Activities of *Datura stramonium* Extracts against clinical pathogens

ABSTRACT

AIM: The antimicrobial activities of the ethanolic extracts of *D. Stramonium* pulp, seed and leaf against some medically important pathogenic microorganisms were studied. **METHODOLOGY:** The antimicrobial activities of the ethanolic extracts of *D. Stramonium* pulp, seed and leaf were assessed on *Bacillus subtilis, Streptococcus pneumoniae* and *Staphylococcus aureus* (Gram-positive bacteria) and *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* (Gram-negative bacteria).
RESULT: The highest percentage recovery at 50% ethanolic extract of leaf was 5.6±0.1 and lowest in Pulp with 3.9±0.1. The 50% ethanolic extracts showed significant activities against tested pathogens more than the 75% ethanolic extracts which, may be due to the effect of heat generated by water bath during extraction process. The plant extracts exerted highest zones of inhibition in pulp and seed extracts against *P. aeruginosa* with 21±1.0 and 17±2.0 respectively and least in *K. pneumoniae* with 10±0.5 from seed extract. The antimicrobial activities observed in this study were due to the presence of certain phytochemials that have bactericidal or inhibitory effects on test organisms. These phytochemicals include alkaloids, tannins, flavonoids, saponins, terpenoids, phenol and glycosides.
CONCLUSION: *D. stramonium* extracts revealed very promising results with health-promoting potentials that could be applied in the treatment of ailments caused by these pathogens.

Keywords: *Datura stramonium*, Pathogens, Ethanol extracts, Phytochemicals

1. INTRODUCTION

Plant extracts contain extensive range of chemical compounds that can be used to treat long-lasting diseases as well as transmittable infections [1-3]. “These chemical compounds which include tannins, alkaloids, terpenoids and flavonoids exhibit antimicrobial, antioxidant, anti-infectious and antitumor activities” [1-4]. “During the past decades, infectious diseases have been the leading cause of death throughout the globe, particularly in the developing countries” [5]. “Some of the pathogens have developed resistance to multiple antibiotics as a result of the mutagenic characteristics of the bacterial genome, rapid multiplication, and transformation of bacterial cells. Consequently, numerous surveys have been carried out in search of novel medicinal plants with potent antibacterial effects against these pathogens” [6].

“Plants have always been a source of drugs for the treatment of human ailments, and millions of people in the world rely on medicinal plants for primary health care, income generation, and livelihood improvement”. [7]. “Demand for medicinal plant is on the increase due to the availability, affordability, reliability, accessibility and low side effects in therapeutic use. There are nearly 1000 medicinal plants that constitute about 10% of the entire flora available in the state” [8]. “Medicinal plants contain some organic compounds which provide definite physiological action on human body. For several years, the bulk of these plant materials have been employed by the local community as an alternative medicine to treat many diseases, even though most of them are not well characterized scientifically” [9]. Today, uses of plant

extracts are still employed in treating diseases since they are traditionally practical, harmless to health when used with considerable dose.

Datura stramonium in the family, Solanaceae, is a well-known medicinal plant [10] mostly found in the tropical and warm temperate regions of the world including Nigeria. It is widely distributed and can be found in Africa, Australia, America, Europe and Asia [11] either as native or adventive plants. This aggressive invasive weed is known predominantly for its alkaloidal contents, most important of which are the tropane alkaloids namely hyoscyamine, hyoscine and atropine [12-13]. "Due to the presence of these significant bioactive components, *D. stramonium* has been used for centuries in some cultures as a poison and hallucinogen" [14]. "It is also considered to be important in treating heart disease, dental and skin infections, ulcer, asthma, bronchitis, leukoderma, fever and piles, sinus infections; it has antimicrobial, anticholinergic, anti-inflammatory, anti-fungal, antioxidant, hypolipidemic, anti-asthmatic, analgesic, insecticidal, neurological, antirheumatoid and hypoglycemic properties" [15-18]. "Of the ten species of *Datura* found all over the world, *D. anoxia* and *D. stramonium* are the most important drug plants" [19]. "All parts of the plant are toxic, but the highest amount of alkaloids is contained in the ripe seeds" [20-21]. "Many cases of accidental poisoning by *D. stramonium* have been reported when these plants were eaten accidentally" [22].

Many researches have been carried out on *D. stramonium* such as its antibacterial and antifungal activities [23], its antibacterial activities and phytochemical analysis of the leaves and seed extracts [24], pharmacological properties [19], the antimicrobial investigation of its leaf extract against different microorganisms [25] etc., none has combined the antimicrobial effects of the leaves, seeds and pulp of *D. stramonium* on some selected microorganisms. Hence, this study is aimed at investigating the antibacterial activities of *D. stramonium* leaf, pulp and seed extracts as well as to determine its inhibitory concentration on some selected pathogenic microorganism.

2. MATERIALS AND METHODS

Fresh plants of *D. stramonium* were harvested from the Federal Polytechnic Ado-Ekiti forest and transported to the laboratory. The leaves, seeds and the pulps were separated and air dried at 27^{o}C for about 15 days. They were ground to a fine powder using blending machine. The powdered forms were respectively extracted by soaking in 50% and 75% ethanol, with constant stirring for 72 hours. The extracting solvents were evaporated to dryness.

2.1 Phytochemical analysis of the ethanol extract of *D. stramonium*

Qualitative preliminary phytochemical screening tests were carried out for 80% methanol root extract of *D. stramonium* using standard procedures [26-27], to determine the presence or absence of alkaloids, phenols, flavonoids, tannins, saponins, anthraquinones, terpenoids, glycosides, and steroids. Antimicrobial activities of the mushroom extracts were determined by agar well diffusion method. The bacterial strains used as indicator organisms were cultivated on Nutrient Agar Medium at 37 ± 1^{o}C for 24 hours while the fungal strains were cultivated on Potato Dextrose Agar at 26 ± 1^{o}C for 48 to 72 hours. The inoculums suspension were standardized before use and then tested against the effect of the mushroom extracts. A 100µl of the aliquot was aseptically pour plated in sterile Petri dishes. NA and PDA (20ml) were poured into the sterilized Petri dishes and gently stirred for

even distribution of the inoculums. Wells of 5mm diameter were bored in the agar with sterile cork-borers. For the investigation of the antibacterial and antifungal activities, the dried mushroom extracts were dissolved in sterile distilled water and sterilized by filtration through 0.22Åm membrane filter. A 100µ volume was introduced into wells of agar plates directly. The plates were incubated at 37± 1^{o}C (for bacteria) for 24 hours and 26 ± 1^{o}C for 48 to 72 hours (for fungi). At the end of incubation period, inhibition zones formed on the medium were evaluated in mm. Amoxicillin, streptomycin and chloramphenicol were used as standard antibacterial agents while ketoconazole was used as antifungal standard under standard conditions respectively. The diameter of the inhibition zones were measured in milliliters (mm). Inhibition zones were measured in triplicates. Agar wells with distilled water were used as negative control. The inhibitory action of negative control was not visible. Studies were performed in triplicate.

2.2 Media preparation

Moller Hinton agar was prepared according to manufacturer specification, the agar was poured into a sterile petri dish and was allowed to solidify.

2.3 Preparation of test microorganisms

In this study, *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the bacterial strains, used. The bacteria were seeded into each plate and cork-borer of 6mm was used to make a hole on the plate and each extract solution was totaled to the hole and a sensitive disk was placed on extra six plates that have been seeded with each organism to serves as control. The plates were incubated at 37^{o}C for 24 hours for bacterial growth to occur as well as for zone of inhibition to be observed.

2.4 Phytochemical Analysis

Standard biochemical methods were followed for phytochemical analysis of the ethanolic extract for the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, phenol and glycosides [28].

Test for tannin

To 0.5 ml extract solution, 1 ml distilled water and 1-2 drops of ferric chloride solution was added and observed for blue black colouration which indicates the presence of tannin.

Test for saponin

0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing shows the presence of saponin.

Test for flavonoid

0.2 g of the extract was dissolved in 10% NaOH solution, yellow colouration indicates the presence of flavonoid.

Test for phenol

To 2 ml of extract solution, 2 ml of alcohol and few drops of ferric chloride solution were added and observed for change in colour.

Test for cardiac glycoside
5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the present of cardiac glycoside. (A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed).

Test for alkaloid
0.5 g extract was boiled with concentrated HCl and filtered. A different test tube was used to add 0.5 ml of picric acid and 1 ml of the filtrate, and the contents were then checked for coloured precipitate or turbidity.

Test for anthraquinone
To 0.2 g of extract, 5 ml of chloroform and 5 ml diluted ammonia were added. The presence of bright pink colour in the aqueous layer indicated the presence of anthraquinone.

Test for terpenoid and steroid
To create a layer, 5 ml of extract solution was combined with 2 ml of chloroform and 3 ml of strong sulphuric acid. The interface developed a reddish brown coloration to signify the presence of terpenoids. The presence of steroids is indicated by the lower surface's red colour.
Test for reducing sugar
To 0.5 ml of extract solution, 1 ml of water was added and heated after adding 5 to 8 drops of Fehling's solution. Brick red precipitation indicated the presence of reducing sugar.

2.5 Statistical Analysis
The data obtained during the investigations were subjected to Analysis of Variance and inferences made at $P<0.05$ using the SPSS 23.0 software package. Duncan's New Multiple Range Test was used to separate means. The test provides significance levels for the difference between any pair of means.

3. RESULTS AND DISCUSSION

3.1 Results
The result in table 1 shows the percentage recovery of the extracts. The highest value of percentage recovery for *D. stramonium* was observed in the Leaf extract (5.6±0.1) from 50% concentration of the extracting solvent while it was 5.12±0.02 in 75% ethanol extract. This was followed by seed extract with the values of 5.4±0.1 and 4.9±0.1 respectively. The least observed recovery value was seen in the pulp extract with 3.9±0.1. Generally, active ingredients were easily extracted from leaf and seed extracts when compared with the pulp.

Table 1: Percentage recovery of all the extracts

	Ethanol 50%	Ethanol 75%

Pulp	3.9±0.1	4.4±0.1
Leaf	5.6±0.1	5.12±0.02
Seed	5.4±0.1	4.9±0.1

Table 2 shows the antibacterial activity of *D. stramonium* extract on selected pathogenic organisms at 75% and 50% ethanolic extract. At 75% ethanolic extract, it was observed that *Pseudomonas aeruginosa* had the highest zone of inhibition with (14mm) while *Klebsiella pneumoniae* had the lowest inhibition of 11mm using the seed extract. The pulp extract inhibitory concentration ranges from 8mm – 12mm, *Streptococcus pneumoniae* had the highest inhibitory concentration (12mm) while *S. aureus* and *Escherichia coli* had the lowest inhibitory concentration of 8mm. 75% ethanol extract of leaf shows that *Klebsiella pneumoniae* has highest inhibitory concentration with 11mm and the lowest inhibitory concentration was seen in *Streptococcus pneumoniae* with 9mm. However, 75% ethanol extract of pulp did not inhibit the growth of *Bacillus subtilis*, 75% ethanol of seed extract was resistance on *Streptococcus pneumoniae, Bacillus subtilis, S. aureus* and *Escherichia coli,* also 75% ethanol of leaf extract shows resistance to *Pseudomonas aeruginosa, Escherichia coli*, *Bacillus subtilis* and *S. Aureus.*

Table 2 also shows that 50% ethanol extract of pulp, seed and leaf of *D. stramonium* inhibited all the tested bacteria with inhibition zone ranging from 10mm to 21mm except the leaf extract that is resistance to *Escherichia coli*. The highest inhibition zone of 50% ethanol extract (21 mm) was recorded for *Pseudomonas aeruginosa* from the pulp sample, while that of seed is (17mm) on *Pseudomonas aeruginosa* and the leaf recorded (12mm) on *Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively. The phytochemicals present in the extracts of pulp, seed and leaf of *D. stramonium* were presented in Table 3. The result indicated that the seed contains more phytochemicals than the leaf and pulp. The entire phytochemicals screened were found to be present in both the seed and pulp of the plant except terpenoids and phenol which were absent in pulp. However most of the phytochemicals were absent in the leaf (except tannins and saponins which were present in low quantity).

Table 2: Antimicrobial activity of 0.6g/mL of 50% and 75% ethanolic extract of *D. stramonium* against test organisms

Organism	**zones of inhibition 75% ethanolic extract (mm)**			**zones of inhibition 50% ethanolic extract (mm)**		
	Pulp	**Seed**	**Leaf**	**Pulp**	**Seed**	**Leaf**
Streptococcus pneumoniae	12±2.0	0±0.0	9±0.1	18±2.0	14±1.0	12±1.0

Klebsiella pneumonia	10±1.0	11±2.0	11±0.5	19±2.0	10±0.5	12±0.5
Pseudomonas aeruginosa	11±1.0	14±1.0	0±0.0	21±1.0	17±2.0	12±2.0
Escherichia coli	8±0.1	0±0.0	0±0.0	18±0.5	12±0.0	0±0.0
Bacillus subtilis	0±0.0	0±0.0	0±0.0	19±1.0	14±0.5	11±0.2
Staphylococcus aureus	8±0.5	0±0.0	0±0.0	15±1.0	13±1.0	11±0.5

Key: mean ± standard deviation

Table 3: Phytochemical screening of pulp, seed and leaf of *D. stramonium*

Phytoconstituent	Seed	Pulp	Leaf
Tannins	+++	++	+
Flavonoids	+++	+	-
Alkaloid	+++	+	-
Saponins	+	+	+
Terpenoids	+	-	-
Phenol	++	-	-
Glycosides	++	+	-

Key +++ve = High, ++ve = Moderate, +ve = Low, -ve = Absent

3.2 Discussion

"Plants have always been a source of drugs for the treatment of human ailments, and millions of people in the world rely on medicinal plants for primary health care" [1-3]. In this study, the antibacterial activities of the extracts of *D. stramonium* pulp, seed and leaf using 50% and 75% ethanol as extraction solvents were conducted against some clinically isolated human pathogenic microorganisms. The study revealed that 50% ethanol extracts showed antibacterial activity against tested pathogenic microorganisms. This support the findings of El safey and Salah [29] who used water as an extraction solvent for finding active antibacterial components. It was also revealed that the pulp, seed and leaf extracts of 50% ethanol *D. stramonium* extract inhibited the growth of human pathogenic bacteria *S. aureus, E. coli, Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *K. pneumoniae* (clinical isolate) which is in line with the outcomes obtained by Obi *et al*. [30]. The leaf extracts of *D. stramonium* showed antibacterial activity against *E. coli* which is compatible to Baynesagne *et al*. [31] who found that ethanol extract did not inhibit the growth of *E. coli*. This might be due to high antimicrobial activity against this

microorganism. In addition, higher antibacterial activity was obtained against *S. pneumoniae* and *S. aureus* and lower antibacterial activity against *E. coli* clinical isolate which is partially in line with the results obtained by Benito *et al*. [32] who found higher antibacterial activity against *S. aureus* and *E. coli* clinical isolate. Moreover, *D. stramonium* 75% ethanol extracts showed some antibacterial activity against the entire organism used which supported the results of Eftekhar *et al*. [33]. Finally, the result is in congruent with recent reports that showed antibacterial activities of 80% methanol extract of *D. stramonium* against standard bacterial strains (*B. licheniformis, B. subtilis, S. aureus, S. Typhimurium, S. flexneri, P. aeruginosa, E. coli* and *P. mirabilis*) [1 and 3].

Phytochemical constituents in the plant sample are known to be biologically active compounds and they are responsible for different activities such as, antimicrobial, antioxidant, antifungal and anticancer. Hence, the antibacterial activity of *D. stramonium* (pulp, seed and leaf) extracts is due to the presence of phytochemicals that includes, flavonoids, phenols, tannins, saponins, terpenoids and alkaloids. The seed contains more phytochemicals than the leaf and pulp. This agreed with the work of Almalki [34] who reported that plant seed extracts documented good antimicrobial and antioxidant activities. Due to the presence of these fundamental phytochemicals, *D. stramonium* is considered as treasured medicine and useful in the treatment of many diseases.

4. CONCLUSION

The study revealed that ethanol extract of *D. stramonium* possesses considerable antibacterial activity that supports the use of the plant in treating some infectious diseases. *D. stramonium* extracts revealed very promising results with health-promoting potentials that could be applied in the treatment of ailments caused by pathogens such as *B. subtilis, S. pneumonia, S. aureus* and *Escherichia coli* which was largely inhibited by pulp extract. This Antibacterial activity of the plant was due to the presence of secondary metabolites such as flavonoids, phenols, tannins, saponins, terpenoids and alkaloids. However, advance studies are required to identify and characterize the bioactive compounds responsible for these activities which are necessary to validate the uses of this plant to treat infections and identify their mode of action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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