Evaluation of the antibacterial and chromatographic activity of the stem bark of *Andira Inermis* (Cabbage Tree)

Quazim B. Adebisi*, Andrew D. Gosomji, Bwede D. Danbok and Emeka Aniobi
[1]*Department of Chemistry, Federal college of Education Pankshin Plateau State, Nigeria*
*Corresponding Author**

Abstract: **Plants have been used for centuries for better health care management of mankind. Modern science is now re-focusing its attention on natural plant therapies as a source of dealing with recent health challenges. The aim of this study focuses on the chromatographic and antibacterial activity of the chloroform and ethyl acetate extracts of Andira Inermis (cabbage tree). The Thin layer chromatography test detected 11 components with retention factors between 0.16 and 0.95 proving the presence of secondary metabolites as phytochemicals present within the plant. Antibacterial effects of these extracts were tested against clinical isolates of bacillus subtilis, Escherichia coli, staphylococcus aureus and Pseudomonas aeruginosa at concentrations of 20, 30 and 40mg/ml. The extracts showed inhibitory effects on the microorganisms with inhibition diameter ranges of 8-18mm. However, the chloroform extract showed greater inhibitory action against staphylococcus aureus at the concentration of 40mg/ml. Therefore, the study justifies the use of Andira inermis as a potential source of antibiotics, and suggests the use of more highly effective organic solvents for its extraction.**

Keywords: **antibacterial activity; andira inermis; phytochemistry; inoculation; thin layer chromatography**

I. 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. Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of many people living in Northern Nigeria. From the very beginning of human civilization, people have used different plant parts to treat various diseases. This reality has forced many researchers to study the toxicity levels of many plants with specific medicinal activity. One of such plants includes "*Andira Inermis*".

Andira inermis (A. Inermis) also referred to as "Cabbage tree" is a beautiful tree grown mostly in tropical America and West African countries including Nigeria. The plant is known for its many secondary metabolites with therapeutic importance (Sanmugapriya and Venkataraman, 2006). Many plants have been subjected to phytochemical screening to determine the phyto-constituents present therein, *A.Inermis* is no exception. However, research on the antibacterial potentials of the plant have been scanty and far-fetched.

Antibacterial are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterial is generally considered low and safe for prescription. The discovery, development and use of antibacterial during the 20^{th} century has reduced mortality from bacterial infections. Antibacterial are among the most commonly used drugs and among the drugs commonly misused by physicians, for example, in viral respiratory tract infections. However, micro-organisms are becoming resistance to antibiotics making treatment of infectious diseases difficult. Therefore, the need to synthesize and modify natural compound with biological activities is imperative. Most natural products are in limited supply therefore, synthetic route is used to provide sufficient quantity of natural product.

The beneficial effects of plant materials typically result from the combinations of phytochemicals that have several biological effects on microorganisms. These phytochemicals differ from plant to plant and examples include: Anthraquinones, flavonoids, alkaloids, steroids, tannins (Bohm and Kocipai, 1994), saponins, glycosides, and terpenoids. The presence of such a phytochemical of interest may lead to its further isolation, purification and characterization (Oomah, 2003), then it can be used as the basis for a new pharmaceutical product. It is against this background that the study is aimed at evaluating the chromatographic (using Thin layer Chromatography technique) and antibacterial activities of *Andira Inermis* against clinical isolates of bacteria.

II. EXPERIMENTAL

2.1 Sample collection and processing

The *Andira Inermis* stem bark were collected from an uncultivated land at Pankshin Local Government Area of Plateau State, Nigeria located between Latitude $9^{o}19'$ 31.48'' N and Longitude $9^{o}26'$ 6.72'' E. The sample was identified in the Department of Biology, Federal College of Education Pankshin, Plateau State, Nigeria. Using mortar and pestle, the stem barks were grinded into powder and stored for further analysis.

2.2 Preparation of Test Organisms

The test organisms were clinical isolates of *bacillus subtilis (B. subtilis), Escherichia coli (E. coli), staphylococcus aureus (S. aureus)*and *Pseudomonas aeruginosa (P. aeruginosa)* obtained from the department of pharmacognosy, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto, Nigeria. They were characterized based on the standard procedure adopted from Cheesbrough (2006).

2.3 Extraction

Extraction was carried out by maceration in batches using ethyl acetate and chloroform as extraction solvents. Two batches of 50g of powdered *A.Inermis* each was dissolved in 200ml of ethyl acetate and chloroform in two separate conical flasks. Each of the flask contained one of the two solvents. After 2hrs of stirring, a sheet of foil paper was used to cover the flask and left for 24hrs. It was then filtered twice and the marc was allowed to concentrate for two days (Deepika *et al*., 2016).

2.4 Thin Layer Chromatography (TLC) Analysis

Using a capillary tube, a small spot of solution containing the samples (that of ethyl acetate and chloroform extract of *andira inermis*) was applied to a plate, about 1.5cm from the bottom edge. The specific amount of an appropriate solvent (N-hexane, chloroform and ethyl acetate) were used in different ratios, starting from 9:1,8:2 and 7:3 by pouring it into a TLC tank to a depth of less than 1cm. The TLC plate was then placed in the chamber so that the spots of the sample do not touch the surface of the eluent in the chamber, and the lid was closed. The solvents moved up the plate by capillary action, met the sample mixture and then carried it up the plate. The plate was removed from the chamber before solvent front reached the top of the stationary phase. Without delay, the solvent front, the furthest extent of solvent up the plate was marked.

2.5 Antibacterial Activity

Prior to the screening 0.5% McFarland equivalent standard was prepared (European Committee on Antimicrobial Susceptibility Testing, 2012). 20 ml of Nutrient agar plates that has been checked for sterility were seeded with 2 ml of an overnight broth culture of each bacterial isolate in a sterile Petri-dish. The seeded plates were allowed to set after a uniform distribution of the bacterial isolate following slow rotation of the Petri dish. A standard sterile cork borer of 6 mm diameter was used to cut uniform wells on the surface of the agar. The wells filled with 0.5 ml of each prepared extract concentrations of (20, 30 and 40 mg/ml) with the aid of a sterile syringe. The plates were then allowed to stand for 1 hour at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 37°C for 24 hours and observed for zones of inhibition. A zone of clearance round each well signifies inhibition and the diameter of such zones were measured in millimetre (mm).

III. RESULTS AND DISCUSSION

3.1 Thin Layer Chromatography

For the chloroform extract using ethyl acetate and n-hexane solvent in the ratio of 9:1, the retention factor (Rf) was calculated to be 0.32 and 0.45 for the two components detected as presented in Table 1. It can also be seen from Table 1, the retention factors of the chloroform extract using ethyl acetate and n-hexane solvent in the ratio of 8:2 as 0.22 and 0.33, as well as the solvent ratio of 7:3 as 042 and 0.95. The ethyl acetate of *A.Inermis* was run through the TLC technique with use of chloroform and n-hexane as solvents in the ratios of 9:1, 8:2 and 7:3. The Rf of the solvent ratios were 0.24, 0.32 and 0.49 for ratio 9:1, 0.16 for ratio 8:2 and 0.25 for ratio 7:3 as seen in Table 2.

The Rf values obtained from the phytochemicals provide important information about their polarity and important clues for the separation of these phytochemicals in the separation process. Different Rf values of the compound also reflect an idea about their polarity. The use of the various solvent systems for TLC studies could be important for the selection of the appropriate solvent system.

The conducted TLC was used to separate the constituents of secondary metabolites of plant extracts. The chromatograms have validated the presence of several types of saponins, glycosides, carbohydrates, alkaloids relative to phytochemical reactions in *A.Inermis* stem bark (Adebisi *et al., 2020*).

Table 1: Result of TLC analysis of the chloroform extract of *Andira Inermis* using ethyl acetate and N-hexane as solvents

No. of Component	Ratio	Distance of Spot (cm)	Solvent Front (cm)	Retention factor
1.	9:1	1.4	4.4	0.32
2.	9:1	2.0	4.4	0.45
3.	8:2	1.1	4.9	0.22
4.	8:2	1.6	4.9	0.33
5.	7:3	1.8	4.3	0.42
6.	7:3	4.1	4.3	0.95

Table 2: Result of TLC analysis of the ethyl acetate extract of *Andira Inermis* using chloroform and N-hexane as solvents

No. of Component	Ratio	Distance of Spot (cm)	Solvent Front (cm)	Retention factor
1.	9:1	1.0	4.1	0.24
2.	9:1	1.3	4.1	0.34
3.	9:1	2	4.1	0.49
4.	8:2	0.7	4.5	0.16
5.	7:3	0.9	3.6	0.25

3.2 Antibacterial Activity

The extracts of *Andira Inermis* stem bark showed zones of inhibition ranging from 8-18mm as shown in Table 3. As can be seen from Figures 1 and 2, the chloroform extract of *A.Inermis* was significantly active against *staphylococcus aureus* and *pseudomonas aeuroginosa* at concentrations above 30mg/ml. The ethyl acetate extract of *A.Inermis* was also active against *staphylococcus aureus* and *pseudomonas aeuroginosa* at concentrations above 30mg/ml, however its effects was significantly low against *bacillus subtilis and E.coli* at concentrations between 20-40mg/ml.

It can be seen that the extracts were more active against the gram negative test organisms (*E.coli* and *pseudomonas aeruginosa*) than they were when used against the gram positive test organism (*B.subtilis*). The activity of the extracts on *E.coli* and other gram negative microorganisms is in line with the work carried out by Aliero and Wara (2009), despite the difference in concentration and solvent of extraction.

Table 3: Result of the preliminary screening of the antibacterial activity of ethyl acetate and chloroform extracts of *Andira Inermis*.

Test Organisms	Concentration (mg/ml)	Inhibition Zone (mm)	
		Chloroform	Ethyl Acetate
Bacillus Subtilis	20	10	8
	30	11	9
	40	13	11
Escherichia coli	20	9	11
	30	11	12
	40	13	14
Staphylococcus aureus	20	14	12
	30	16	14
	40	17	15
Pseudomonas aeruginosa	20	13	13
	30	15	15
	40	18	18

IV. CONCLUSIONS

The present study has shown that the stem bark of *Andira inermis* examined has significant antibacterial activity. The inhibition level of the chloroform extract showed significant activity against the four test organisms (*staphylococcus aureus, pseudomonas aeuroginosa, bacillus subtilis and E.coli*) at concentrations above 30mg/ml. The results of the TLC conducted on the plant also provided evidence to prove the presence of secondary metabolites with diverse therapeutic importance. The stem bark of *A.Inermis* can be used as an antibacterial agent in treating bacteria-related diseases.

REFERENCES

[1] Adebisi, Q.B., Ogori, B.O., and Dakup, N.D. (2021) Phytochemical screening and toxicity profile of chloroform and ethyl acetate extract of *Andira inermis. Chemistry Research Journal*, 6(2):1-7

[2] Aliero, A. A. and Wara, S.H. (2009). "Validating the medicinal potential of *Leptadenia hastata". African Journal of Pharmacy and Pharmacology*, 3:335 - 338.

[3] Bohm, B.A. and Kocipai A.R. (1994) Flavonoids and condensed Tannins from leaves of *Hawaiian Vaccinium vaticulatum* and *V. Calycinium. Pac Sci.*, 48(4):458–463.

[4] Cheesbrough, M. (2006). "District laboratory practice in tropical countries." Part 2. Cambridge University Press, Cambridge, UK, 12(1): 137-150.

[5] Deepika, K., Kumar, M.V., Radhika, J.S., and Rajagopal, S.V. (2016). Studies on Isolation, Purification and Structural Elucidation of Chemical Constituents from Methanolic Flower Extract of *Blepharis Molluginifolia Pers.* and their Biological Activities. *IJPSR*; Vol. 7(12):4873-4904

[6] European Committee on Antimicrobial Susceptibility Testing (EUCAST). (2012). "Disc diffusion method for antimicrobial susceptibility testing". Version 2. 1.

[7] Oomah, D.B. (2003). Isolation, characterization and assessment of secondary metabolites from plants for use in human health. *PBI Bull*;13–20.

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