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Antibacterial activity of *Daniellia oliveri* leaf extract on enteric bacteria

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Abstract

Plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs. The aim of this study was to investigate the antibacterial activity of *Daniellia oliveri* leaf extract on enteric bacteria. The leaves of *Daniellia oliveri* plant were obtained around the Joseph Sarwuan Tarka University, Makurdi and were packaged in polyethene bags and transported to the Microbiology Laboratory Joseph Sarwuan Tarka University. The leaves were air dried and pulverized to obtain the powder from which the ethanolic and aqueous extracts were made. The concentrations prepared were 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml, respectively. The antibacterial activity was carried out using agar well diffusion method while the minimum inhibitory concentration (MIC) of the aqueous and ethanolic *Daniellia oliveri leaf* extracts was determined by macro broth dilution method. The phytochemical screening showed that the ethanolic extract had alkaloids, glycosides, cardiac glycosides, flavonoids, phenols, tannins, saponins, terpernoids and quinones while phytosterols were absent. The aqueous extract had alkaloids, cardiac glycosides, flavonoids, phenols, tannins, saponins, terpernoids, quinones and phytotosterols while glycosides were absent. The effect of the ethanolic leave extract of *Danellia oliveri* was highest (28.67±2.08) on *Salmonella* spp at a concentration of 250 mg/ml and lowest (13.67±1.53) at a concentration of 31.25 mg/ml. On *Klebsiella* spp, the effect was highest (22.00 ±2.62) at a concentration of 250 mg/ ml and lowest (9.33±0.58) at a concentration of 31.25 mg/ml, while the effect of the ethanolic leave extract of *Danellia oliveri* as observed on *Shigella* sp was highest (21.33±1.53) at a concentration of 250 mg/ ml and lowest (0.00±0.00) at a concentration of 31.25 mg/ml. The effect of the aqueous extract was highest (28.67±2.08) at a concentration of 250 mg/ml and lowest (0.00±0.00) at a concentration of 31.25 mg/ml on *Salmonella* spp, highest (22.00 ±2.65) at a concentration of 250 mg/ml and lowest (9.33±0.58) on *Klebsiella* sp while it was highest (7.22±0.18) at a concentration of 250 mg/ml and lowest (0.00±0.00) at a concentration of 31.25 mg/ml on *Shigella* spp. The findings of this study suggest the use of *Daniellia oliveri* leaf extracts in synthesized antibiotics which could be effective in the treatment of gastrointestinal disorders.

Keywords: *Daniella oliveri*; Antibacterial; Ethanolic extracts; Aqueous extracts; Minimum inhibitory concentration; Agar well diffusion; Phytochemical screening

1. Introduction

Plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs [1]. Finding new naturally active components from plants or plant-based agricultural products have been of interest to many researchers. Hence, a great deal of attraction has been paid to the antibacterial activity of *Daniellia oliveri* as a potential and promising source of pharmaceutical agent [2]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [3].

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Daniellia is a genus of legumes in the Fabaceae family. The trees reach heights of 30 to 40 metres. *Daniellia oliveri* which produces pea-shaped flowers has been reported to possess anti-malarial, anti-diarrhoeal, anti-inflammatory, antinociceptive, antipyretic, aphrodisiac, antidiabetic and antimicrobial activities [4].

There are ethnobotanical claims that several parts of *Daniellia oliveri* have biological activities. For example, young leaves are used to treat wounds and relieve general body pains [5]. Despite the effective, simple and cheap treatment of oral dehydration therapy, majority of the local populace still rely on herbs to treat diarrhea especially when it is persistent. Medicinal plants frequently used for treating diarrhoeal infections in Nothern Nigeria include: *Daniellia oliveri* (Fabaceae) and *Ficus sycomorus* Linn (Moraceae). In addition to diarrhoea, the leaves of *D. oliveri* are used traditionally in Northern Nigeria to treat diabetes, gastrointestinal disturbances, diarrhoea, as diuretic and aphrodisiac [6].

1.1 Statement of Problem

The increasing problem of multidrug resistance among pathogens had further necessitated the need to search for newer antibiotics. Aside the problems of multidrug resistance exercised by human pathogenic microorganisms, there is also a side effect to the use of commercial antibiotics which has led to research by scientist for alternatives such as medicinal plants for treatment of some bacterial related infections. There is a drawback in the use of major antibiotics which includes antimicrobial spectrum that results in serious side effects and high incidence of resistance in bacteria. Also, the high cost of the synthetic antibiotics is a major problem that has necessitated the use of natural antibiotics.

1.2 Justification of the Study

Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic antibiotics, there has been increasing interest in the pursuit of natural alternatives. The result of this study could help bridge the gap between the use of traditional/natural antibiotics and commercially or synthetically produced antibiotics used in the treatment of enteric diseases, and it could also lead to cutting down the high cost associated with synthetic antibiotics.

Aim of the Study

The aim of this study is to investigate the antibacterial activity of *Daniellia oliveri* leaf extract on enteric bacteria.

Objectives of the Study

- To determine the antibacterial activity of *Daniellia oliveri* leaf extracts on the enteric bacteria using the agar well diffusion method.
- To determine the minimum inhibitory concentration (MIC) of the extract using micro broth dilution method.
- To screen the leaf extracts for phytochemical properties.

2. Materials and methods

2.1 Study Area

This study was carried out in Microbiology Laboratory, Joseph Sarwuan Tarka University Makurdi. Makurdi is the capital of Benue State. The city is situated on the south bank of the Benue River. In 2016, Makurdi and the surrounding areas had an estimated population of 365,000 [7]. The area is characterized by two seasons, the dry season (October to April) and rainy season (April to October). About 45% the total population are civil servant and business people. While about 25% are famers, 30% vocational workers and students.

2.2 Sample Collection

The leaves of *Daniellia oliveri* plant were obtained around the university premises and were packaged in polyethene bags and transported to Microbiology Department Joseph Sarwuan Tarka University. The plant sample were authenticated in the department of Botany Joseph Sarwuan Tarka University. The leaves were be air dried and pulverized to obtain the powder from which the extract was made.

2.3 Sterilization and Disinfection of Materials

Work benches were properly disinfected with sodium hypochloride. All glass wares (Petri dishes, test tubes, conical flasks) were washed during the bench work with detergents and rinsed with clean water and sterilized at 121 °C for 15 minutes in an autoclave**.**

2.4 Preparation of Plant Extracts

2.4.1 Preparation of Ethanolic and Aqueous Extracts of Daniellia oliveri

Exactly 200g of *Daniellia oliveri* powdered leaves were mixed with 400 mL of 75% ethanol and distilled water in a glass container to obtain a homogenous mix by stirring it occasionally for three (3) days at 35 ℃. The mixtures were filtered and further centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurities. Thus, the obtained alcoholic extract was concentrated by heating to evaporate ethyl alcohol and to obtain the crude extract. A similar procedure was followed to obtain the aqueous leave extract using distilled water.

2.4.2 Preparation of Different Concentrations of the Plant Extract.

Double standard dilution method by Olafadehan [8], was used to obtain five different concentrations of the crude extracts, these were 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml, respectively. This was done by diluting appropriate milligrams of the extracts into corresponding volumes of solvents.

2.4.3 Media Preparation

All media were prepared according to manufacturers' instruction. They were sterilized by autoclaving at 121 ℃ for 15minutes. These media were allowed to cool before pouring into Petri dishes (20mL each). This was done under aseptic conditions to avoid contaminations.

2.5 Bacterial Isolation

The isolates used in this study were obtained from Microbiology Joseph Sarwuan Tarka university laboratory. They were activated and used to test for the antibacterial activity of the extracts from leaves of *Daniellia oliveri* extract on them.

2.6 Inoculum *Preparations* Using Macfaland Standard

The colonies were transferred from the plates to the nutrient broth with a sterilized straight nichrome wire. The turbidity was visually adjusted with nutrient broth to equal that of a 0.5 McFarland unit turbidity standard that was freshly prepared by reacting Barium chloride with sulfuric acid [9].

2.7 Antibacterial Susceptibility Testing

The antibacterial susceptibility test was carried out using agar well diffusion method according to Kirby Buer. After adjusting the inoculum to a 0.5 MacFarland unit turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of Muller Hinton agar plate was swabbed three times, rotating plates approximately 60 °C between streaking to ensure even distribution. The inoculated plates were allowed to stand for at least 3 minutes but not longer than 15 minutes before the wells were punched in the agar plate. A hollow tube of 5 mm diameter was taken and heated. It was pressed on the inoculated agar plate and removed immediately after making a well in the plate. Likewise, six wells were made on each plate and the crude extracts introduced into the wells. This was allowed to stand for 1 hour and then incubated at 37 ℃ for 24 hours. After 24 hours of incubation, the plates were examined for zones of inhibition which were measured in millimeter using a meter rule as diameter zone of inhibition [10].

2.8 Determination of the Minimum Inhibitory Concentration of the Extract.

The minimum inhibitory concentration (MIC) of the aqueous and ethanolic *Daniellia oliveri leaf* extracts was determined by macro broth dilution method. The dilutions of 250 mg/mL, 125 mg/mL, 65.5 mg/mL and 31.25 mg/mL were used. In each test tube containing 4ml of nutrient broth, 1ml of the extract was added, a loopful of the test organisms that was equivalent to 0.5 McFarland standards was introduced into the appropriate broth media.

A set of tubes containing only 1ml of the broth medium without the diluted extract were incubated and used as the control. The lowest concentration of the extract that completely inhibited the growth of the organisms was considered as the minimum inhibitory concentration (MIC).

2.9 Phytochemical Screening of Extracts

The extracts were subjected to standard phytochemical qualitative and quantitative screening for secondary metabolites.

2.10 Test for Alkaloids

2.10.1 Mayer's Test

Exactly 1ml of the extract was added to 4 drops of Mayer's reagent (Along the side of the test tube). A creamy white or yellow precipitate indicates the presence of Alkaloids.

2.10.2 Iodine Test

Exactly 1ml of extract added to few drops of iodine solution. A blue colour which disappears on boiling and reappears on a cooling indicates the presence of Alkaloids

2.11 Test for Saponins

2.11.1 Froth Test

Exactly equal volume of the extract and water was boiled. An appearance of creamy mix of small bubbles indicates the presence of saponins [11].

2.12 Test for Glycosides

2.12.1 Borntrager's test

Exactly 1ml of the extract was added to 3ml of chloroform and shaken well, 10% ammonia solution is added. Pink color solution indicates the presence of glycosides. (Mohammad *et al.,* 2011).

Exactly 1ml of dilute H_2SO_4 was added to 1ml the extract and boiled for 15 minutes and allowed to cool and neutralized with 10% NaOH + 1ml Fehling's A and B. a yellow color indicates the presence of glycosides [12].

2.13 Test for Cardiac Glycosides

2.13.1 Keller-Killani Test

Exactly 1ml of filtrate was added to 2ml glacial acetic acid and 1 drop of 5% $FeCl_3$ and concentrated H_2SO_4 (Along the side of the test tube). A blue colored solution in acetic acid indicates the presence of Cardiac Glycosides.

2.14 Test for Flavonoids

2.14.1 Alkaline Test

Exactly 1ml of the extract was added to 2% of NaOH solution and a few drops of dilute HCl. An intense yellow color that becomes colorless when dilute acid is added-flavonoids.

2.14.2 Lead acetate Test

Exactly 1ml of the extract was added to a few drops of lead acetate solution. A yellow precipitate indicates the presence of flavonoids.

2.14.3 Ferric Chloride Test

Exactly 1ml of the extract was added to few drops of $FeCl_3$ solution (10%). A green precipitate indicates the presence of flavonoids.

2.15 Test for Phenolic Compounds

2.15.1 Iodine Test

Exactly 1ml of the extract was added to a few drops of dilute iodine solution. A transient red colour indicates the presence of phenol.

2.15.2 Ferric Chloride Test

Exactly 1ml of the extract was added to a few drops of 5% of $FeCl_3$ solution. A dark green color or bluish black indicates the presence of phenol.

2.15.3 Lead Acetate Test

Exactly 1ml of the extract is added to few drops of lead acetate solution. A white precipitate indicates the presence of phenol

2.16 Test for Tannins

2.16.1 Braymer's Test

Exactly 1ml of the extract was added to few drops of 10% of $FeCl_3$ solution. A blue green color indicates the presence of Tannins.

2.16.2 Lead Acetate Test

Exactly 1ml of the extract was added to few drops of lead acetate solution. A creamy gelatinous precipitate indicates the presence of Tannins.

2.17 Test for Phyotosterols

2.17.1 Salkoskis' Test

Exactly 1ml of the extract was added to few drops of concentrated H_2SO_4, shaked and allowed to stand. A red color in lower layer indicates the presence of sterols.

2.17.2 Hene's Test

Exactly 1ml of the extract was added to 2ml of Chloroform and 2ml of concentrated H_2SO_4. A pink ring or red color in lower Chloroform layer indicates the presence of sterols.

2.17.3 Test for Terpinoids

Exactly 1ml of Chloroform is added to 1ml of the plant extract and evaporated on water bath, then few drops of concentrated H_2SO_4 was added and boiled on water bath. A grey color solution indicates the presence of Terpinoids.

2.17.4 Test for Quinones

Exactly 1ml of the extract was added to few 1ml of KOH. A red to blue color and concentrated HCL was added and a green color indicates the presence of Quinones.

2.18 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for the statistical analysis. Descriptive statistics, that is mean ± standard error values ware expressed for log. One way analysis of variance was carried out at P=<0.05 and Tukey HSD statistics was used for mean separation.

3. Results

Table 1 shows that the extracts had alkaloids, glycosides, cardiac glycosides, flavonoids, phenols, tannins, saponins, terpernoids and quinones while phytotosterols were absent. Table 2 presents the phytochemical screening of aqueous extract of *Daniellia oliveri*. The analysis showed that the extract had alkaloids, cardiac glycosides, flavonoids, phenols, tannins, saponins, terpernoids, quinones and phytotosterols while glycosides were absent.

Table 3 showed that the zones of the ethanolic leave extract of *Daniellia oliveri* were highest zone (28.67±2.08) at a concentration of 250 mg/ml and lowest (13.67±1.53) at 31.25 mg/ml on *Salmonella* spp. The zones shown on *Klebsiella* spp were highest (22.00 ±2.62) and lowest (9.33±0.58) at concentrations of 250 mg/ml and 31.25 mg/ml respectively. On *E. coli*, the zones were highest (10.33±0.58) and lowest (10.11±1.02) at the concentrations of 250 mg/ml and 31.25 mg/ml respectively and finally on *Shigella* spp, the zones were highest (21.33±1.53) and lowest (0.00±0.00) at concentrations of 250 mg/ml and 31.25 mg/ml respectively.

Table 4 showed that the aqueous extract of *Danielia oliveri* extract where highest (28.67±2.08) and lowest (0.00±0.00) at concentrations of 250 mg/ml and 31.25 mg/ml respectively on *Salmonella* spp. The zones shown on *Klebsiella* spp were highest (22.00 ±2.62) and lowest (9.33±0.58) at concentrations of 250 mg/ml and 31.25 mg/ml respectively. On *E. coli*, the zones were highest (10.33±0.58) and lowest (0.00±0.00) at the concentrations of 250 mg/ml and 31.25

mg/ml respectively and finally on *Shigella* spp, the zones were highest (7.22±0.18) and lowest (0.00±0.00) at concentrations of 250 mg/ml and 31.25 mg/ml respectively.

Table 1 Phytochemical Component of Ethanolic Extract *of Daniellia oliveri*

S/no	Compounds	Reactions
1	Alkaloids	+
2	Glycosides	+
3	Cardiac glycosides	+
4	Flavonoids	+
5	Phenols	+
6	Tannins	+
7	Saponnins	+
8	Phytotosterols	-
9	Terpenoids	+
10	Quinones	+

Key: + = present, - = absent

Table 2 Phytochemical Screening of Aqueous Extract of *Daniellia oliveri*

S/no	Compounds	Reactions
1	Alkaloids	+
2	Glycosides	-
3	Cardiac glycosides	+
4	Flavonoids	+
5	Phenols	+
6	Tannins	+
7	Saponnins	+
8	Phytotosterols	+
9	Terpenoids	+
10	Quinones	+

Key: + = present, - = absent

Table 3 Antibacterial Activity of Ethanol Extract of *Daniellia oliveri*

Organisms	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
Salmonella spp	28.67±2.08	21.33±1.53	18.67±1.53	13.67±1.53
Klebsiella spp	22.00 ±2.62	17.00±1.00	13.00±1.00	9.33±0.58
E. coli	10.33±0.58	13.67±1.53	18.67±1.53	10.11±1.02
Shigella spp	21.33±1.53	9.33±0.58	7.22±0.28	0.00±0.00

Table 4 Antibacterial Activity of Aqueous Extract of *Daniellia oliveri*

Organisms	**250 mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**
Salmonella spp	28.67±2.08	21.33±1.53	18.67±1.53	0.00±0.00
Klebsiella spp	22.00 ±2.65	17.00±1.00	13.00±1.00	9.33±0.58
E. coli	10.33±0.58	11.67±1.51	7.22±0.18	0.00±0.00
Shigella spp	7.22±0.18	0.00±0.00	0.00±0.00	0.00±0.00

Table 5 presents the minimum inhibitory concentration of the ethanolic extract of *Daniellia oliveri* on the tested isolates. The MIC observed on *Salmonella* spp was 62.5 mg/ml, 31.25 mg/ml on *Klebsiella* spp, 31.25 mg/ml on *E. coli* and lastly 62.5 mg/ml on *Shigella* spp.

Table 6 presents the minimum inhibitory concentration of the aqueous extract of *Danillia oliveri* on the tested isolates. The MIC observed on *Salmonella* spp was 62.5 mg/ml, 31.25 mg/ml on *Klebsiella* spp, 62.5 mg/ml on *E. coli* and lastly 250 mg/ml on *Shigella* spp.

Table 5 Minimum Inhibitory Concentration (MIC) of Ethanolic Extract of *Daniellia oliveri*

Organisms	**MIC**
Salmonella spp	62.5 mg/ml
Klebsiella spp	31.25 mg/ml
E. coli	31.25 mg/ml
Shigella spp	62.5 mg/ml

Table 6 Minimum Inhibitory Concentration (MIC) of Aqueous Extract of *Daniellia oliveri*

Organisms	**MIC**
Salmonella	62.5 mg/ml
Klebsiella spp	31.25 mg/ml
E. coli	62.5 mg/ml
Shigella spp	250 mg/ml

4. Discussion

Daniellia oliveri has a wide spectrum of antibacterial activity, affecting *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Clostridium*, *Mycobacterium*, and *Helicobacter* species [13]. Previous reports have shown a synergistic antibacterial effect when *Daniella oliveri* extract and antibiotics are combined [14].

In this study, the antibacterial activity of ethanolic and aqueous *Daniellia oliveri* leave extracts on enteric bacteria showed a reasonable antibacterial activity on *Salmonella* spp, *Klebssiella* spp, *E. coli* and *Shigella* spp. The trend in the antibacterial activity of this extract showed that the effect of the ethanolic extract was dependent on the concentration as the effect kept decreasing form highest concentration (250m/ml) to lowest concentration (31.25 mg/ml). This therefore implies that the higher the extract concentration the higher the antibacterial activity and vice versa. However, the effect observed on *E. coli* was not dependent of the concentration of the extract as the effect was highest at a concentration of 62.5 mg/ml and lowest at a concentration of 31.25 mg/ml. The effect of the ethanolic extract of *Daniellia oliveri* extract observed in this study is in agreement with the study by Coker *et al* [9], who stated that *Daniellia oliveri* exhibited broad spectrum antibacterial activity even on multi-drug resistant pathogens.

This effect of the aqueous leave extract of *Daniellia oliveri* was as well dependent on the concentrations of the extract like in the ethanolic extract but was not dependent on the concentration of the extract in the case of *E. coli* as the effect was highest at a concentration of 250 mg/ ml and lowest at a concentration of 31.25 mg/ml. The effect of the aqueous extract of *Daniellia oliveria* observed in this study is also in agreement with the work of [15] who reported that *Daniellia oliveria* also inhibited the growth of the test organisms with the most active against *K. pneumoniae* with MIC of 0.6 µg/ml and also very active against *S. aureus* with MIC of 0.8 µg/ml and his sample was also effective against *E. coli*, *P. aeruginosa* and *E. faecalis* with MICs of 1.6, 2.6, 5.4 and 5.8 µg/ml respectively but not as effective as *K. pneumoniae* and *S. aureus*.

5. Conclusion

Daniellia oliveri exhibited inhibitory activity on enteric pathogens including *Salmonella* sp, *Klebsiella* sp., *E. coli* and *Shigella* spp. This discovery is necessary because the use of *Daniellia oliveri* extract in synthetic antibiotics could be helpful in the treatment of gastrointestinal disorders caused by enteric pathogens.

Recommendations

- Further research should be carried out using higher concentration to clearly establish the relationship between the inhibitory activities of *Daniellia oliveri* and extract concentration.
- Other parts of *Daniellia oliveri* such as roots, bark and seeds should also be investigated for antibacterial activity, this should be in the form of a comparative analysis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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