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**PHYTOCHEMICAL AND *IN VITRO* ANTIMICROBIAL POTENTIAL OF THE LEAF AND STEM OF *DATURA STRAMONIUM***

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**ABSTRACT**

*Datura stramonium* is a widely grown plant and is well known for its potent pharmacological activities in medicine. This study was carried out to determine the phytochemical components of *D. stramonium* and its antimicrobial potential on three selected micro-organisms (*Staphylococcus aeurus*, *Shigella dysenteria*, and *Salmonella typhi*). The maceration method was employed for the methanol extraction of the leaf and stem. The extracts were subjected to qualitative and quantitative phytochemical screening. Agar well diffusion method was used to assay for the antibacterial property while micro broth dilution method was employed for microbial inhibitory concentration of the crude extracts of *D. stramonium* (leaf and stem). The qualitative phytochemical screening showed the presence of phenols, flavonoids, tannins, saponins, and alkaloids in both extracts. However, the quantitative phytochemical screening showed that the leaf extract was found to contain more phenols, flavonoids, tannins, saponins, and alkaloids than the stem extract. The extracts were observed not to exert inhibitory effects against *S. aureus*. The extracts showed inhibitory effects on *S. typhi* and *S. dysentria* with maximal activities at 100 mg/mL. However, leaf extract showed higher zone of inhibition of 27.00 ± 0.80 mm and 20.00 ± 0.82 mm against *S. typhi* and *S. dysentria* respectively than stem extract (10.00 ± 0.21 mm and 7.00 ± 0.28 mm). The leaf extract showed MBC of 200 and 250 mg/mL against S. dysenteria and S. typhi respectively while stem extract showed presence of bioactive secondary metabolites in the both extracts, which can be responsible for its antimicrobial activities against the tested microorganism. *D. stramonium* can serve as potential sources of antimicrobial agents.

**Keywords:** *D. stramonium*, phytochemicals, antioxidant, antimicrobial

**INTRODUCTION**

**Background of the Study**

Plants have been employed in the treatment or management of various human diseases worldwide [1]. The interest in natural medicine is expanding in both advanced and developing countries mainly because of the growing recognition of the advantages of herbal medicine [1]. *Datura stramonium* is a widely grown plant and it is well known for its enormous pharmacological activities. The water and methanol extract of *D. stramonium* contains saponins, tannins, steroids, flavonoids, alkaloids, phenol and glycosides. The leaf extract of *D. stramonium* is reported to be useful in the treatment of pain and skin diseases [2]. For various human ailments, *D. stramonium* is often described as a helpful and useful remedy [3]. Some other phytochemicals have additionally been found in *D. stramonium* and the principal phyto-constituent is alkaloid [4].

*D. stramonium* is one of the most significant medicinal herbs utilized worldwide due to its calming properties, though it is globally considered a toxic plant when taken in enormous portions, prompting trance state, and even death [5]. The seed of the plant has been attributed to intoxication [6]. Antimicrobial, antioxidant and phytochemical screening of this plant have been reported earlier in a lot of scientific studies [7]. *D. stramonium* is a rich source of alkaloids among its secondary metabolite, containing atropine which is known to have more exciting properties, while scopolamine has more relaxing and hallucinogenic characteristics [8].

It has been accounted for that all parts of *D. stramonium* are noxious whenever ingested directly by humans or domesticated animals. However, for medicinal purposes, a small amount is required [9]. Cautious thought of the harmfulness of this plant is needed before use because oral administration may lead to severe anti-cholinergic symptoms which include dizziness, hallucination, nausea, blurred vision and possibly coma which may last for several hours or days depending on the quantity consumed [10]. This study aimed to evaluate the antimicrobial properties of the leaf and stem extracts of *D. stramonium* against selected microorganisms.

**MATERIALS AND METHODS**

**Reagents and chemicals**

The chemicals and reagents used in this study included but not limited to hydrochloric acid, ferric chloride, Meyer’s reagent, chloroform, anhydride, sulphuric acid, lead acetate, acetic anhydride, ninhydrin reagent, acetic acid, Folin-Ciocalteu reagent, gallic acid, DPPH ethanol solution, ascorbic acid, phosphate buffer and hydrogen peroxide.

**Collection of plant samples**

The leaf and stem of *D. stramonium* were collected from the field of Umar Farouk Primary School Keterengwari Minna, Niger state. The plant specimens were botanically authenticated by a taxonomist at the plant biology department, Federal University of Technology, Minna and stored for future reference in the herbarium. Plant samples were washed under tap water to remove any debris. The collected plant parts were rinsed with distilled water to eliminate dust and dried at ambient conditions (for 21 days) until a constant weight was obtained for each plant part. After drying, the leaf and stem were pulverized into powder using an electric blender. The bacteria were isolated in the Department of Microbiology Laboratory Federal University of Technology Minna.

**Preparation of extracts**

The pulverized plant parts (200 g each for leaf and stem) were separately extracted by cold maceration, mixing each plant part with 1000 mL of methanol for 72 hours at ambient temperature (27 ± 5.00 ºC). After 72 hours, the extracts were separately filtered into clean beakers using No 1 Whatman filter paper and concentrated by air-drying until a constant weight was obtained for each of the extracts. The dried extracts were then stored in separate air-tight containers until use [11].

**Phytochemical Screening**

**Quantitative analysis**

**Total phenol measurement**

The total phenols composition of the methanolic crude extracts leaf and stem of *D. stramonium* was estimated [12]. Using this method, 0.01 g of each of the extracts was dissolved in 10 mL of distilled water and 0.5 mL of each was oxidized with 2.5 mL of 10% Folin-reagent Ciocalteu's reagent, and subsequently neutralized with 2 mL of 7.5% sodium carbonate. The reaction mixtures were then allowed to stand for 40 minutes at 45 ºC. The absorbance was read at 765 nm using a Double-beam Shimadzu UV-visible spectrophotometer (UV-1800 series). Gallic acid was used for the preparation of the calibration curve.

**Total flavonoids determination**

The flavonoid content of the extracts was determined using the aluminum chloride method. Also, 0.01 g of each of the extracts was dissolved in 10 mL of distilled water and 0.5 mL was added to 1.5 mL absolute methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M sodium acetate and 2.8 mL distilled water, and incubated for 30 minutes at room temperature. The absorbance was read at 415 nm using a Double-beam Shimadzu UV-visible spectrophotometer (UV-1800 series). Quercetin was used for the preparation of the calibration curve [13].

**Total alkaloids determination**

The total alkaloid content of each of the extracts was estimated [14]. Briefly, 0.5 g of each of the extracts was dissolved in 5 mL of 96% ethanol/20% H2SO4 (1:1) solution before being filtered. Afterwards, 1 mL of the filtrate was added to a test tube containing 5 mL of H2SO4 60% and allowed to stand for 5 minutes. Subsequently, 5 mL of 0.5% formaldehyde was introduced and set aside to sit for 3 hours at room temperature. Utilizing a wavelength of 565nm, absorbance was measured with a UV spectrophotometer [14].

**Determination of tannin**

To determine the tannins content of the crude extracts, the procedure of AOAC was utilized. Briefly, 0.2 g of each of the extracts was dissolved in 20 mL 50% methanol, which was then enclosed with parafilm and heated in a water bath at 80 ºC for an hour. All of the reaction mixtures were shaken together to ensure homogenous mixtures. Once the extract had been filtered into the flask, it was combined with 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of sodium carbonate. Afterward, the reaction mixtures were incubated at ambient temperature for 20 minutes. To create the calibration curve, 760 nm of absorbance was reported using a double-beam UV- spectrophotometer, standard tannic acid was employed [15].

**Total saponins determination**

Saponins content was determined [14]. In this method, 0.5 g of each of the extracts was mixed with 20 mL 1 N HCl and heated at 80 ºC for 4 hours. The reaction mixture was cooled and 50 mL of petroleum ether was added to separate the fat-soluble constituents. The ether layer was collected and dried. Subsequently, 5 mL of mixture of acetone-ethanol (1:1), and sulphuric acid was added and allowed to stand for 10 minutes before being diluted with distilled water. The absorbance at 490 nm was measured. To create the calibration curve, standard saponin was used [14].

**Evaluation of antibacterial potentials**

**Diffusion method utilizing agar well**

Using the agar well diffusion technique, three test micro-organisms viz., *Salmonella typhi, Staphylococcus aureus* and *Shigella dysenteriae* were isolated by the lab technologist, in the Department of Microbiology laboratory Federal University of Technology Minna. The isolates were resuscitated for 24 hours [16]. Muller Hinton agar was set based on the manufacturers’ instructions and autoclaved at 121°C for 15 min. The media was allowed to cool and then it was dispensed into each Petri dish and allowed to gel. After the media had gelled, a standardized population of 1.0×106 bacteria/mL of each isolate was inoculated on the gelled Mueller-hinton agar using a swab stick. A cork borer of 8mm in diameter was used to bore a well on the pre-inoculated agar plate. The agar well bottom was then sealed using liquid Mueller-hinton agar. The concentration (20, 40, 60, 80 and 100 mg/ml) of the leaf and stem of *D. stramonium* crude extract was taken and introduced in the well respectively and chloramphenicol (It is a broad-spectrum antibiotic and has good activity against certain pathogens) at the same concentration was used as control. For 30 minutes, the agar plate was allowed to rest and incubated at 37oC for 24 hours. Each concentration was in triplicate experiment and the zone of inhibition was achieved by measuring the diameter of the plate [17].

**Determination of minimum inhibitory concentration (MIC) of the extracts**

To determine the minimum inhibitory concentration (100 mg/mL) of extracts that inhibit microbial growth using the macro-tube dilution technique. Eight milliliters (8 mL) of nutrient broth was diluted to each labeled concentration, and 2 mL of 1.0×106 bacteria/mL of the suspension of test isolate was added. Without any inoculation of test isolates control was developed. The micro-tube was incubated at 37°C for 24 h. Growth was inspected by checking the turbidity of bacteria on each tube before and after inoculation and the results were compared with the control tube [18].

**Determination of the minimum bactericidal concentration (MBC)**

Dilutions with no turbidity were obtained and subcultured on Muller Hinton agar to carry out MBC before being incubated for 24 hours at 37°C. Finally, the outcomes that showed no growth were chosen as MBC values [19].

**Analyzing Data**

The data were analyzed by One-way Analysis of Variance (ANOVA) using Statistical Product and Service Solutions (SPSS). The results were expressed as mean ± SD (standard deviation). The differences in means among the extracts were compared using the “Duncan multiple range test”. A *p*-value less than 0.05 was considered significant (p < 0.05).

**RESULTS**

**Quantitative Phytochemical Component**

Table 1 shows the phytochemical composition of methanolic crude extracts of *D. Stramonium* (leaf and stem). The crude leaf extract contained higher amounts of all the assessed phytochemicals namely; phenols, flavonoids, tannins, and alkaloids. However, it was observed that phenols were highest in leaf extract with an amount of 121.82 ± 0.42mg/100 g while stem recorded saponins (76.84 ± 0.37 mg /100 g) as the highest phytochemical. The least present phytochemical in both leaf and stem extracts was flavonoids, with 4.12 ± 0.10 mg/100 g and 1.42 ± 0.04 mg/100 g for leaf and stem respectively.

**Table 1:** Quantitative phytochemical composition of *D. stramonium*

| **Phytochemical** | **Leaf**  **(mg/100 g)** | **Stem**  **(mg/100 g)** |
| --- | --- | --- |
| Phenols | 121.82 ± 0.42 b | 64.93 ± 0.12 a |
| Flavonoid | 4.12 ± 0.10b | 1.42 ± 0.04b |
| Tannins | 76.84 ± 0.67b | 35.30 ± 0.56a |
| Saponins | 100.45 ± 0.71b | 76.84 ± 0.37a |
| Alkaloids | 27.88 ± 0.37b | 13.73 ± 0.22a |

The values are the mean ± standard deviation of three replicates. Values with different superscripts in a row are significantly different at p < 0.05.

**Antibacterial potentials of methanolic crude extracts of *D. Stramonium***

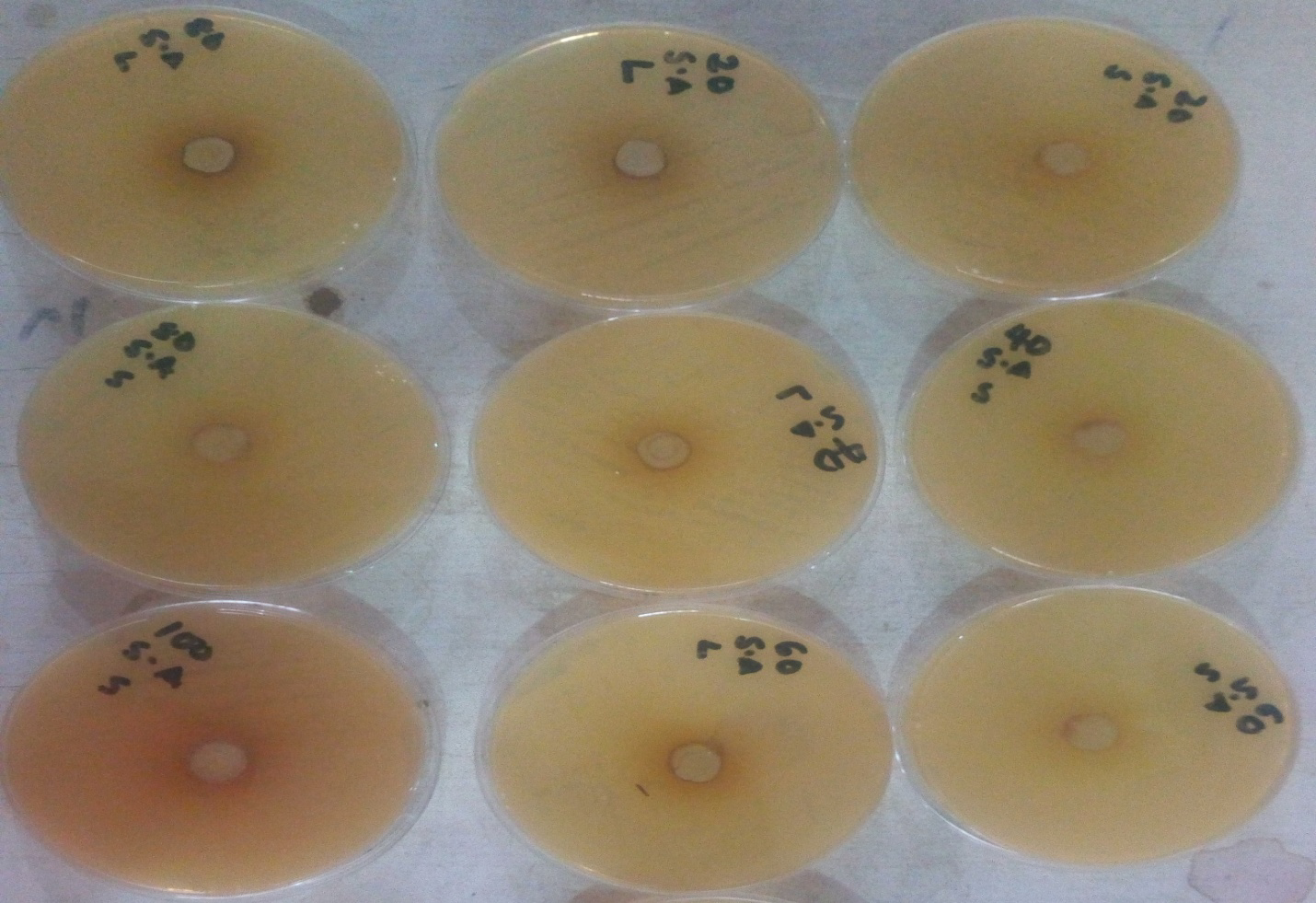
**Antibacterial potentials against *S. aureus***

Antimicrobial potentials of crude leaf and stem extracts of *D. stramonium* against *Staphylococcus aureus* is shown in Table 2 below. Therein, it was observed that both extracts were not able to inhibit the growth of test organism (*Staphylococcus aureus*) as compared to chloramphenicol that exhibited appreciable inhibitory effect against it. This can also be seen clearly in plate 1.

**Table 2:** Antibacterial activity of the leaf and stem of *D. Stramonium* crude extracts against *staphylococcus aureus*

| **Conc.**  **(mg/mL)** | **Zone of Inhibition (mm)** | | |
| --- | --- | --- | --- |
| **Leaf** | **Stem** | **Chloramphenicol** |
| 20 | 0.00 | 0.00 | 02.00 ± 0.17a |
| 40 | 0.00 | 0.00 | 06.00 ± 0.16b |
| 60 | 0.00 | 0.00 | 09.00± 0.31c |
| 80 | 0.00 | 0.00 | 12.00 ± 0.25d |
| 100 | 0.00 | 0.00 | 13.00 ± 0.20e |

The values are expressed in mean ± standard deviation (SD) of three replicates. Values with different superscripts are significantly different at p < 0.05.

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**Plate 1:** Zone of inhibition of *Datura Stramonium* crude extract against *Staphylococcus aureus*.

**Antibacterial Activity against *Salmonella Typhi***

Unlike the effect of the extracts against *Staphylococcus aureus*, the extracts exhibited substantial inhibitory activities against *Salmonella typhi* which is comparable to that of chloramphenicol. The inhibitory effect of the leaf extract was observed to be in a concentration-dependent manner as the highest tested concentration of 100 mg/mL gave the highest zone of inhibition (27.00 ± 0.80 mm) which was significantly higher than that of chloramphenicol (23.00 ± 0.45 mm). The zone of inhibition for *Salmonella typhi* is shown below in plate 2. Contrarily, stem extract was found not to be concentration-dependent and the highest zone of inhibition was recorded at 40 mg/mL (12.00 ± 1.22 mm) though not significantly different from 60 mg/mL (12.00 ± 1.29).

**Table 3:** Antibacterial activity of leaf and stem of *D. Stramonium* crude extracts against *Salmonella typhi*

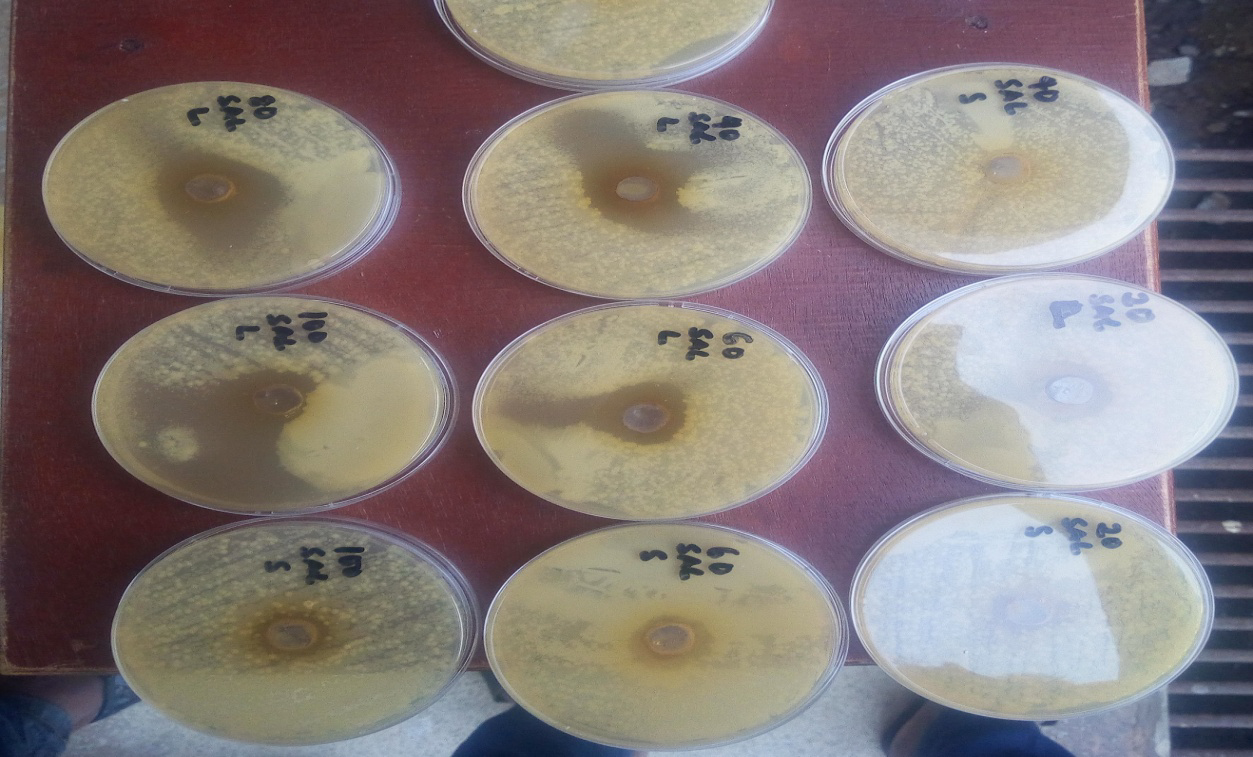
| **Conc.**  **(mg/mL)** | **Zone of Inhibition (mm)** | | |
| --- | --- | --- | --- |
| **Leaf** | **Stem** | **Chloramphenicol** |
| 20 | 15.00 ±0.82c | 10.00 ± 0.82a | 11.00 ± 0.30b |
| 40 | 12.00 ± 1.29b | 12.00 ± 1.22b | 16.00 ± 0.25c |
| 60 | 17.00 ± 1.00b | 12.00 ± 1.29a | 20.00 ± 0.32c |
| 80 | 22.00 ± 0.81b | 9.00 ± 0.28a | 21.00 ± 0.52b |
| 100 | 27.00 ± 0.80c | 10.00 ± 0.21a | 23.00 ± 0.45b |

The expressed value is in mean standard error of bar replicate. Same color of bar and superscript have no significant differences at (p<0.05).

**Table 4:** Antibacterial activity of the leaf and stem of *D. Stramonium* crude extracts against *Shigella dysenteria*

| **Conc.**  **(mg/mL)** | **Zone of Inhibition (mm)** | | |
| --- | --- | --- | --- |
| **Leaf** | **Stem** | **Chloramphenicol** |
| 20 | 7.00 ± 0.82c | 3.00 ± 0.82a | 8.00 ± 0.12c |
| 40 | 14.00 ± 0.21e | 5.00 ± 1.24b | 12.00 ± 0.26d |
| 60 | 17.00 ± 1.00f | 8.00 ± 1.22c | 15.00 ± 0.22e |
| 80 | 18.00 ± 1.24f | 6.00 ± 0.82b | 20.00 ± 0.37g |
| 100 | 20.00 ± 0.82g | 7.00 ± 0.28c | 23.00 ± 0.45h |

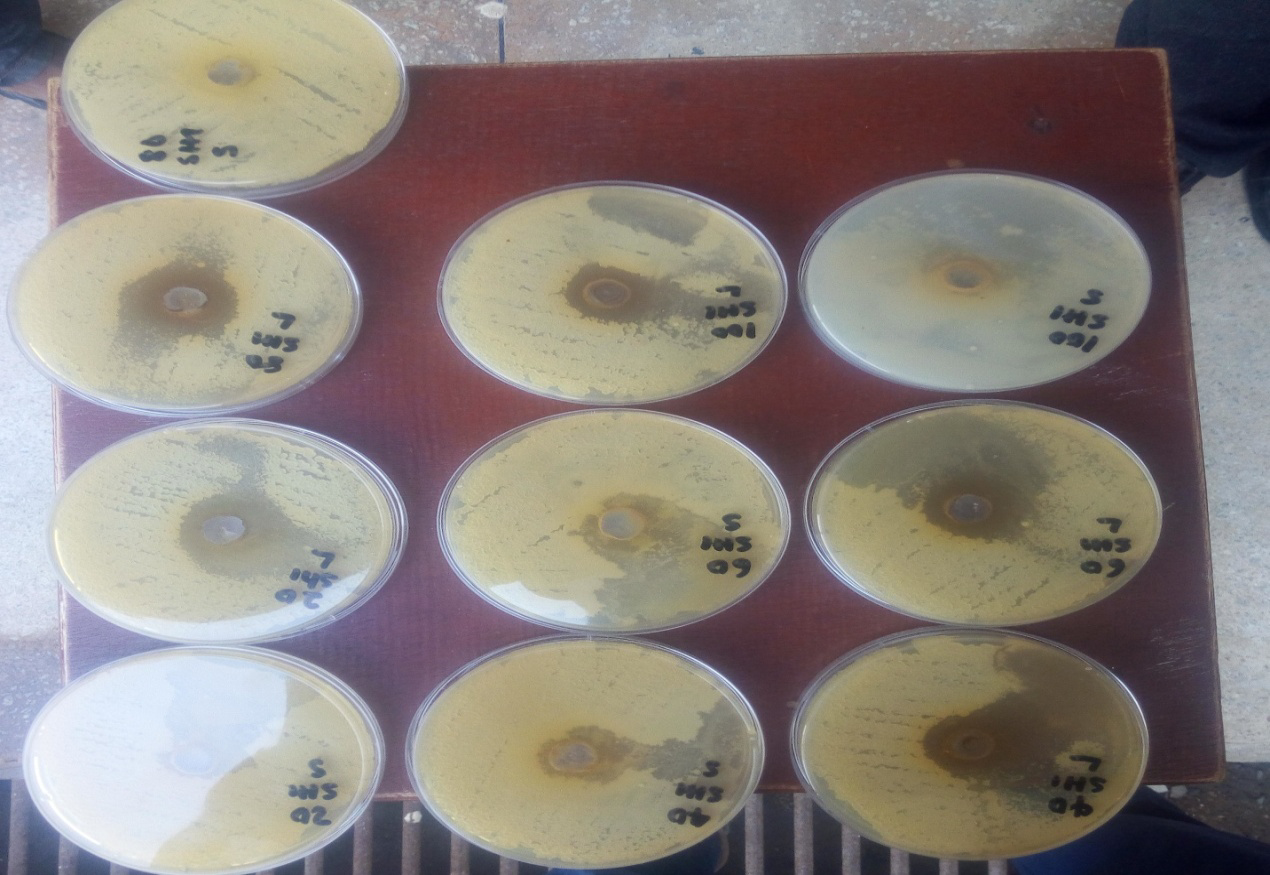
Values are presented as mean ± standard deviation (SD) of three replicates. Values with different superscripts in a row are significantly different at p < 0.05.

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**Plate 2:** Zone of inhibition of *Datura Stramonium* crude extract against *Salmonella Typhi*.

**Antibacterial activity against *Shigella dysenteria***

As shown in Table 4, the extracts inhibited the growth of *Shigella dysenteria* substantially especially the leaf extract. The leaf extract exhibited an inhibitory effect against the test organism in a concentration-dependent manner with the highest zone of inhibition being observed at 100 mg/mL. The stem extract on the other hand did not behave in a concentration-dependent manner and possessed a lower zone of inhibition (8.00 ± 1.22 mm) than the leaf extract (20.00 ± 0.82 mm). The activities of the two extracts were lower than that of chloramphenicol (23.00 ± 0.45 mm). This is also shown in plate 3.

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**Plate 3:** Zone of inhibition of *Datura Stramonium* crude extract against *Shigella dysenteria*

**Minimum inhibitory concentration of *D. Stramonium* Methanol extract**

Table 5 below shows the minimum inhibitory concentrations (MIC) of the extracts against the three test bacteria. Both extracts did not inhibit the growth of *Staphylococcus aureus* as such no MIC was recorded. The leaf extract recorded MIC of 20 mg/mL and 25 mg/mL against *Shigella dysenteria* and *Salmonella Typhi* respectively while the MIC of the stem extract was found to be 15 and 20 mg/mL respectively.

**Table 5: Minimum inhibitory concentrations of the extracts against microorganisms**

| **Plant extract** | **MIC (mg/mL)** | | |
| --- | --- | --- | --- |
| ***Staphylococcus aureus.*** | ***Shigella dysenteria*** | ***Salmonella typhi.*** |
| Leaf | - | 20 | 25 |
| Stem | - | 15 | 20 |

(-) no antimicrobial activity, (MIC) minimum inhibitory concentration

**Table 6:** Minimum bactericidal concentrations of the extracts against microorganisms

| **Plant extract** | ***Staphylococcus aureus*** | ***Shigella dysenteria*** | ***Salmonella typhi.*** |
| --- | --- | --- | --- |
| Leaf | - | 200 | 250 |
| Stem | - | 100 | 100 |

(-) no antimicrobial activity, (MBC) minimum bactericidal concentration

**Minimum bactericidal concentration of *D. Stramonium* Methanol extract**

The minimum bactericidal concentration (MBC) of the extracts is depicted in Table 6. Therein, it was observed that the extracts did not show inhibitory effects against *Staphylococcus aureus*. However, the leaf extract recorded MBC of 200 and 250 mg/mL against *Shigella dysenteria* and *Salmonella Typhi* while the stem extract had an MBC of 100 mg/mL for the two bacteria.

**DISCUSSION**

The studies of natural plants that have biologically active phyto-constituents which exhibit important therapeutic effects have generated a lot of interest in our world today [19]. The quantitative phytochemical screening revealed the presence of phenols, flavonoids, tannins, saponins and alkaloids in appreciable amounts in the two extracts. These phytochemical components are known to be biologically active and are responsible for different activities such as antimicrobial, antioxidant, antifungal, anticancer and anti-diabetic [2]. Different phytochemicals have been established to own a wide variety of pharmacological activities, which may help in protection against chronic diseases. Tannins, glycosides, saponins and flavonoids have hypoglycemic and anti-inflammatory activities. Saponins are involved in plant defense systems because of their antimicrobial activity [20]. Tannin-like compounds are found in plant leaves

and pulp and inhibit bacteria growth [21]. Saponins are a bioactive compound with antibacterial action that plays a role in the defense

system of the plant. The saponins concentration of the stem extract was significantly different from that of the *D. stramonium* leaf extract. As a result, the pharmacological activities exhibited by these extracts could be attributed to the presence of the phytochemicals in both extracts.

The leaves and stem extracts of *D. stramonium* have also been shown to contain the aforementioned phytochemicals but in different amounts by other studies using different solvents [22, 23, 24]. However, it has been established that different solvents used for extraction can result in different phytochemical compositions of the same extract. The polarity of the solvent plays a crucial role in determining which phytochemicals are extracted [25, 26]. Solvents with higher polarity, such as water and methanol, are more effective in extracting phenolic, flavonoid, and alkaloid compounds [26]. On the other hand, solvents with lower polarity, such as chloroform and n-hexane, may extract different classes of compounds, such as resins and steroids [27]. The choice of solvent can also affect the overall antioxidant activity of the extract, with methanol often showing greater antioxidant activity [27]. Therefore, the selection of solvent for extraction is crucial in obtaining specific phytochemicals with desired properties.

This study shows that the leaf and stem of *D. stramonium* extracts were efficient against the species of tested bacteria. As such, the extracts can be employed for the treatment of infections associated with *Shigella dysenteria* and *Salmonella typhi.* However, the leaf extract exerted more inhibitory effects against the test bacteria than the stem extract making leaf extract a better antibacterial agent against the test bacteria than the stem extract. The higher antibacterial activity exhibited by the leaf extract could be attributed to the higher phytochemicals present in it [28]. The findings in the study correlate with another study that reported *D. stramonium* to possess antibacterial activity against the test bacteria [29]. However, the absence of the activity against *S. aureus* contradicts their findings. The inhibitory effect of plant extracts on the growth of microorganisms can vary depending on several factors. Environmental factors such as pH, temperature, and nutrient availability can influence the susceptibility of microorganisms to plant extracts [30]. Additionally, the choice of solvent used for extraction, as well as the concentration and period of extraction, can also affect the inhibitory activity of plant extracts [32]. Furthermore, the biochemistry, physiology, and adaptation strategies of the microorganisms, as well as the bioactive constituents present in different plant species, can contribute to variations in susceptibility [33]. As such it is important to consider these factors when studying the antimicrobial activity of plant extracts [34]. As a result, the differences between the two findings could be traceable to one or more of the factors mentioned above.

**CONCLUSION**

Conclusively, the leaf and stem extracts of *D. stramonium* possessed antibacterial activities against the test bacteria except *S. aureus*. However, the inhibitory effects of leaf extract on susceptible bacteria were more than the stem extract. As such, the leaf extract of *D. stramonium* can be employed in the treatment and/or management of diseases caused by susceptible bacteria.

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