Original Research Article

SCREENING *ASCLEPIAS SYRIACA* FOR ANTIMICROBIAL ACTIVITY

ABSTRACT

Antibiotic resistance, as well as the evolution and spread of new strains of disease causing agents are of great concern to the global health community. The high cost of antibiotics has made it inevitable to search for cheaper sources of antimicrobials. There has been considerable interest in the use of plant materials as an alternative method of controlling pathogenic microorganisms.

Asclepias syriaca is one of such plants that need to be investigated in view of its acclaimed medicinal uses. It has been reported to contain certain bioactive chemicals which could be antimicrobial in action. This work investigated *Asclepias syriaca* stem for antimicrobial activity. Cold water, Hot water and Methanolic extracts of Asclepias stem were prepared using standard methods. The extracts were used to carry out susceptibility test, determine minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the stem extract on *Salmonella typhi, Salmonella typhimurium,* and *Pseudomonas aeruginosa*. The extracts were found to be effective on the three test organisms, with the methanolic extract being most active, followed by hot water extract, then cold water extract. Inhibitory zone diameter ranged between15 to 26mm for Methanolic extract, 14 to 23mm for Hot water extract, and 12 to 20mm for Cold water extract. The extracts were most active on *Pseudomonas aeruginosa,* then *Salmonella typhi*, and lastly *Salmonella typhimurium.* The MIC of the extract on *Salmonella typhimirium* was 200mg/mL for both the Cold and Hot water extracts, but 100 mg/mL for methanolic extract. The MIC of the extract on *Salmonella typhi* was 12.50mg/mL for Methalonic extract and for both Cold and Hot water extracts. For *Pseudomonas aeruginosa,* the MIC was 3.125mg/mL for all the extracts, i.e Cold and Hot water extracts, as well as Methanolic extract. MIC of the standard antibiotic was 3.125mg/mL.

The MBC result showed no inhibition by all the extracts on *Salmonella typhimurium*, even at 400 mg/mL. All the extracts had MBC at 400 mg/mL for *Salmonella typhi*. All the extracts had MBC at 200 mg/mL on *Pseudomonas aeruginosa*, while MBC of the standard control on all test bacteria was 6.25 mg/mL. From our results, it is evident that *Asclepias syriaca* stem extracts possesses antimicrobial property which can be exploited to treat *Salmonella typhi* infection as used traditionally, as well as infections caused by *Pseudomonas aeruginosa*.

Key words: Asclepias syriaca, Extracts, Antimicrobial, Methalonic extract, Salmonella typhi

1.0 INTRODUCTION

Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of drugs is exploitation of plants for their medicinal value [1].

Recently, there has been considerable interest in the use of plant materials as an alternative method of controlling pathogenic microorganisms [2], and many compounds from plants have been shown to be effective against resistant pathogenic bacteria [3]. According to [4], medicinal plants are the best sources to obtain a variety of new herbal drugs. About 80% of individuals from developing countries use traditional medicine, which has substances derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [4].

Finding healing powers in plants is an ancient idea. People on all continents long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. Different parts of different plants such as the roots, bark, leaves, flowers, and fruits are composed of phytochemicals, which when used, either possess a healing effect to the symptoms of a disease, or clear up infection [5]. This knowledge has led to this research, which seeks to investigate the antimicrobial property of *Asclepias syriaca* with a view to exploit it for large scale production.

Asclepias syrica (milkweed) has been known for many years for its folklore uses, being used to cure many diseases. Of particular importance is its ability to cure diseases caused by microorganisms. The leaves are also described as having anticancer effect, wound healing, diuretic, anti-asthmatic, being also used to treat bronchitis, pneumonia, rheumatism, and kidney stones [6]. The root is anodyne, diaphoretic, diuretic, emetic, expectorant and purgative. It has been used in the treatment of asthma, kidney stones and venereal disease [7].The plant is also said to contain phytochemicals tannins, saponins, glycosides, alkaloids and phenols, which are known to have antimicrobial activities [8].

In view of the high cost of orthodox antimicrobials and microbial resistance against them, milkweed could be a good candidate for the production of natural, cheap and effective antimicrobials against several deadly diseases. This work is, therefore, set up to investigate the antimicrobial activity of the stem of this important age long plant.

2.0 MATERIALS AND METHODS

2.1 Area of Study

The study was carried out in Bingham University located at AutaBalefi, Karu, Nasarawa state. It has a tropical climate with two distinct seasons; rainy and dry seasons. The university covers a land mass of 200 square meters and is geographically located at latitude $8^{o}50^{o}$N and longitude $7^{o}52^{o}$E. It is found 26km away from Abuja, the capital of Nigeria [9].

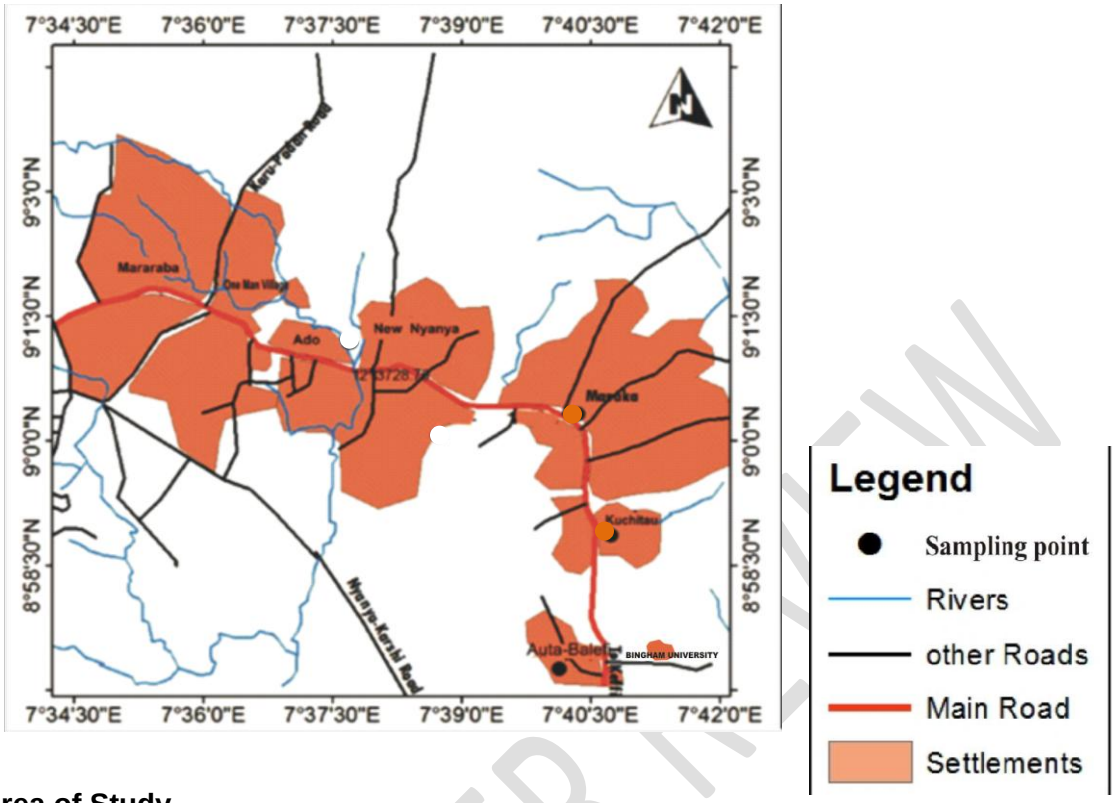

Fig 1: Area of Study

2.2 Sample Collection

The plant was collected and dried at room temperature for about two weeks; it was then separated into leaves, fruits, stem and roots. The stems were pounded using a pestle and mortar and further pulverized using an electric blender to reduce it to powder form it was then stored until required for analysis.

2.3 Preparation of Plant Extracts

Three different extracts were prepared, hot water extract according to the local method used, cold water extract and methanol extract. These were carried out according to the methods described by [10].

2.3.1 Hot Water Extraction

200g of powdered leaves was suspended in 1000ml of distilled water and allowed to boil for 15 mins, it was then left to stand for about 6hours to ensure maximum extraction of metabolites. The mixture was then sieved using a sterile sieve and subjected to freeze drying and stored in air tight containers.

2.3.2 Cold Water Extraction

200g of the powdered leaves was weighed into a sterile jar and mixed with 1000ml of cold water, it was left to stand for 24 hours at room temperature to allow for maximum extraction. It was then filtered using a sterile sieve and subjected to freeze drying and stored in air tight containers.

2.3.3 Methanol Extraction

200g of the powdered leaves was soaked in 1000ml of methanol for 48 hours, it was then extracted using a soxhlet apparatus and subjected to drying using a rotary evaporator to evaporate the methanol leaving the extract residue behind. It was then stored in airtight containers.

2.4 Source of bacterial isolates

The isolates were gotten from the Bacteriology division of National Veterinary Research Institute, Vom, Plateau State, they were transported to Bingham University using the appropriate medium and sub-cultured unto nutrient agar plates and incubated at 37^0C for 24 hours. These plate agar cultures were subsequently use for testing the antimicrobial activity of the plant extract.

2.5 Preparation of McFarland Turbidity Standard

Barium sulphate suspension at 1.0% w/v was prepared as follows: One percent (1% w/v) solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 in 99ml of water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of barium chloride in 50ml of distilled water. Barium chloride solution (0.6ml) was added to 99.4ml of sulphuric acid solution to yield 1.0% w/v barium sulphate suspension. The turbid solution formed was transferred into a test tube as the standard for comparison

2.5.1 Preparation of inoculum

A colony of the microorganism was dissolved in sterile normal saline solution and its turbidity was compared to that of the McFarland standard, its turbidity was adjusted until it was equal to that of the McFarland standard.

2.6 Preparation of dilution of different extracts

0.8g of the hot, cold and methanolic extracts were dissolved in 8ml of Dimethyl Sulphate ($DmSO_4$) respectively to give a stock solution of 800mg/ml, doubling dilution was then carried out to give concentrations of 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml.

2.6.1 Preparation of control

For the control, ciprofloxacin was used, because it is a broad spectrum antibiotic and a first line drug for typhoid fever.

10g of Ciprofloxacin was dissolved in 10ml of distilled water from which 100 $\mathrm{mg/mL}$ was taken into the first well of the positive control microtitre plate.

2.7 Susceptibility test

For the susceptibility test, plates of Mueller Hilton agar were uniformly seeded with the test bacteria separately. Sterile Number 1 Whattman filter paper discs soaked in 200mg/ml concentration of the methanolic extract (with excess extract drained at the wall of the bottle),

were placed on the seeded agar surface. The standard antibiotic, Ciprofloxacin (10μg) was also placed at one edge on the agar surface for each test organism. This was to compare the activity of the extract with the standard antibiotic. The plates were allowed to rest on the work bench for 15 minutes after which they were incubated in a preset incubator at 37^0C for 24 hours. After incubation plates were examined and inhibition zone diameter around each disc was recorded against each test organism.

2.8 Determination of Minimal Inhibitory Concentration (MIC) using 96 well microtitre plate method

The minimum inhibitory concentration (MIC) for plant extract was evaluated using 96-well microtitre plates, two microtitre plates were used. For each plate, 100μl of Mueller Hilton Broth MHB) was placed in each well followed by 100 μl of plant extract (which contained 400 mg/ml of plant extract) added to the first column of the microplates. This made each well of the first column have a total volume of 200 μl. Serial dilution (doubling dilution) was carried out starting from the first column to the eighth column by taking out 100μl from the first well to the next until the eighth column well, after which 100 μl mixture in the eighth column well was taken and discarded. After the dilution 50 μl of the bacterial suspension which was compared to Mcfarland standard with density of $1.5x10^8$colony forming unit per ml (cfu/ml) was added to each of the wells. Subsequently, the plates were incubated for 24hrs at 37◦C incubator. The minimum inhibitory concentration (MIC) was determined by adding 30 μl (0.005g mg/ml) of resazurin dye and incubated at37◦C for one hour. Resazurin dye was used as an indicator for bacteria growth; bacteria metabolized it and changed from purple to pink color. The well that had no change in color after the addition of resazurin dye indicated no growth of the microorganisms. The concentration of the first well in which there was no growth for each organism was taken as the MIC value [11].

2.9 Determination of Minimal Bactericidal Concentration

The MBC is defined as the lowest concentration where no bacterial growth is observed upon sub-culturing. This was determined by aseptically sub-culturing the contents of wells with no growth from the MIC results for individual bacterium on Mueller Hilton agar and incubated for 24hrs, the concentration of the plates which recorded no growth were taken as the MBC.

3.0 RESULTS

3.1 Susceptibility test

The extracts were found to be effective on the three test organisms, with the methanolic extract being most active, followed by hot water extract, then cold water extract. Inhibitory zone diameters ranged between15 to 26mm for Methanolic extract, 14 to 23mm for Hot water extract, and 12 to 20mm for Cold water extract. The extracts were most active on *Pseudomonas aeruginosa,* then *Salmonella typhi*, and lastly *Salmonella typhimurium* (Table 1).

Table 1: Susceptibility test result of Asclepias extracts on *S. typhi, S. typhimurium, P. aeruginosa*

Zone of inhibition (mm)

	Hot water extract	Cold water extract	Methanol extract	Control (Ciprofloxacin)
S. typhimurium	14	12	15	16
S. typhi	16	15	18	18
P. aeruginosa	23	20	26	25

3.2 Minimum Inhibitory Concentration (MIC)

The MIC of the extract on *Salmonella typhimirium* was 200mg/mL for both the Cold and Hot water extracts, but 100 mg/mL for methanolic extract. The MIC of the extract on *Salmonella typhi* was 12.50mg/mL for Cold and Hot water extracts as well as Methalonic extract. For *Pseudomonas aeruginosa* the MIC was 3.125mg/mL for all the extracts, i.e Cold and Hot water extracts, as well as Methanolic extract (Table 2).

Table 2: Minimum Inhibitory Concentration of *Asclepias syriaca* extracts on *Salmonella typhi, Salmonella typhimurium* and *Pseudomonas aeruginosa*

Name of microorganisms	**MIC (mg/mL)**			
	Hot extracts	Cold extracts	Methanol extracts	Control
Salmonella typhimurium	200	200	100	3.125
Salmonella typhi	12.50	12.50	12.50	3.125
Pseudomonas aeruginosa	3.125	3.125	3.125	3.125

3.3 Minimum Bactericidal Concentration (MBC)

The MBC results showed no bactericidal on *Salmonella typhimurium*, even at 400 mg/mL which was the highest concentration used. All the extracts (cold water, hot water and methanol extracts) had MBC at 400 mg/mL for *Salmonella typhi*. All the extracts had MBC at 200 mg/mL on *Pseudomonas aeruginosa*, while MBC of the standard control on all test bacteria was 6.25 mg/mL.

Table 3: Minimum Bactericidal Concentration of *Asclepias syriaca* extracts on *Salmonella typhimurium*, *Salmonella typhi* and *Pseudomonas aeruginosa*

Name of microorganisms	MBC (mg/mL)			
	Hot extracts	Cold extracts	Methanol extracts	Control
Salmonella typhimurium	-	-	-	6.25
Salmonella typhi	400	400	400	6.25
Pseudomonas aeruginosa	200	200	200	6.25

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

Table 1 shows that the extracts were effective on the three test microbes, with the methanolic extract showing most of the antimicrobial activity, followed by hot water extract and then the cold water extract. Inhibitory zone diameters ranged between15 to 26mm for Methanolic extract, 14 to 23mm for Hot water extract, and 12 to 20mm for Cold water extract.

Table 2 shows the MIC values of the extract against the test microbes. From the table, the extracts showed weak antimicrobial activity on *Salmonella typhimurium*, with MIC at 200 mg/mL for both hot water and cold water extracts while the methanolic extract was at 100 mg/mL. The extracts showed more antimicrobial activity against *Salmonella typhi* with an MIC of 12.50 mg/mL for the hot water extract, cold water and methanolic extract separately. This means that irrespective of the method of extraction, the plant proved to have antimicrobial activity against *Salmonella typhi,* the causative agent of human typhoid. However, the plant showed a remarkable activity against *Pseudomonas aeruginosa* with all the extracts having an MIC of 3.125 mg/mL, just as the standard/control drug. The antimicrobial activity of the plant extract is attributable to its phytochemical components of which most of them are known to have antimicrobial activity.

From table 3, the extracts were not inhibitory to *Salmonella typhimurium,* even at 400 mg/mL which was the highest concentration used for MBC determination. The extracts, however, showed bacteriocidal activity at 400 mg/mL against *Salmonella typhi.* For *Pseudomonas aeruginosa* the MBC was 200mg/mL. The high MBC value obtained could suggest that the plant had a bacteriocidal effect on the test organisms used except *Salmonella typhimurium*.

The MIC was recorded at 3.125 mg/mL for all the microorganisms and the MBC at 6.25 mg/mL. The MIC was the same for both the plant extracts and the standard/control antibiotic against *Pseudomonas aeruginosa.* The plant extracts studied compared favorably well with the standard antibiotic used with MIC ranging between 100mg/mL and 3.125mg/mL for the organisms used.

Just as [12] said, *Asclepias* has for a very long time been used to treat ailments by the native Canadians. Furthermore a research by [13] showed *Asclepias syriaca* at different concentrations showed antimicrobial property against methicillin resistant *Staphylococcus aureus* and *Streptococcus faecium*. The work of [14] also reported the antimicrobial activity of *Asclepias syriaca against Escherichia coli* and *Pseudomonas aeruginosa*. This research further proved the antimicrobial activity of *Asclepias syriaca* as it has now been shown to have a high antimicrobial activity against *Pseudomonas aeruginosa* and *Salmonella typhi*. Its low antimicrobial activity to *Salmonella typhimurium* is unique and may be attributed to the fact that it is not a human pathogen. Further studies may expose, in the nearest future other reasons why the extract is not inhibitory to *Salmonella typhimurium*.

4.2 CONCLUSION

Asclepias syriaca has for a very long time been used traditionally as a medicine to cure ailments. This research focused on finding scientific backing for claims of its healing property. Its antimicrobial activity has been determined on certain drug resistant pathogenic microrganisms. The plant has shown weak antimicrobial activity on *Salmonella typhimurium,* but relatively high antimicrobial activity against *Salmonella typhi and Pseudomonas aeruginosa.* The plant could be further assayed for its antimicrobial properties on other pathogens.

4.3 RECOMMENDATIONS

- Based on the results from this study, *Asclepias syriaca* has been proved to contain antimicrobial properties; therefore it should be further exploited as a means of treatment for drug resistant microorganisms.
- Further study should be done on the toxicity aspect of *Asclepias syriaca* so as to ascertain its safety.

- **COMPETING INTERESTS DISCLAIMER:**
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- Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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