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Research Article

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Phytochemical Screening and Toxicity Profile of Chloroform and Ethyl Acetate Extract of *Andira inermis*

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Abstract *Andira inermis* stem barks are used by Nigerian herbal medicine as purgative to expel intestinal worms. To determine tolerance levels, we conducted toxicity profiling of its stem bark using the dose method and then we analysed the phytochemicals present in *A. inermis* responsible for its medicinal activity. The results showed that there was no mortality case detected when within the range of 1600 mg/kg – 5000mg/kg. However, some observable signs on the inoculated animals were diarrhoea, restlessness and fatigue for doses above 2900mg/kg weight by weight. Phytochemical analysis revealed the presence of alkaloids and carbohydrates in the chloroform extract with absence of saponins and glycosides. Meanwhile carbohydrates, glycosides and saponins were present in the ethyl acetate extract.

Keywords *Andira inermis*, Toxicity, Phytochemistry, *In vivo* studies

Introduction

Plants have been subject of research as a result of their therapeutic and medicinal uses. Almost 70% of the people living in developing countries rely mostly on traditional medicine in treating common diseases. This reality has forced many researchers to discover the toxicity levels of many plants with medicinal activity. One of such plants includes "*Andira inermis*".

Andira inermis also referred to as "Cabbage tree" is a beautiful tree grown mostly in tropical America and West African countries including Nigeria. The purple flowers are highly fragrant and the tree serves mostly as shade and other medicinal uses. *Andira inermis* is a multiple use tree that has not been extensively used in agro forestry or other reforestation programs because of relatively slow growth rates; however, it offers refuge for wildlife year-round and could be used as fodder for ruminants and other domestic animals. In countries where *andira inermis* are planted, they are used as purgative in expelling intestinal worms. The bark is used in the Brazilian Amazon as a purgative and vermifuge, it is poisonous in large doses [1]. Fresh seeds are toxic and cannot be eaten. They are used as an antihelmintic to reduced vomiting [1]. Herbal healers use the bark of the tree to cure constipation, reduce fever and as a strong laxative.Some of the bioactive constituents found in cabbage tree bark include: andirine, adinermal A, B and C, andirol A and B, N-methyltyrosine, prunetin [2].

An excessive dose can cause vomiting, fever, insomnia and even death. African populations are confronted with chronic diseases emergence whose treatment and follow-up constitute more economic problem [3]. Many individuals abuse the use of medicinal plants with purgative and laxative potentials. They tend to increase usage as

their bodies become accustomed to the additional assistance derived from these laxatives. The body's reliance on laxatives can lead to far more devastating medical complications. Hence it becomes paramount that the bioactive phytochemicals in *Andira inermis* are subjected to research, while also subjecting them to toxicity profiling.
The objectives of the study were:

a) To phytochemically (both qualitative and quantitative) screen the selected plants extracts using standard laboratory procedure.
b) To investigate the toxicity profile of the plants extracts using *in vivo* study.

Experimental

Materials

All chemicals used were of standard reagent grade. They were used without further purification.

Reagents

Methanol, Chloroform, N-Hexane, Ethylacetate, Ferric chloride, Potassium iodide, Mercuric chloride Sodium hydroxide, Conc. hydrochloric acid, Fehling solution, Magnesium powder.

Methods

Collection and identification of plant materials

The *Andira inermis* stem bark was collected from Zuru Local Government of Kebbi State, Nigeria. The plant was air dried until it became brittle and dry. The dried plant materials were turned to powdered using pestle and mortar and kept each in a separate plastic rubber container for extraction. The powdered plant material was kept in an air tight container at room temperature until further analysis.

Preparation of Reagents

The following reagents were prepared for the extraction to be carried out on the plants.

1. **Hager's Reagent**

Picric acid (25g) was dissolved in volumetric flask (50cm^3) and filled up to the marc with distilled water. The solution was thoroughly shaken.

2. **Dragendorff's reagent**

Potassium iodide (5g) was dissolved in volumetric flask (50cm^3) and filled up to the marc with distilled water. Bismuth nitrate (8g) was dissolved in nitric acid (20cm^3). The solution was mixed and diluted with water (100cm^3).

3. **Mayer' reagent**

Potassium iodide (5g) was dissolved in volumetric flask (10cm^3) and filled up to the marc with distilled water. Mercury (1.3g) was dissolved in volumetric flask (60cm^3) and filled up to the marc with distilled water. The two solutions were mixed and diluted with distilled water (100cm^3).

4. **Ferric Chloride solution (1%)**

Ferric chloride (1g) was dissolved in volumetric flask (100cm^3) and filled up to the marc with distilled water.

Extraction

Using maceration extractor, the coarsely powdered crude drug was placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of 3days with frequent agitation until the soluble matter dissolved. The mixture then was strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration after standing.

Chloroform Extract

50g of powdered sample was weighed before being placed into a 250ml conical flask. 200ml of chloroform was added into the conical flask that contained the powdered sample and then stirred for 2hours using a mechanical stirrer. The 250ml conical flask containing the mixture was covered using an aluminium foil paper and it was allowed to stand for 24hours. It was stirred again in order to have a good mixture and then filtered using a filter paper. The filtrate showed a brown colour. The marc was rinsed twice using 50ml of the same solvent (chloroform) and then it was stored in a beaker to stand for 2 days to dry off the solvent. The dried extract was then called 'chloroform extract'.

Ethylacetate Extract

50g of powdered sample was weighed before being placed into a 250ml conical flask. 200ml of ethylacetate was added into the conical flask that contained the powdered sample and then stirred for 2hours using a mechanical stirrer. The 250ml conical flask containing the mixture was covered using an aluminium foil paper and it was allowed to stand for 24hours. It was stirred again in order to have a good mixture and then filtered using a filter paper. The filtrate showed a brown colour. The marc was rinsed twice using 50ml of the same solvent (ethylacetate) and then it was stored in a beaker to stand for 2 days to dry off the solvent. The dried extract was then called 'ethylacetate extract'.

Phytochemical screening

The preliminary phytochemical tests were performed according to the standard procedures as used by Evans [4], Sofowora [5] and Harborne [7], and the results are summarized in Tables 1 and 2 .Various portions of the extract were used for the following tests.

Detection of Saponins- Frothing Test

The extract (2ml) was diluted with twice its volume of water and shaken in a test tube for 5 mins. The occurrence of a honeycomb frothwhich lasts for about 45 minutes indicates the presence of saponins.

Detection of Tannins

a) Lead acetate test

To 1 ml of the extract, 2 drops of lead sub acetate solution was added. A colored precipitate indicates the presence of tannins.

b) Bromine water test

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

Detection of Glycosides

5 ml of the concentration H_2SO_4 was added to 0.5 g of the plant extract and was boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Few drops of $FeCl_3$ (ferric chloride) solution was added to one of the portions, and a green to black precipitate indicate the presence of phenolic aglycone as a consequence of hydrolysis of glycoside.

Detection of Alkaloids

Extract was dissolved in dilute Hydrochloric acid and filtered. The filtrate was divided into 3 portions and the following reagents were used to test for the presence of alkaloids.

a) Mayer's Test

The filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow colored precipitate indicated the presence of alkaloids.

b) Wagner's Test

The filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown/reddish precipitate indicated the presence of alkaloids.

c) Dragendroff's Test

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

Detection of carbohydrates

Extract was dissolved in 5mls of distilled water and filtered. The filtrate was divided into 2 portions and was used to test for the presence of carbohydrates using the following reagents:

a) Molisch's Test

The filtrate was treated with 2 drops of alcoholic α- naphthol solution in a test tube. The formation of the violet ring at the junction indicates the presence of carbohydrates.

b) Fehling's Test

The filtrate was hydrolyzed with diluteHCl, and then neutralized with alkali before being warmed with Fehling's A & B solutions. The formation of red precipitate indicates the presence of reducing sugars.

Toxicology Study

Toxicology study was carried out according to modified Lorke's method. The study was conducted in two phases using a total of twenty-one animals. The albino rats were fasted overnight prior administration of plant extract. In the first phase, twelve animals were divided into 4 groups of 3 mice each. Groups 1, 2 and 3 animals were given single dose of 10, 100 and 1000 mg/kg of the extract orally, respectively, to establish the possible range of doses producing any toxic effect. Group 4, the control group received distilled water. In the second phase, the first three animals received 1600, 2900 and 5000 mg/kg separately, while the forth (the control) received distilled water. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity. Body weights of the albino rat were recorded on study days 0 (initiation), 7 and 14 (termination).

Results and Discussion

Results

Table 1: Results of phytochemical screening of chloroform extract of stem bark

S/No	Phytochemicals	Chloroform
1.	**Alkaloids**	
	a. Mayer's test	++
	b. Dragendroff's test	++
	c. Wagner's test	++
2.	**Tannins**	
	a. Ferric chloride	-
	b. Strong lead acetate	-
3.	**Carbohydrates**	
	a. Fehling test	++
	b. Molish test	++
4.	**Saponins**	
	a. Froath's Test	-
5.	**Glycosides**	
	a. Killer Test	-
	b. Lumber test	-

Table 2: Results of phytochemical screening of ethylacetate extract of stem bark

S/No	Phytochemicals	Chloroform
1.	**Alkaloids**	
	a. Mayer's test	++
	b. Dragendroff's test	++
	c. Wagner's test	++
2.	**Tannins**	
	a. Ferric chloride	++
	b. Strong lead acetate	-
3.	**Carbohydrates**	
	a. Fehling test	+
	b. Molish test	+
4.	**Saponins**	
	a. Froath's Test	++
5.	**Glycosides**	
	a. Killer Test	+++
	b. Lumber test	+

KEY: +++= present in high quantity ++ = present in moderate quantity + = present in little quantity - = not present.

Table 3: Effects of oral administration of chloroform extract of *Andira inermis* on the Body Weight changes of albino rat

Experiment	Dose (mg/kg)	Initial weight (g) 0 days	Weight gain (g) After 7 days	Weight gain (g) After 14 days
Phase 1	10	117.3±1.85	119±0.57	125.3±1.76
	100	115.0±4.04	117.3±2.19	117.0±1.76
	1000	116.7±3.53	123.3±2.19	121.3±1.76
Control	0	117.7±2.96	125.3±2.03	128.7±1.20
Phase 2	1600	133.3±0.89	136.7±0.67	143.3±3.38
	2900	141.7±1.45	132.7±1.67	144.0±2.08
	5000	132.7±1.27	140.7±0.88	143.0±2.65

Values are expressed as Mean±Standard Error of Mean (n=3).

Table 4: Effects of oral administration of chloroform extract of *Andira inermis* on the Mortality rate of albino rat

Experiments	Dose (mg/kg)	Mortality of albino rat After 24 hours of administration	Mortality at 14 days of observation
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase 2	1600	0/3	0/3
	2900	0/3	0/3
	5000	0/3	0/3

Table 5: Effects of oral administration of chloroform extract of *Andira inermis* on the Behaviour Changes of albino rat

Experiments	Dose (mg/kg)	Behavioural Changes
Phase 1	10	None
	100	None
	1000	None
Control	0	None
Phase 2	1600	Erection of hair coat
	2900	Restlessness, increase respiratory rate in first 5 minutes
	5000	Refusal to eat after 4 hours of extract administration, salivation and fatigue.

Table 6: Effects of oral administration of ethylacetate extract of *Andirainermis* on the Body Weight changes of albino rat

Experiments	Dose (mg/kg)	Initials (g) 0 day	Weight gain (g) After 7 days	Weight gain (g) After 14 days
Phase 1	10	119.3±1.87	121±0.59	127.3±1.78
	100	117.0±4.06	119.3±2.21	119.0±1.78
	1000	118.7±3.55	125.3±2.21	123.3±1.78
Control	0	119.7±2.98	127.3±2.05	130.7±1.22
Phase 2	1600	135.3±1.01	138.7±0.69	145.3±3.40
	2900	143.7±1.47	134.7±1.69	146.0±2.10
	5000	134.7±1.29	142.7±1.00	145.0±2.67

Values are expressed as Mean±Standard Error of Mean (n=3).

Table 7: Effects of oral administration of ethylacetate extract of *Andira inermis* on the Mortality rate of albino rat.

Experiments	Dose (mg/kg)	Mortality of albino rat After 24hours of Administration observation	Mortality at 14 days
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	0	0/3	0/3
Phase 2	1600	0/3	0/3
	2900	0/3	0/3
	5000	0/3	0/3

Table 8: Effects of oral administration of ethylacetate extract of *Andira inermis* on the behaviour changes of albino rat

Experiments	Dose (mg/kg)	Behavioural Changes
Phase 1	10	None
	100	None
	1000	None
Control	0	None
Phase 2	1600	Salivation, fatigue after 3 hours of extract administration
	2900	Diarrhoea, restlessness after 5 hours of the extract administration
	5000	Refusal to eat and drink after 3 minutes of extract administration

Discussion

The percentage yields of chloroform and ethylacetate extracts after extraction were 0.22% and 1.274% maceration respectively.

The preliminary phytochemical analysis of the stem bark of *Andira inermis* showed the presence of alkaloids and carbohydrates in both the chloroform and ethyl actetate extracts. Though saponins and tannins were absent in the chloroform extracts, they were all present in the ethyl actetate extract. The presence of the tannins proves the purgative activity of the stem bark of *A. inermis.* They may also be subjected to the use of managing asthma, cough [7]. The presence of saponins could suggest that the plant can be used as antihypercholesterol agents as a cardiac depressant medicine. The presence of these secondary metabolites suggests the plant can also be used for its antibacterial activity [8-9].

Toxicity level study of chloroform and ethylacetate extracts on albino rat showed that no animal died within 24 hours and 14 days after oral administration of the extract shown in table 6 and 9 respectively, and the LD_{50} is greater than 5000 mg/kg. The major signs of toxicity noticed within 24 hours include diarrhoea, slight sound, fatigue, restlessness as can be seen in table 7 and 10. These signs were not seen in 10 mg/kg dose group but progressed and became increasingly pronounced as the dose increased toward 5000 mg/kg body weight (b.w.). The body weight changes were calculated and were compared to the control group as can be seen in table 5 and 7. There was a significant difference in body weight gained on day7 ($p< 0.05$) among dose group up to 1000 mg/kg body weight. The albino rat in all experimental groups gained weight over the course of the study especially those albino rats that were administered higher dose. The LD_{50} being greater than 5000 mg/kg body weight is thought to be safe as suggested by Lorke [10]. Again the absence of death among the albino rat in all dose groups throughout the 2 weeks of the experiment seems to support this claim. The LD_{50} value more than 5000 mg/kg, showed that the extract is practically safe.

Finally, the result obtained from this research work may differ from the research carried out by others on the same plant. This may be due to some factors which may include period of collection.

Conclusion

The chloroform and ethylacetate extract of *Andira inermis* reveals the presence of some secondary metabolites like, Alkaloids, which is believed to be most significant and play a metabolic role in the living system and it involved in the protective functions in animals. The chloroform and ethylacetate extracts of *Andira inermis* appear non-toxic by oral administration at the tested doses as indicated by the high oral median lethal dose.

Studies on natural products are becoming more prevalent especially in pharmaceuticals. The products have numerous therapeutic benefits. *A. Inermis* cannot only function as a trado-medicine but also possess immense ethno-botanical importance. However, it is recommended that further toxicity studies are required using different animals. The antibacterial activity of *A. Inermis* could be subjected to further research.

Reference

[1]. Schultes, R.E. and Raffauf, R.F. (1990). An Ethnobotanical Study of the Traditional Medicine of Plant. *Journal of Ethnopharmacology*, 53, 163-171.

[2]. Sanmugapriya E, Venkataraman S (2006). Toxicological investigations on *Strychnos potatorum* seeds in experimental models. *J. Health Sci.,* 52: 339–343

[3]. Konkon, N.G., Adjoungoua, A.L., Manda, P., Simaga, D., N'Guessan, K.E., Kone, B.D. (2008). Toxicology and phytochemical screening study of *Mitragyna inermis* (Willd) O Ktze (Rubiaceae) anti diabetic plant. *Journal of Med Plants Res* Vol. 2(10), pp.279-284.

[4]. Evans, W.C., Trease, G., (2008). *Pharmacognosy*. BrailliarTiridel. Can. Macmillian Publishers, 11th Ed.

[5]. Sofowora, A. (1993). *Medicinal plants and Traditional medicine in Africa*. Spectrum Books. Ltd. Ibadan Nig. pp. 270-289.

[6]. Harborne, J.B. (1973). *Phytochemical methods, London*. Chapman and Hall Ltd. pp. 49-188.

[7]. Gills, L.S. (1992). *Ethnomedical uses of Plants in Nigeria*. University of Benin Press, Nigeria P. 276.

[8]. Lewis, K., Ausubel, F.M. (2006). Prospects for plant derived antibacterials. *Nat. Biotechnol*., 24(12): 1504-1507.

[9]. Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbial*. Rev., 12(4): 564-582.

[10]. Tijani, A., Pomtantz, A., Khawar, R., Fusi, M. (1986). Community acquired infections in children on maintenance cyclosporine therapy. *International Journal of Pediatry Nepthrol*, 7, 141-144.