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Antibacterial, Phytochemical and Preliminary Toxicity Profile Studies of N-hexane Chloroform, Ethyl Acetate and Methanol Extracts of *Andira inermis* Stem Bark (Leguminosea)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Phytochemical screening remains the most effective method for identifying and screening medically active components of plant. This research work studied the phytochemical screening, toxicity profile and antibacterial activity of the stem bark of *Andira inermis* using four different extracts by varying the polarity of the solvents; n-hexane, chloroform, ethyl acetateand methanol. The screening revealed the presence of alkaloids and carbohydrates in all extracts, tannins and saponins were not detected in chloroform and n-hexane extract, glycoside was also not detected in the chloroform extract. The toxicity profile in vivo studies using the Lorke's method revealed no significance changes in the body weight of the albino rats, and the LD50 was higher than 5000mg/kg. However, there was a significant changes in behavior of the albino rats such as, fatigue, diarrhea, restlessness etc at 1600 mg/kg, 2900mg/kg and 5000mg/kg doses of the extracts respectively. Antimicrobial studies was evaluated with four (4) each of different bacteria and fungi. The results

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revealed ethylacetate and chloroform (moderately polar solvents) extracts to be more active against both bacteria and fungi but the methanol (highly polar) and hexane (highly non polar) extracts possessed less activities as compared with the other extracts at 15, 20, 30 and 40% concentrations.

Keywords: Andira inermis; phytochemicals; toxicity profile; antibacterial studies; extracts.

1. INTRODUCTION

Andira inermis (Leguminosea) is a nitrogen-fixing plant and one of the numerous plants used in traditional medicine in many part of the world. It is mainly found in the lowland forests of Southern Mexico and Central America among others, its tree has a good shape, beautiful flowers and has been used as an ornamental [1]. The plant was reported to be used solely or in combination with other plants for the treatment of different diseases including cancer, malaria, anaemia, snake bites and skin diseases etc [2]. The stem bark is used in the Brazilian amazon as a purgative, antihelmintic and a vermifuge, it is poisonous in large doses [1]. Freixa *et al.*[3] reported the methanol and dicholoromethane extracts of the stem bark of for its potential antifungal activity against the fungal strains of *Fusarium oxysporum var pinaster, Microsporum gypseum and Tricophyton mentagrophytes.* The liphophilic extracts of the leaves and the stem bark were also reported to possess an Isoflavones including *calyosin and genistein* which have shown a vitro activity against the chloroquine-sensitive strain poW of *Plasmoduin falciparum* [4]. The roots was also reported to contain Biochanin A, a triglycosideiso flavonol which was presumed to be a cancer inhibitor [5].

In the present study, we screened the stem bark of the plant for more phytochemicals, toxicity effect and antibacterial analysis using four extracts; methanol chloroform, ethyl acetate, and n-hexane. To the best of our knowledge, we came across no report on the antibacterial and toxicity effect of the stem bark of this plant, hence making this report the first of its kind.

2. METHODOLOGY

2.1 Sampling and Sample Treatment

2.1.1 Collection, identification and drying of the sample

The stem bark of the plant was collected from Zuru local government of Kebbi state, Nigeria. The harberium sample of the plant leaves and flower was identified as *Andira inermis* by a taxonomist at the taxonomy unit of the Usmanu Danfodiyo University Sokoto. The sample was air dried under shade, grounded into powder and stored in appropriate container for further use.

2.1.2 Extraction of the sample

Batch extraction using the Soxlet apparatus was perforedto obtain four (4) extract of different polarities. Briefly, powdered sample (100g) was taken in a cotton temble into the extraction unit of the Soxlet, then n–hexane (500mL) was taken into the Round bottom flask and the system refluxed for about 24 hours with continues stirring. The extract was concentrated with rotary evaporator. The marc was treated afterward with chloroform, ethyl acetate and methanol independently using the same method above to obtain the chloroform, ethyl acetate and methanol extracts respectively, inorder of polarity {17].

2.2 Quantitative Phytochemical Screening

The phytochemical screening was conducted using standard procedures according to Trease and Evans [6] cited in Halilu et al [7] to identify the presence of the following metabolites: alkaloids, saponins, tannins, carbohydrates and glycosides.

2.2.1 Test for 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.

2.2.1.1 Mayer's test

The filtrate was treated with Mayer's reagent (potassium mercuric iodide).The formation of a yellow colored precipitate confirmed the presence of alkaloids.

2.2.1.2 Wagner's test

The filtrate was treated with Wagner's reagent (Iodine in potassium iodide). The formation of brown/reddish precipitate indicates the presence of alkaloids.

2.2.1.3 Dragendroff's test

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

2.2.2 Test for tannins

2.2.2.1 Lead acetate test

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

2.2.2.2 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

2.2.3 Test for carbohydrates

The extract was dissolved in 5 mL 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.

2.2.3.1.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.

2.2.3.2 Fehling's test

Filtrate was hydrolyzed with dil. HCl, and then neutralized with alkali and warmed with Fehling's A & B solutions. The formation of red precipitate indicates the presence of reducing sugars.

2.2.4 Test for saponins

2.2.4.1 Frothing test

The extract (2 mL) was diluted with twice its volume of water and shaken in a test tube for 5 minutes. The formation of a honeycombs froth, which lasts for about 45 minutes, indicates the presence of saponins.

2.2.5 Test for cardiac glycosides

2.2.5.1 Keller- Kiliani's test

The extract (1 mL) was diluted with 20 mL of water, 1mL of strong lead sub-acetate solution was added to precipitate pigments, which were filtered off. The filtrate obtained was shaken with equal volume of chloroform and allowed to separate into two layers in a separating funnel. The chloroform layer was removed and evaporated to dryness over a water bath. The residue was dissolved in 3ml of ferric chloride in glacial acetic acid, and then transferred to a dry test tube. Few drops of conc. H_2SO_4 was added by the wall of the test tube, on standing, a brown colour at the interphase (due to deoxy sugars) and a pale green colour in the upper layer (due to steroidal nucleus) represent a preliminary test for digitoxose [7].

2.3 Toxicity Studies

The toxicological studies was conducted according to the modified Lorke's method [8] and Hassan [9], using a total of 21 albino rats. The albino rats were divided into two phases and a control group: phase1 and 2 with 9 albino rats each, and the control group with 3 albino rats. Prior to the oral administration of plant extract, the albino rats were fasted overnight and weighed. The albino rats in Phase 1 (9 mice) were divided into 3 groups of 3 mice each, and were administered with single dose of 10, 100 and 1000 mg/kg (for group 1,2 and 3 respectively) of the extract orally, in other to establish the possible range of doses producing any toxic effect. For the Phase 2, the albinos were also group into 3 (3 albinos per group) and were administered with a single dose 1600, 2900 and 5000 mg/kg of the extract for group 1, 2, and 3 respectively. In both cases (Phase 1 and 2), the control group remain the same and received no dose of the extract, only a distilled water. The albinos were carefully and frequently observed throughout the day of the extract administration and over the period of 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 recorded at 0, 7 and 14 days of the study.

2.4 Antimicrobial Activities of the Extracts

2.4.1 Test organism

Clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Aspergilusniger, A.flavus, A.tricorder* and *A.fumigatus*were obtained from the Department of Microbiology, UsmanuDanfodiyo University Sokoto. The isolates were identified using standard

microbiological procedure as described by Ganapathi et al [10].

2.4.2 Screening for antibacterial and antifungal activities

The antibacterial and antifungal activities of the extracts were assayed as described by Adebayo-Tayo[11] and Adekunle [12]. The standardized suspensions were used to inoculate the surfaces of Muller Hinton agar plates (90 mm in diameter) using sterile cotton swab. 6 mm diameter wells were bored using sterile cork borer in agar and filled with the desired concentrations of the plant extract (15, 20, and 25, 30 and 40%). Commercial antibiotics (ampicillin 30%) was used as reference standard to determine the sensitivity of the isolates. The plates were allowed to stand for 5 hours at room temperature for extracts to diffuse into the agar and then incubated at 37ºC over night. The zone of inhibition was measured using metric rule [13].

3. RESULTS AND DISCUSSION

3.1 Results

Table 1. Result of phytochemical screening of stem bark of *Andira inermis*

S/N	Plant constituents	N-hexane extract	Ethyl acetate extract	Chloroform extract	Methanol extract
1	Alkaloids				
	- Mayers test	+	+	+	N.D
	- Drangendroffs test	+	+	+	+
	- Wagners Test	+	+	+	+
2	Tannins				
	- Ferric Chloride	N.D	+	N.D	+
	- Lead Acetate	N.D	N.D	N.D	N.D
3	Carbohydrates				
	- Fehling test	+	+	+	+
	- Molish test	+	+	+	+
4	Saponins				
	- Froath test	N.D	+	N.D	+
5	Glycosides				
	- Killer kilani test	+	+	N.D	+
	- Lumber test	+	+	N.D	+

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

Table 2. Effect of oral administration of chloroform extract on the Body Weight changes of Albino rat

Experiments	Dose (mg/Kg b.w.)	Initial (g) 0 days	Weight gain (g) After 7 days	Weight gain (g) After 14 days
	10	117.3±1.85	119±0.57	125.3±1.76
Phase 1	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
	1600	133.3±0.89	136.7±0.67	143.3±3.38
Phase 2	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). Values with different superscript in the same rows are significantly different from control (P<0.05), values with the same superscript are not significantly different (P>0.05) using One way ANOVA followed by DunnettMultiple Comparison test using Graph Pad In Stat Version 3.

Table 3. Effect of oral administration of chloroform extract on mortality rate and behavior changes of albino rats

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	1600	0/3	Slight sound, erection of hair coat
	2900	0/3	Restlessness, increase respiratory rate in first 5 minutes
	5000	0/3	Refusal to eat after 4hours of administration, salivation and fatigue.

Table 4. Antibacterial activity of ethyl acetate extract

	Inhibition zone (mm)		
	20%	30%	40%
P.aeruginosa	13	15	18
S.aureus	12	14	15
E.coli	11	12	14
B.subtilis	8	9	11

Table 5. Antibacterial activity of chloroform extract

	Inhibition zone (mm)		
	20%	30%	40%
P.aeruginosa	13	15	18
S.aureus	14	16	17
E.coli	9	11	13
B.subtilis	10	11	13

Table 6. Antibacterial activity of hexane extract

	Inhibition zone (mm)			
	15%	20%	25%	30%
P.aeruginosa	-	-	-	-
S.aureus	2	8	10	12
E.coli	-	2	12	14
B.subtilis	4	10	-	-

Table 7. Antibacterial activity of methanol extract

	Inhibition zone (mm)			
	15%	20%	25%	30%
P.aeruginosa	1	4	6	7
S.aureus	4	2	1	11
E.coli	-	-	-	-
B.subtilis	1	4	2	6

3.2 Discussion

Andira inermis(Leguminusae) is widely employed in folk medicine to treat a number of diseases; skin, anaemie, malaria, snake bites, antihelmintic and cancers. The family Leguminusae are known to be very in reach in isoflavones and other secondary metabolites such as alkaloids and flavonoids [14], which were believed to play a significant role in the protection of the plant

material and its medicinal properties.As a well-known fact, using solvents of different polarity in extraction enable the removal of all/most of the metabolites that could be present in the plant material under extraction, we conduct the extraction using different solvents with increasing polarity (n-hexane, chloroform, ethyl acetate and methanol). The extracts were screened for the following metabolites: Alkaloids, tannins, carbohydrates, saponins and glycosides. Table 1 summarized the results obtained from the screening of the stem bark of *A.inermis*. Alkaloids and carbohydrates were the only metabolites common in all the four extracts (Table 1). Glycosides were present in three of the extracts (ethyl acetate, n-hexane and methanol), except in the chloroform extract. Tannins and saponins were also not detected in chloroform and N-hexane extracts.Overall, the results in Table 1.revealed that the stem bark of *A.inermis* possessed a wide variety of phytochemicals, all the five metabolites tested (alkaloids, tannins, carbohydrates, saponins, and glycosides) are present in the stem back of *A.inermis* either in one extract or the other. It is notable that, we decided not to screen for the presence of flavonoid because most of the reports we came across already focuses mainly on it. [5,15]

Toxicity lethal studies of chloroform extract on albino rats are reported in Table 2 and 3.Table 2 shows the effect of oral administration of the 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 (1600mg/kg, 2900mg/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 shows a progressive changes in the body weight (g) of the albino rats over the course of the study as the doses increases. The effect of oral administration of the chloroform 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 chloroform extract of different doses on the albino rats, no mortality case was recorded, this shows that the LD_{50} is greater than the highest tested dose (5000 mg/kg) administered. Therefore, the dose of 5000 mg/kg of body weight is thought to be a safe dose. It is noteworthy, although the higher doses (1600, 2900, and 5000mg/kg b.w respectively) of the extract could be safe, they could have some significant effect on the behaviors of the albino rats as observed during the time of the study.

The toxicity lethal studies was further investigated for the ethyl acetate, methanol and n-hexane extracts, (Table 1-6 supporting documents). Like in the case of the chloroform extract, the ethyl acetate, methanol and the n-hexane extracts also showed no significant difference in the body weight of the albino rats in the phase 1 (10, 100, 1000 mg/kg b.w) from those in the control group ($p>0.05$). On the other hand, the albino rat in phase 2 (1600, 2900 and 5000mg/kg b.w) showed a significant difference in their body weight from those in the control group at the initial day, 7th day and the 14th day of administering the extract.

Antibacterial activity of the plant extracts revealed that, the extracts possessed significant activity against clinical isolates of *P.aeruginosa, S.aureus, E.coli*and *B.subtilis*. The ethylacetate and chloroform extracts showed highest activity from 8 to 18mm zone of inhibition. This is in agreement with the result of phytochemical screening since tannins, flavonoids and saponins were detected in ethylacetate and chloroform extract. It is generally believed that tannins, flavonoids and saponins containing compounds possessed biological activity [7]. Very little or no activity against bacteria was detected with n-haxane and methanol extracts and again is due to absence of microbiologically active compounds like saponins, tannins and flavonoids.

4. CONCLUSION

Phytochemical screening of the extractsdetectthe presence of Alkaloids, carbohydrates, glycosides, tannins, and saponins in the stem bark of *Andira inermis* (Legeminusea). The toxicity lethal studies revealed that the LD50 of the stem bark in greater than 5000mg/kg and has no significant effect on the body weight of the albino rats after 14 days. Antibacterial activity showed that the ethylacetate and chloroform extracts possessed significant activity against clinical isolates of *P. aeruginosa, S. aureus, E. coli* and *B. subtilis*. But n-hexane and methanol extracts revealed little or no antibacterial activity. It is concluded that ethylacetate and chloroform extracts showed activity mainly due to presence of tannins and saponins and lack of these

phytoconstituents is the reason why n-hexane and methanol extracts show very little activities.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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