

Phytochemical Screening and Toxicity Profile of Methanol Extract of *Andira inermis* Stem Bark (Gwaska)

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Abstract

The best method for finding medicinally active ingredients in plant material is to screen for phytochemicals in the plants. The toxicity profile of a methanol extract of the stem bark of *Andira inermis* (Gwaska) and a phytochemical screening study were conducted in this study. Alkaloids, carbohydrates, cardiac glycosides, saponins, tannins, flavonoids, and steroids were all detected in the methanol extracts during the screening process, with the exception of tannins and flavonoids for lead acetate and Shinoda's test, which were not detected. The albino Wister rats' body weight did not significantly alter, and the LD₅₀ was more than 5000 mg/kg, according to research using Lorke's method of toxicity profile in vivo. At 1500, 2500, and 5000 mg/kg doses of the extracts, respectively, the albino Wister rats also showed notable behavioural changes, including restlessness, erection of the hair coat, tiredness, and diarrhea. The findings showed that even at a high dosage of 5000 mg/kg, the *Andira inermis* methanol extract was not harmful to the experimental animals.

Keywords: *Andira inermis*; phytochemicals; methanol; toxicity profile; extracts.

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1.0 INTRODUCTION

Many herbal medicines are frequently sold openly in public spaces in developing nations, such as markets, parks, bus stations, traveling cars, and even on the streets. This is an indication of their acceptance. The majority of these herbal products have not yet been subjected to toxicity assessments or scientific examination. Additionally, studies on the safety and effectiveness of several of these commercial herbal products have been published (Obi, *et al.*, 2006). Drug development depends on pharmacological and toxicological analyses of medicinal plants, and there are many unidentified compounds in nature's bank that can be used to treat a wide range of human disorders (Maxwell, 2019). Cabbage Tree, Cabbage Bark, and Angelin are just a few of the common names for *Andira inermis*, a well-known species in the Papilionoidea sub-family of the Fabaceae family. Almendro de México in El Salvador, Guacamayo in Honduras, and Gwaska in Nigeria are just a few of the many local names that vary greatly over its wide geographic span. This nitrogen-fixing tree is a large plant that may grow to remarkable heights of 35 meters and trunk diameters of greater than

90 centimetres (Bruneton, 1999). The tree is distinguished by its evergreen foliage, which has a dense, spreading crown and brilliant tan juvenile leaves that develop into glossy green leaves with full margins. Its striking pink to purplish-red blooms, which are frequently terminal and axillary, are grouped in well-branched racemes and are exceedingly fragrant. The trunk can often produce buttresses up to three meters tall in some areas, and the stem usually has a rough exterior surface (Karem and Thomas, 2002). Reports of the toxicity of herbal treatments have been made all over the world. For instance, garlic (*Allium sativum*) is typically linked to routine exposures that cause dermatitis or eczema, and there have been a few cases of bleeding issues as a result of consuming a lot of garlic; Ginseng (*Panax ginseng*), often known as "Ginseng abuse syndrome," is linked to hypertension and seemingly hostile behavioural changes in over users (Maxwell, *et al.*, 2020). Fruits are consumed, the bark of the trees is used as a vermifuge, an antihelmintic, a pain reliever, and a purgative, and a leaf decoction is used as a beverage (leaf decoction is also used for laundry in Africa) (Hodges and Minich, 2015). Acute toxicity studies is one

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of the suitable/designated pharmacognostic studies in the assessment of plants herbal products undergoing medical scientific scrutiny, aside from reports and knowledge of toxicity of herbal products (OECD, 2008). Therefore, after an earlier acute toxicity study of *Andira inermis* (AI) stem bark (reported to be 5000 mg/kg), this study aims to evaluate the toxicity (subacute) of the methanolic *Andira inermis* stem bark extract in adult Wister rats in order to provide additional information for future research on the efficacy study on the stem bark (Etuk, *et al.*, 2009). The herb *Andira inermis* is being examined for its pharmacognostic therapeutic function, which is its antihyperglycemic action. Because this effect requires everyday usage and dosages, a subacute toxicity study is currently underway. Additionally, using herbal medical plants without first assessing their safety profile may harm species' organs, most frequently the liver and kidney, which are involved in the metabolism and excretion of substances consumed (Nashwan, *et al.*, 2018). Depending on whether the extract directly damages other organs or systems (such as the heart, spleen, and lungs) (Maxwell, *et al.*, 2021). *Andira inermis* is widely distributed throughout a variety of tropical biomes, and its innate capacity to fix nitrogen highlights its versatility and resilience. A plant's ability to produce a wide range of secondary metabolites is frequently strongly correlated with its ecological resilience. These substances are produced by plants either for advantageous ecological interactions or as a defence against environmental stressors, pests, and diseases (Nawaz, *et al.*, 2020). A complex array of bioactive chemicals is therefore likely to have developed in a species like *A. inermis*, which thrives in a variety of settings and demonstrates remarkable resilience. In turn, this vast diversity of phytochemicals offers a convincing explanation for its wide range of traditional therapeutic uses in various cultures. Its wide geographic range further highlights its significance in ethnobotanical activities by making it an easily accessible natural resource for people dependent on traditional medicine (Chudnoff, 1984)

2. EXPERIMENTAL PROCEDURES

2.1 Sample and Treatment of Sample

2.1.1 Collection, identification and drying of the sample

A freshly stem bark of the plant was collected from Danko Wasagu local government of Kebbi State, Nigeria. The herbarium sample of the plant leaves and flower was identified as *Andira inermis* by a taxonomist at the taxonomy unit of the Abdullahi Fodiyo University of Science and Technology Aliero Kebbi State. The sample was dried under shade for two weeks grounded into powder with motor and pistole and stored in an air tied bottle for further use.

2.1.2 Sample extraction

Extraction using the Soxhlet apparatus was performed to obtain the methanol extract. (100g) of the powdered sample was taken in a cotton tumble into the

extraction unit of the Soxhlet, then methanol (500mL) was taken into the round bottom flask and the system refluxed for 24 hours with continues stirring. The extract was concentrated with rotary evaporator. The marc was treated afterward with methanol, brown colour methanol extract of *Andira inermis* was obtained (Halilu, *et al.*, 2012).

2.1.3 Qualitative Phytochemical Screening

Qualitative phytochemical screening of the plant methanol extract was conducted using standard procedures according to Trease and Evans (1989) as cited in Halilu *et al* (2012) to identify the existence of the following primary and secondary metabolites: alkaloids, carbohydrates, cardiac glycosides, tannins, saponins, flavonoids and steroids.

2.1.4 Test for alkaloids

After dissolving 3 mL of the extract in diluted hydrochloric acid, it was filtered. Three sections of the filtrate were separated, and the presence of alkaloids was checked using the following reagents.

2.1.4.1 Mayer's test

Mayer's reagent (potassium mercuric iodide) was applied to the filtrate. The presence of alkaloids was verified by the production of a yellow precipitate.

2.1.4.2 Wagner's test

The filtrate was subjected to Wagner's reagent (iodine in potassium iodide). Alkaloids are present when a brownish-reddish precipitate forms.

2.1.4.3 Dragendroff's test

The filtrate was treated with (solution of Potassium Bismuth Iodide) Dragendroff's reagent. The formation of reddish precipitate indicates the presence of alkaloids.

2.1.5 Test for carbohydrates

After dissolving 3 mL of the extract in 5 mL of distilled water, the mixture was filtered. The following chemicals were employed to test for the presence of carbohydrates in the filtrate, which had been separated into two sections.

2.1.5.1 Molisch's test

Two drops of alcoholic α -naphthol solution were added to the filtrate in a test tube. Carbohydrates are present because of the violet ring that forms at the junction.

2.1.5.2 Fehling's test

Fehling's A & B solutions were used to warm the filtrate after it had been hydrolyzed with dilute HCl and neutralized with alkali. The presence of reducing sugars is shown by the production of red precipitate.

2.1.6 Test for cardiac glycosides

2.1.6.1 Keller- Kiliani's test

After diluting 2 milliliters of the extract with 40 milliliters of water, 2 milliliters of a potent lead subacetate solution was added to precipitate the pigments, which were then filtered out. After shaking the resulting filtrate with an equivalent volume of chloroform, it was left in a separating funnel to separate into two layers. Over a water bath, the chloroform layer was eliminated and dried by evaporation. After dissolving the residue in three milliliters of ferric chloride in glacial acetic acid, the mixture was moved to a dry test tube. As a preliminary test for digitoxoside, a few drops of concentrated H₂SO₄ were applied to the test tube wall. When the tube was left standing, the interphase turned brown from the deoxy sugars and the upper layer turned pale green from the steroidal nucleus. (Halilu *et al.*, 2012).

2.1.7 Test for saponins

2.1.7.1 Frothing test

In a test tube, the extract (2 mL) was agitated for five minutes after being diluted with twice as much water. Saponins are present when a honeycomb-like foam forms, which lasts for roughly 45 minutes.

2.1.8 Test for tannins

2.1.8.1 Lead acetate test

Two drops of lead subacetate solution were added to one milliliter of the extract. The presence of tannins is indicated by a colored precipitate.

2.1.8.2 Bromine water test

The plant extract was treated with 3 drops of bromine water. Non-formation of coloured precipitate indicates the presence of hydrolysable tannins

2.1.9 Test for flavonoids

2.1.9.1 Alkaline reagent test

Plant extract was treated with 3mL of dilute ammonia solution followed by addition of concentrated sulfuric acid. The presence of flavonoids was confirmed by a yellow colouring that disappeared upon standing.

2.1.9.2 Shinoda's test

2mL of the methanol plant extract was treated with (Magnesium-hydrochloride reduction test), which yields a pink to crimson colour with magnesium ribbon and HCl.

2.1.10 Test for steroids

2.1.10.1 Salkowski test

A little amount of plant extract was mixed with two milliliters of chloroform, and then concentrated sulfuric acid was applied along the test tube's side. The presence of steroids was indicated by a crimson or reddish-brown color at the site of contact.

3.0 Acute Oral Toxicity Studies

The toxicological studies were conducted according to the modified Lorke's (1983) method and Hassan (2005), using twenty-one (21) healthy male and female albino Wister rats. The albino rats were divided into two (2) phases and a control group: phase I and II with nine (9) albino Wister rats each, and the control group with 3 albino Wister rats. Prior to oral administration of plant extract, albino rats were allowed to familiarized with the environment for two weeks and a day to oral administration there were fasted overnight and weighed. The albino Wister rats in Phase 1 (nine rat) were divided into 3 groups of three rat each, and were administered with single dose of 10, 100 and 1000 mg/kg (for group I, II and III respectively) of the extract orally, in other to establish the possible range of doses producing any toxic effect. For the Phase 2, the albinos' rats were also group into 3 (three albinos per group) and were administered with a single dose 1500, 2500 and 5000 mg/kg of the extract for group I, II, and III respectively. In both cases (Phase I and II), the control group remain the same and received only a distilled water. The albinos were carefully and frequently observed throughout the day of the extract administration and over the period of 14 days (2 weeks) for the signs of acute toxicity. The behavioral changes were carefully observed over the period of the study and the body weight changes were also recorded at 0, 7 and 14 days of the study.

4.0 RESULTS AND DISCUSSION

4.1 Results

Table 1: Result of the phytochemical screening of stem bark of *Andira inermis*

S/N	Plant constituents	Methanol extract
1.	Alkaloids - Mayers test - Wagners Test - Drangendroffs test	+ + +
2.	Carbohydrates - Molish test - Fehling test	+ +
3.	Glycosides - Killer-Kiliani's test	+
4.	Saponins - Frothing test	+

S/N	Plant constituents	Methanol extract
5.	Tannins - Lead Acetate test - Bromine water test	N.D +
6.	Flavonoids - Alkaline reagent test - Shinoda's test	+ N.D
7.	Steroids - Salkowski test	+

KEY: + = present, N.D = not detected.

Table 2: Impact of oral methanol extract treatment on Albino Wister rat body weight variations

Experiments	Dose (mg/Kg b.w.)	Initial (g) 0 days	Weight gain (g) After 7 days	Weight gain (g) After 14 days
Phase 1	10	119.4±1.75	123.1±1.67	127.3±1.79
	100	120.0±5.04	122.3±2.07	123.0±1.66
	1000	120.7±2.73	121.3±3.18	123.3±2.67
Control	0	120.5±1.86	123.4±2.04	128.4±2.30
Phase 2	1500	124.6±1.68	128.7±0.66	131.3±3.36
	2500	126.8±1.55	129.6±1.57	134.0±2.06
	5000	128.6±1.38	132.5±0.86	136.2±2.45

Values are expressed as Mean±Standard Error of Mean (n=3) at (P>0.05) using One way ANOVA followed by Dunnett Multiple Comparison test using Graph Pad in Stat Version 3.

Table 3: Impact of oral methanol extract treatment on Albino Wister rats' mortality rate and changes in behavior

Experiments	Dose (mg/kg b/w)	Mortality after 14 Days	Behavior changes
Phase 1	10	0/3	None
	100	0/3	None
	1000	0/3	None
Control	0	0/3	None
Phase 2	1500	0/3	Small sound, erection of hair coat
	2500	0/3	Restlessness, breathing rate increases in first 4 minutes
	5000	0/3	Denial to eat after 5 minutes of oral administration, salivation, fatigue and diarrhea.

4.2 DISCUSSION

The plant *Andira inermis* (Leguminosae) is extensively employed in traditional medicine to treat a number of ailments; skin, anaemia, malaria, snake bites, antihelmintic and cancers. The family Leguminosae are known to be very rich in isoflavones and other secondary metabolites such as alkaloids, flavonoids and saponins (Cocker, *et al.*, 2022). Which were believed to play a substantial role in the protection of the plant material and its medicinal properties. As a well-known fact, methanol was used in extraction for removal of almost all the metabolites that could be present in the plant material. The extract was screened for the following metabolites: Alkaloids, carbohydrates, glycosides, tannins, saponins, flavonoids and steroids. Table one summarized the results obtained from the phytochemical analysis of the stem bark of *A. inermis*. A positive result was shown for all the metabolites except tannins and flavonoids for lead acetate and Shinoda's test which were not detected. Overall, results revealed that the stem bark of *A. inermis* possessed a wide variety of phytochemicals, all the seven metabolites tested are present in the stem bark methanol extract of *A. inermis*

(Isah, *et al.*, 2022). Toxicity lethal studies of methanol extract on albino Wister rats were reported in Table two and three respectively. Table two shows the impact of oral administration of the stem bark extract on the body weight changes of the albino rats. The results revealed no significant difference in the body weight of the albino rats in the control group and those in phase 1 at the initial day of the extract administration and after 7 and 14 days respectively. On the other hand, the albino rats administered with higher doses (1500mg/kg, 2500mg/kg, and 5000mg/kg) of the extract showed a significant difference (P>0.05) from the control group at the initial, 7 and 14 days of the extract administration respectively. The results show a substantial change in the body weight (g) of the albino rats over the course of the study as the doses increase. The impact of oral administration of the methanol extract on the mortality rate as well as the behavioral changes of the albino rats were also studied (Table 3). After 14 days of administering the methanol extract of different doses on the albino rats, no mortality case was recorded, this shows that the LD₅₀ is greater than the highest tested dose of 5000 mg/kg administered. Consequently, the dose of

5000 mg/kg of body weight is thought to be a safe dose. It is notable, although the higher doses (1500, 2500, and 5000mg/kg b.w) respectively of the extract could be safe, they could have some substantial effect on the behaviors of the albino rats as observed during the time of the study.

4.3 CONCLUSION

Phytochemical screening of the methanol extract of *Andira inermis* revealed the presence of several secondary metabolites, including alkaloids, saponins, steroids, and flavonoids, which are thought to be the most important and have metabolic roles in the living system as well as protective roles in animals. The high oral median lethal dose LD₅₀ suggests that *Andira inermis* methanol extracts are not harmful when taken orally at the studied dosages. Research on plant-based natural products is growing in popularity, particularly in the pharmaceutical sector. There are many therapeutic applications for the goods. *A. Inermis* has great ethnobotanical significance in addition to its use as a traditional medicine. It is also advised that additional toxicity profile investigations be conducted with various solvents and animals.

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