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ANTIMICROBIAL ACTIVITIES OF *DATURA STRAMONIUM* (DEVIL'S WEED) LEAVES AND ROOT EXTRACT ON *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA*

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Abstract: *Datura stramonium (D. stramoniun)* is an annual herb of the Solanaceae family which is commonly known as Thorn apple, Jimson weed and locally as 'Haukata Yaro' in Hausa, 'Miniaramuo' in Igbo and 'Apikan' in Yoruba. This study was to assess the antimicrobial activity of Ethanolic and Aqueous extracts of the leaf and root of *Datura stramonium*, against some selected pathogenic organisms namely, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The concentrations of the extracts used were between 100mg/ml, 80mg/ml, 60 mg/ml, 40mg/ml and 20mg/ml for each. Antimicrobial activity with Ethanolic leaf extract of *Datura stramonium* produced inhibitions zone of 21mm and 17mm of 100mg/ml, and 20mg/ml against *Pseudomonas aeruginosa* and a zone of 20mm and 16mm for concentration of 100mg/ml%, and 20mg/ml% against *Staphylococcus aureus*. The Aqueous leaf extract produced inhibition zone of 21mm and 16mm against *Pseudomonas aeruginosa* as well as 22mm and 18mm against *Staphylococcus aureus*. Ethanol roots extract produced inhibition zone of 18mm, and 14mm against *Pseudomonas aeruginosa*, while 22mm and 17mm against *Staphylococcus aureus*. The Aqueous root extract produced inhibition zone of 21mm, and 19mm against *Pseudomonas aeruginosa* as well as 21mm, and 17mm against *Staphylococcus aureus*. The Minimum Inhibitory Concentration (MIC) of *Datura stramonium* ethanol leaf extract of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 50mg/ml and 25mg/ml respectively. The MIC of aqueous leaves extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 50mg/ml. The MIC of ethanol roots extracts of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 12.5mg/ml and 25mg/ml respectively. The MIC for Aqueous roots extract of *Pseudomonas aeruginosa* was 50mg/ml, 12.5mg/ml and *Staphylococcus aureus* was 25mg/ml. Therefore the demonstration of antimicrobial activity against test organisms is an indication that the plant is a potential source for the production of drugs with a broad spectrum of activity.

Key word: *Datura stramonium, Pseudomonas aeruginosa*, *Staphylococcus aureus*, Antimicrobial, Ethanolic, Aqueous, extracts, leaf, and root

Introduction

An antimicrobial agent (also known as an antibiotic) is a naturally or artificially synthetic or a semi synthetic chemical agent which when released in low concentrations, is able to inhibit the growth of bacteria. The effects of these agents can either be bactericidal, fungicidal etc. (if they cause the death of the target agent), or bacteriostatic or fungicidal etc. (if they inhibit the growth of the target organism for a period of time) (Cheesbrough, 2006). It is important to note that not all antimicrobial agents at the concentration required to be effective are completely non-toxic to human cells (Cheesbrough, 2006). Generally, Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too (El-Mahmood *et al.*, 2010). Plant like *Datura* species have been exploited time immemorial for their ability to inhibit or killed microorganism.

Datura stramonium (D.stramonium) is an annual herb from the Solanaceae family which is commonly known as Thorn apple, Jimson weed, prickly burr, moon flower, devil's weed, devil's cucumber, devil's trumpet. And locally as 'Miniaramuo' in igbo, 'Haukata Yaro' in Hausa, 'Zakedi' in Kanuri and 'Apikan' in Yoruba (Gidado *et al*., 2001; Shobha *et al*., *2014*). According to Shagal *et al.* (2012) plants usually contain bioactive/biochemical constituent which are active substances technically referred to as drugs, and over the years these drugs have been exploited as traditional medicine for the treatment of various ailments afflicting man, many Infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Phytochemical analysis reported by Evans and Evans (2003) reveals that *D. stramonium* composes of different types of bioactive or biochemical constituent which include Tannins, Saponins, Alkaloids, Glycoside, Flavonoids, Steroids and Phenols. According to Chavhan *et al*. (2018) the phytochemical screening of different plant part reveal that the root consist of 3α, 6β-ditigloyloxytropane, 3α, 6β- ditigloyloxytropan-7β-ol, tigloidine, apohyoscine, hyoscine, 3α-tigloyloxytropan, norhyoscine, meteloidine, hyoscimine, cuscohygrine, and tropine. The phytochemical screening of aqueous extract of *D. stramonium* leaves revealed the presence of carbohydrates, tannins, steroidal glycosides, phenols and saponins (Shobha *et al.*, 2014).

The development of resistance to most of the available antimicrobial agents and the high costs of treatment consequent upon this resistance and has necessitated a search for new, safe, efficient and effective agents for the management of infections. Several pathogens have evolved immunity to multiple antibiotics as a result of the mutagenic characteristics of the bacterial genome, rapid multiplication, and transformation of bacterial cells. As a result, it has become imperative to combat the emerging and re-emerging infectious disease with a view to discovering and inventing new agents of greater therapeutic profile to mitigate frequent outbreaks of diseases has posed threat to global health. The aim of this study is to determine the antimicrobial activities of the aqueous and ethanol extracts of *Datura stramonium* root and leaves extracts on *Staphylococcus aurues* and *Pseudomonas aeruginosa*.

Materials and Method

Study Area and Population

This study was carried out in Federal University Wukari, Nigeria. Wukari Metropolis is a large town which is the Headquarters of Wukari Local Government Area of Taraba State. The River Donga and River Benue passes through this area (Blench, 2012). The Local Government Area shares boundary with Benue and Nasarawa state to the south and west respectively. Geographically it is between latitude $9^0$53'42'' North and longitude $9^0$47'59'' East (Blench, 2012, Agwaranze *at al.,* 2024). It is one of the major towns in Taraba state and has an area of 4,308km^2 and a population of 241,546 in the 2006 census .The major spoken languages include, Jukun, Hausa, Fulani and Tiv. The predominant occupations of the people are agriculture, commerce and civil service (Brown *et al*., 2023).

Collection of Samples

The *D. stramonium* leaf and root were collected from different regions (mission quarter, new site, yam market and ibi roundabout) of Wukari town, Taraba State, Nigeria. The plant was authenticated by a botanist from the Department of Biological Sciences, Federal University Wukari Nigeria. It was rinsed with tap water to remove some adhered salts, organism or dust (Baynesagne *et al*., 2017).

Preparation and of extract of D. stramonium leaf and root

The *D. stramonium fresh* leaf and root were cut into pieces, pulverized with a sterilized mortar and pestle, and air dried at room temperature for one week to ensure that the sample lost most of their moisture content. After which the samples were blended into fine powder using electric blender. The blended paste was packed into an air tight container and kept in a cool dry place. The 200grams of fine powder of *D. stramonium* leaf and root was weighed separately into each 500ml reagent bottle and 400ml of extraction solvent was added and left to macerate for 72hours at room temperature. This was done using two extraction solvents (water and ethanol). The extract solution was filtered aseptically into another 500ml reagent bottle using a watt-man No. 1 filter paper or Muslin cloth, following the methods outlined by Temitope *et al*. (2016), and the filtrates were evaporated using a rotary evaporator. The crude extracts were dried *in vial and* stored at 4°C in a refrigerator until screened for antibacterial activity (Baynesagne *et al*., 2017).

Preparation of Extract Concentrations

The extracts were dissolved in Dimethyl sulfoxide (DMSO). Stock solution of extracts were prepared by weighting 5mg of extract and dissolved in 10ml of (DMSO) in plastic container to make 100% concentration, and a sequential dilution were made to obtain various concentration

Standardization of the Test Organisms

The inoculums were standardized using 0.5 McFarland standards. The organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were standardized using the Mc Farland spectrophotometer at wavelength of 625.0. A loopful of the confirmed test isolates was picked using a sterile wire loop and emulsified into 2 ml of sterile normal saline to match the 0.5 McFarland Standard as described by Magashi and Abdulmalik (2018).

Determination of Antimicrobial Effects of Leaf and Root Extract

The antimicrobial effects of root and leaf extracts on test organisms were determined using the agar well diffusion method, as outlined by Vinay *et al*. (2017). Sterile petri plates were filled with 20 ml of Muller's Hilton Agar media and allowed to solidify. Following this, 0.1ml of a 24 hour fresh culture containing 10^5 cells/ml of each test organism (*Staphylococcus aureus* and *Pseudomonas* spp.) was spread over the agar plates. Using a sterile cork borer (6 mm diameter), wells was created in the plates. Six holes were bored into each plate, with different concentrations (100%, 80%, 60%, 40%, and 20%) of the extract dissolved in dimethyl sulfuroxide (DMSO) introduced into individual wells. Additionally, one hole in each plate was reserved as a positive control using ciprofloxacin (0.04mg/ml). The plates were allowed to stand for 30 minutes to 1 hour so that the extracts could percolate the medium, and thereafter they were incubated at 37 °C for 24 hours, after which the plates were observed for clear zones of inhibition, indicating the presence of antibacterial activity

Determination of Minimum Inhibitory Concentration of Extracts (MIC)

The MIC assay was conducted using the agar diffusion method detailed by Coker *et al*. (2021a, 2021b). To start, 2 milliliters (2ml) of the extracts at varying concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml) were combined with 20 ml of pre-sterilized molten Muller-Hinton agar at 45°C. This mixture was poured into sterile Petri dishes and left to solidify. The surface of the agar was then inoculated with a standardized amount of bacteria. Subsequently, the plates were placed in an incubator at 37°C for 24 hours. The lowest concentration that prevented or inhibited bacterial growth was identified as the MIC. Negative control agar plates without the extract were also employed for comparison.

Results and Discussion

Plant derived drugs come into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. Various plants have been employed as a result of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the flora. Medicinal plants constitute an efficient beginning for both traditional and advanced medical specialty. The epoch of utilizing Plant as herbal remedies and drugs product have been employed since prehistoric times to treat human and animal diseases, and several countries still rely on plants and herbs as the main sources of drugs (Ogbonnia *et al*., 2008). Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too, due to easy accessibility and price effectiveness of this medicine. In essence the demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural product. Herbal medicine is an important part of both traditional and modern system of medicines (Priyanka *et al.*, 2012).

Table 4.1, displayed the result of the antimicrobial activity of different concentrations of ethanol leaf extract of *Datura stramonium* on the test organisms; *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which indicate that the ethanol leaf extract has inhibitory effect against the test organism (in essence it has a pronounce activity against staphylococcus aureus, and pseudomonas aeruginosa) .This may be as a result of the factors explained by Jun *et al*. (2007).

Table 4.1: Antimicrobial Activity of Ethanol Leaf Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100%	80%	60%	40%	20%	Positive control
Pseudomonas aeruginosa	21mm	18mm	16mm	18mm	17mm	26mm
Staphylococcus aureus	20mm	18mm	21mm	19mm	16mm	25mm

Key: mg/ml = milligram per mile, (-) = No Inhibition

Table 4.2, displayed the result of the antimicrobial activity of different concentrations of aqueous leaf extract of Datura *stramonium* on the test organisms; *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which indicate that the aqueous leaf extract had more inhibitory effect on *Staphylococcus aureus* compared to *Pseudomonas aeruginosa.* This may be as a result of the factors explained by Jun *et al*. (2007).

Table 4.2: Antimicrobial Activity of Aqueous Leaf Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100%	80%	60%	40%	20%	Positive control
Pseudomonas aeruginosa	21mm	16mm	18mm	20mm	16mm	26mm
Staphylococcus aureus	22mm	-	20mm	20mm	18mm	25mm

Key: mg/ml = milligram per mile, (-) = No Inhibition

Table 4.3, displayed the result of the antimicrobial activity of different concentrations of ethanol roots extract of *Datura stramonium* on the test organisms; *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which indicate that the ethanol roots extract has more pronounce effect against *Staphylococcus aureus* compared to *Pseudomonas aeruginosa.* This may be as a result of the factors explained by Jun *et al*. (2007).

Table 4.3: Antimicrobial Activity of Ethanol Root Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100%	80%	60%	40%	20%	Positive control
Pseudomonas aeruginosa	18mm	17mm	15mm	15mm	14mm	26mm
Staphylococcus aureus	22mm	17mm	-	18mm	17mm	25mm

Key: mg/ml = milligram per mile, (-) = No Inhibition

Table 4.4, displayed the result of the antimicrobial activity of different concentrations of aqueous roots extract of *Datura stramonium* on the test organisms; *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* which indicate that the Aqueous roots extract had more effect on *Pseudomonas aeruginosa* compared to *Staphylococcus aureus.* This may be as a result of the factors explained by Jun *et al*. (2007).

Table 4.4: Antimicrobial Activity of Aqueous Roots Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100%	80%	60%	40%	20%	Positive control
Pseudomonas aeruginosa	21mm	19mm	18mm	21mm	19mm	26mm
Staphylococcus aureus	21mm	18mm	20mm	17mm	17mm	25mm

Key: mg/ml = milligram per mile, (-) = No Inhibition

Table 4.5, above displayed the result of minimum inhibitory concentration of ethanol leaf extract of *Datura stramonium* against the test organisms *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The ethanol extract of *Datura stramonium* showed a minimum inhibitory concentration against *Pseudomonas aeruginosa* at 50mg/ml and 25mg/ml on *Staphylococcus aure*

Table 4.5: MIC of Aqueous Leaf Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100	50	25	12.5	6.25	3.125
Pseudomonas aeruginosa	+	+	-	+	-	-
Staphylococcus aureus	+	-	+	-	+	-

Key: mg/ml = milligram per mile, (-) = No Inhibition, (+) = Inhibition

Table 4.6, displayed the result of minimum inhibitory concentration of Aqueous leaf extract of *Datura stramonium* against the test organisms *Pseudomonas aeruginosa* And *Staphylococcus aureus*. The Aqueous extract of *Datura stramonium* showed a minimum inhibitory concentration of 50mg/ml on both *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Table 4.6: MIC of Aqueous Leaf Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100	50	25	12.5	6.25	3.125
Pseudomonas aeruginosa	+	+	+	-	+	-
Staphylococcus aureus	+	+	-	+	-	-

Key: mg/ml = milligram per mile, (-) = No Inhibition, (+) = Inhibition

Table 4.7, displayed the result of minimum inhibitory concentration of ethanol roots extract of *Datura stramonium*. The ethanol extract of *datura stramonium* roots showed a minimum inhibitory concentration of

12.5mg/ml on *Pseudomonas aeruginosa* and 25mg/ml on *Staphylococcus aureus.*

Table 4.7: MIC of Ethanolic Roots Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100	50	25	12.5	6.25	3.125
Pseudomonas aeruginosa	+	-	+	+	-	-
Staphylococcus aureus	+	-	+	-	+	-

Key: mg/ml = milligram per mile, (-) = No Inhibition, (+) = Inhibition

Table 4.8, displayed the result of minimum inhibitory concentration of Aqueous roots extract of *Datura stramonium*. The Aqueous extract of *Datura stramonium* root showed a minimum inhibitory concentration of 50mg/ml, 12.5mg/ml on *Pseudomonas aeruginosa* and 25mg/ml on *Staphylococcus aureus.*

Table 4.8: MIC of Aqueous Root Extract of *Datura stramonium* showing the Zone of Inhibition (mm)

	CONCENTRATIONS (mg/ml)					
ORGANISMS	100	50	25	12.5	6.25	3.125
Pseudomonas aeruginosa	+	+	-	+	-	-
Staphylococcus aureus	+	-	+	-	+	-

Key: mg/ml = milligram per mile, (-) = No Inhibition, (+) = Inhibition

Phytochemical analysis of this medicinal plants reveals that the plants composes of different types of bioactive or biochemical constituent which include Tannins, Saponins, Alkaloids, Glycoside, Flavonoids, Steroids, protein, carbohydrate and Phenols. This could exhibit antimicrobial activity (Evans and Evans, 2003). In this present work, ethanolic and aqueous extracts of *Datura stramonium* leaves and roots were subjected for the antimicrobial analysis against three standard isolates which are *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.*

The result of the antimicrobial analysis shows that the ethanol and aqueous extracts exhibit activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* as compared with Ciprofloxacin standard drug in conformity to Baynesagne *et al*. (2017). But a little deviation occur, this may be as a result of the factors explained by Jun *et al*. (2007).

Conclusions

The demonstration of antimicrobial activity against test organisms is an indication that the plant is a potential source for the production of drugs with a broad spectrum of activity. The result obtained shows that ethanol and aqueous extract of *Datura stramonium* leaf and root has showed more pronounce activities against *Staphylococcus aureus* compared to *Pseudomonas aeruginosa.* These results supports the traditional use of *Datura stramonium* to treat diseases and shows that the plant contains beneficial biological activities such as anticancer, antioxidant, Anti-inflammatory Activity, antimicrobial, skin disorder, Antiasthmatic Activity, and Malaria as well as Impotency activities. But a great caution is advised since excess dose cause severe intoxication and death.

Recommendations

Since the result has shown that *Datura stramonium* leaves and roots can be used in the treatment of infectious diseases, therefore the researcher recommends the following;

1. Traditional herbalists should be educated on the medicinal benefits of the *Datura stramonium* leaves and roots and its appropriate dosage.

2. The government should encourage research programs to enhance other researchers to make more discoveries on the medicinal uses of the *Datura stramonium* plant.

3. Extracts obtained from *Datura stramonium* in this study were tested only against bacteria and further investigation is necessary to validate against fungal species since the local community uses the leaf of this plant to treat bacterial and fungal infections.

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