the mean of triplicate analysis and ± standard deviation. Figures with different superscript down the row are significantly different ($p < 0.05$).

Conclusion

Results of the present study showed that there was an improvement the therapeutic potentials of the individual extracts as agents of glycemic control. However, the ethanolic extract is seen to be more effective in addressing alloxan diabetes in wistar rats. The study demonstrates that extraction method influences the phytochemical profile of *A. montanus*. The significant differences ($p < 0.05$) across extracts underscore the solvent’s selectivity for bioactive compounds. In vivo evaluation confirmed the ethanolic extract’s notable hypoglycemic effect, restoring normoglycemia in alloxan-induced diabetic rats comparable to standard antidiabetic agents. These findings validate *A. montanus* as a potential source of natural antidiabetic phytomedicine and highlight ethanolic

extraction as the preferred method for enriching flavonoids and alkaloids. Further research should focus on isolating active constituents, elucidating mechanisms of action, and optimizing scalable extraction protocols to harness its therapeutic value.

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