COMPARATIVE ASSESSMENT OF ANTIBACTERIAL ACTIVITIES OF *ASCLEPIAS SYRIACA* (MILK WEED) LEAF EXTRACT AND ANTIBIOTIC DRUGS ON METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS, STAPHYLOCOCCUS AUREUS* AND *STREPTOCOCCUS FAECIUM*

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ABSTRACT

This study was carried out to comparatively assess the antibacterial activities of the leaves extract of Asclepias syriaca (Milk weed) with antibiotics commonly used in the study area (gentamycin, tetracycline and ampiclox) on Methicillin Resistant Staphylococcus aureus, Staphylococcus aureus and Stretococcus faecium. The study employed standard procedures to prepare the extract of the Asclepias syriaca, and then at the five concentration levels of 1mg/ml, 5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml, the growth and inhibitions of Methicillin Resistant Staphylococcus aureus (MRSA), Staphylococcus aureus and Stretococcus faecium were examined. The results showed that the three bacteria species were found resistant to the antibacterial properties of the plant and no inhibition occurs at 1mg/ml but only at 25mg/ml for MRSA and inhibition occurs at 5mg/ml for both Staphylococcus aureus and Stretococcus faecium. The results further showed that the zones of inhibition increases as the concentration increases given different zones of inhibition ranging from 8.6mm – 15mm at MIC level and

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27.0mm -31.3mm at 100mg/ml respectively. The control experiment with antibiotics and ethanol showed that gentamycin was very effective on the tested pathogens while tetracycline and ampiclox have reduced inhibitory effects but ethanol was found ineffective. The study therefore suggests that the Asclepias syriaca as a plant is a good source of antimicrobial agents. The ethnobotanical importance of this plant in traditional medicines is also justified and as such further investigation is needed for the isolation of the active compound responsible for the antibacterial activities.

Keywords: *Antibiotics, antibacterial, Staphylococcus, Asclepias syriaca, ethnobotany, herbal, Ilese-Ogun*

1. Introduction

Plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments (Grabley and Thiericke, 1999). The plants contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1982). The discoveries of antimicrobial drugs have proven effective for the control of bacterial infection, because pathogens abase unconditionally but some pathogens rapidly become resistant to some drugs (Howard et al, 1996).

Furthermore, antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics (Iwu at al., 1999). Positive response of plant based drugs (less/ no side effects) might lies in the structure of the natural products which reacts with toxins and/or pathogens in such a way that less harm is done to other important molecules or physiology of host. It is because of this reason that drug designing studies nowadays have come up as new field of research (Sharma and Kumar, 2008).

Emergence of multi-drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants (Iqbal and Arina, 2000). However, some new compounds that inhibit the growth of microorganism have been isolated from plants (Cox, 1994). These plants are effective in the treatment of infectious diseases while

simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids which are capable of producing definite physiological action on body (Joshi *et al.*, 2009). Plants with anti-bacterial effect are rich in polyphenolic substances such as tannins, alkaloids, steroids and polyphenolic acids. These phenolic substances as well as the alkaloids in plants have been listed as the most important bioactive constituents of natural products (Edeoga *et al.,* 2005), which are valuable supplements used for the maintenance of human health (Kumar *et al.,* 2005) and sometimes possessing remarkable therapeutic potentials. Therefore, for the purpose of the study, *Asclepias Syriaca* were tested on the following three bacteria were used (a) Methicillin-Resistance *Staphylococcus Aureus (MRSA)* (b) *Streptococcus faecalis* and (c) *Staphylococcus aureus.*

It should be noted that, the evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine (Frey and Meyers, 2010). Based on the aforementioned the experimental study were carried out to determine the efficiency of *Asclepias Syriaca* (Milk Weed) to isolate and inhibit the growth of the three bacteria considered for the study.

1.1. Aims of the Study

The aim of the study is to investigate the antibacterial effects of the ethanolic *Asclepias syriaca* extracts on the bacteria pathogens of Methicillin Resistant *Staphylococcus aureus*, *Streptococcus faecalis*, and *Staphylococcus aureus* and compare the effectiveness with other common antibiotic drugs

2. Brief Description of the *Asclepias syriaca* (milk weed)

The common milkweed, *A. syriaca*, is the plant that most people associate with the word "milkweed". This is a tall and conspicuous species that sometimes forms large clones. The

umbels bear large balls of pink to purplish flowers that have an attractive odor. This species is known to form hybrids with both *A. exaltata* (in the east) and *A. speciosa* (in the west).. Among the milkweeds, this species is the best at colonizing in disturbed sites. Within its range it can be found in a broad array of habitats from croplands, to pastures, roadsides, ditches and old fields. It has been traditionally reported that *A. syriaca* has a lot of medicinal and therapeutic qualities. Among the different medicinal and healing properties of *A. syriaca* it has been said that the fruit is antiparasitic. Constituents of the plants includes: the root contains a glucosidal principle, Asclepiadin, which occurs as an amorphous body, is soluble in ether, alcohol and hot water. It also contains several resins, and odorous fatty matter, and a trace of volatile oil and it is antispasmodic, diaphoretic, expectorant, tonic, carminative and mildly cathartic (Tierra, 2008), (Annie's Remedy, 2013) and (Wikipedia, 2013).

3. Materials and Methods

The standard examination and methods for detection of bacteria, and preparation of the extraction were employed.

3.1 Procedures for the Plants Extract

Fresh pealed samples of *Asclepias syriaca* were collected in and around Ilese township Ogun State. The fresh pealed samples of *Asclepias syriaca* were dried properly under room temperature for two weeks and then oven dried at 80°C for 20 minutes to facilitate grinding into powdery form. Then, 250g of plants sample was then measured for extraction.

3.1 1. Extraction Procedures

a. The 250g of the powdered sample *Asclepias syriaca* Leaves were measured

b Soaked the measured powdered in Solvent (ethanol).

c. Obtained the Ethanolic extract (Maceration) using 98% ethanol for three days.

d The mixture is filtered (filtration) to produce the filtrate in a water bath which took hours to produce the paste texture crude extract of (*Asclepias syriaca*).

e. The extract was dispensed into a clean sterilized Mcartney bottle and stored in the refrigerator at 10- 20°C.

3.2 Preparation of Media

Two different media were prepared (Nutrient agar and Nutrient broth). Nutrient agar- 7gram of nutrient agar was dissolved in 250ml of distilled water in a conical flask and covered with foil paper which was then sterilized by autoclaving at 121°C for 15 minutes (Cheesbrough, 2006). After autoclaving, it was allowed to cool to about 45°C and subsequently dispensed into Petri-dishes under sprit lamp to avoid contamination. The plates were allowed to gel (solidified) and about 15minutes from already cooled nutrient agar medium was dispensed in each of the Petri-dishes and allowed to gel. Nutrient broth- 2.5g of nutrient broth medium was weigh into 100ml conical flask and autoclaved at 121°C for 15 minutes. This medium was allowed to cool to about 43°C and dispensed into sterile Mc Cartney bottles. (Cheesbrough, 2006)

3.3 Preparation of the Bacteria

Pure culture of the three bacteria species: *Methicillin Resistant Staphylococcus aureus (MRSA) Streptococcus faecalis, and Staphylococcus aureus* were inoculated into the already prepared bottles containing nutrient broth and left to grow overnight. This process was just to grow homogenous culture of the bacteria for biological assay that followed in the next day.14.0g of Nutrient agar medium was weighed into conical flask; distilled water was added to make 500ml solution. This was autoclaved at 121°C for 15 minutes as well. After sterilization, the medium was removed and cooled to about 45°C. The overnight grown broth culture that contained the tested bacteria species with detailed label were brought out from incubator. Sterile swab sticks were used each for a bacterium, by rubbing the stick containing the broth culture of the bacteria over the gelled nutrient agar plates separately. The plates were gently and carefully rubbed, to allow the broth to mix totally with the nutrient agar.

The essence of this was to have medium of homogenous growth for each bacterium, so that the plant extracts at different concentrations will have equal access to the same concentration of the bacterium cells.

3.4. Plant Extract Preparation

Different concentrations prepared from the extracts were made with the aid of digital weighing balance into different plates and diluted into 1mg, 5mg, 25mg, 50mg and 100mg concentrations.

Each of the concentration was diluted with 1ml of ethanol to make the following concentrations 1mg/ml, 5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml concentrations. The plates were carefully surface rubbed with already prepared overnight broth culture of the test pathogens using swab sticks. With the aid of cork- borer four wells were bored on a plate and three plates were assigned to a species of the test bacterium. The different concentrations of the extracts were inoculated into their respective well (see the figures 2, 3 and 4 below) on different plates of the bacteria species. The plates having inoculated them with the plant extract were incubated for another 24 hours. Observation was made after 24 hours. Inhibitory zones were recorded for those concentrations that inhibited the growth of the bacteria. The measurement of the zones of inhibition was taken as shown below according to NCCLS (1998).

4. Results and Discussion

The table 1 below, showed the Minimum Inhibitory Concentration (MIC) of the *Asclepias syriaca* ethanol extract on the three bacterial species: *Methicillin Resistant Staphylococcus aureus (MRSA), Staphylococcus aureus* and *Stretococcus faecium* different concentration of 1mg/ml, 5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml. All the three bacteria species were found resistant to the antibacterial properties of this plant and no inhibition occurs at 1mg/ml and 5mg/ml for *MRSA.* Inhibition occurs at 5mg/ml for both *Stretococcus faecium* and *Staphylococcus aureus.* The zones of inhibition increases as the concentration increases given different zones of inhibition.

Extract of *Asclepias syriaca* at different concentrations have properties that can interfere with the growth of the three bacteria species and that the inhibition is subjected to the concentration of the plant extracts. The control experiment with antibiotics and ethanol showed that gentamycin inhibited the growth of the test organisms while ampiclox and tetracycline has reduced effect on the bacterial species and ethanol was found ineffective.

Table 1: *The Diameter Zones of Inhibition of the Activities of Asclepias Syriaca on the three Bacteria Species at different concentrations*

Conc. mg/ml	Diameter Zones of Inhibition (mm) on the Bacteria											
	MRSA				*S. aureus*				*S. faecium*			
	A	**B**	**C**	**Mean**	**A**	**B**	**C**	**Mean**	**A**	**B**	**C**	**Means**
1	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	10	9	10	9.6	11	11	10	10.6
25	15	16	14	15	15	17	16	16	15	14	16	15
50	25	26	26	25.6	19	21	20	20	25	24	24	24.3
100	30	31	30	31.3	26	27	28	27	27	28	26	27.0

Source: Authors laboratory result, 2013

The Minimum Inhibitory Concentration (MIC) of *A. syriaca* on *MRSA* was noticed at 25 mg/ml given average zone of inhibition of 15mm. The inhibition increases as the concentration increases given the average of 31.3mm at concentration of 100 mg/ml. Extract of *A. syriaca* inhibited *Staphylococcus aureus* at 5mg/ml given MIC of 9.6mm; 16.6mm (50 mg/ml) and at 100mg/ml having 27.0mm. *Stretococcus faecium* was equally inhibited at 5mg/ml giving MIC of 10.6mm; 24.3mm at 50 mg/ml and 27.0mm at concentration of 100mg/ml.

Table 2*: The Zones of Inhibition (mm) of antibiotics on The Tested Bacteria Species at 0.5mg/ml concentrations*

Drugs	**MRSA**	**S. aurerus**	**S.faecium**
Gentamycin	2077	24	23
Ampiclox	17	12	12
Tetracycline	16	10	12
Ethanol 100%	-	-	-

Source: Authors laboratory result, 2013

Fig 1: Showing the *Asclepsia syriaca*

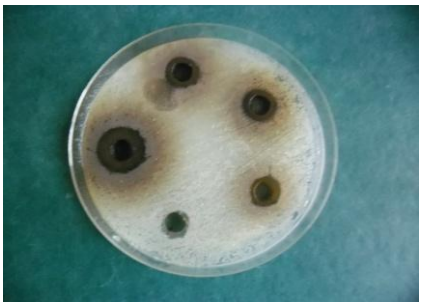

Fig.2 *A. Syriaca* on *S. faecalis*

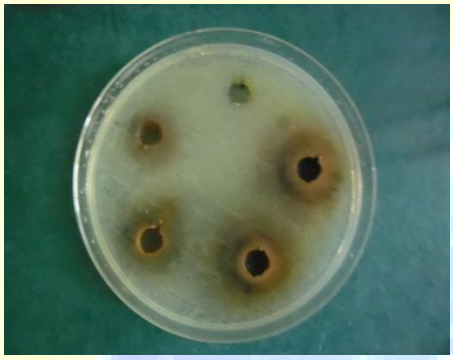

Fig 3: *A. Syriaca* on *S. aureus*

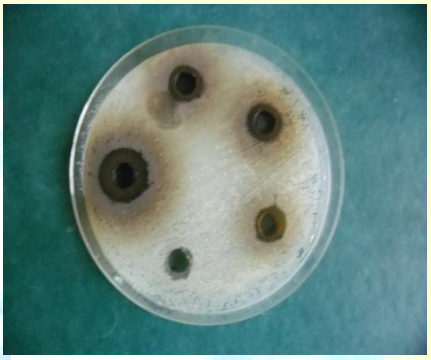

Fig 4: *A. Syriaca* on *MRSA*

Gentamicin, ampiclox and tetracycline were used as the antibiotics for the check on the control responses of the test organisms. Gentamycin belongs to the group of antibiotics called amino glycoside which inhibited the bacterial protein synthesis. This group of antibiotics is effective against many of both gram negative and gram positive organisms. They are most widely effective against Gram negative enteric organisms (Infoplease 2013). Invariably, it was observed that gentamycin proved to be more effective against all the bacteria with inhibitory zone ranging between 20.00mm and 24.0mm. On the other hand, Ampiclox belongs to a group of synthetic antibiotics, a broad-spectrum antibiotic, effective against both Gram positive and Gram negative bacteria, it was observed in this study (Tables 2) that Ampiclox and tetracycline have lower inhibitory potentials when compared with Gentamycin, with average inhibitory zones ranging from 12.0mm to 17.0mm. Since the bacteria were inhibited at 5mg/ml and 25mg/ml with increased zones of inhibition as the concentration increases, this shows that the plant can be used when treating bacterial infections caused by the tested organisms. The leaves of *A. syriaca* could be taken along side with the antibiotics in a patient receiving treatment against the tested organisms implicated diseases. It is recommended that macerating the *A. syriaca* leaves herbal

products or concoctions could be effective and the use in ethnobotany is justified by this study. And as well, the use of this plant with the antibiotics could also be effective.

5. Conclusion

In conclusion, the study has shown that the *A. syriaca* leave extract has properties that inhibited the growth of bacteria and could serve as a source of plant-derived products that modify antibiotic resistance for use against multidrug-resistant bacteria and enteric bacteria species which increasingly posing threat to the health of the people. The study also suggests that the *A. syriaca* as a plant is a good source of antimicrobial agents. The ethnobotanical importance of this plant in traditional medicines is also justified and as such further investigation is needed for the isolation of the active compound responsible for this antimicrobial work.

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