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Extraction, Fractionation, Antimicrobial Screening, Phytochemical Analysis and Fourier Transform Infrared (FTIR) Spectroscopic Characterization of *Daniella Oliveri* Leaf

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Abstract

Medicinal plants have been discovered for decades and used in traditional medicine practices ever since prehistoric times. Numerous phytochemicals with established or potential biological activity have been identified in plants. The objectives of this work is to determine the Antimicrobial Activities, Minimum Inhibitory Concentration (MIC), Phytochemical constituents and the FT-IR of the fractions of *Daniellia oliveri*. The Antimicrobial screening was determined using agar well diffusion method. The Crude methanolic extract obtained was fractionated into N-Hexane, Ethylacetate, Dichloromethane and N-Butanol. The phytochemical screening was done using standard methods. The IR absorption spectra were recorded in the 4000-650 cm^{-1} range on a perkin-Elmer FT-IR spectrometer model 2000 using KBr pellets. The result of the FT-IR analysis shows the presence of different functional groups. The result of the Antimicrobial screening shows that at 10 mg/mL the crude extract was active against *Salmonella typhi* with Zone of inhibition of (±16 mm) which compares favourably with the control (±17 mm), *Escherichia coli* (±17 mm) compares favourably with the control ciprofloxacin (+18 mm). The result of the antimicrobial screening shows that the fractions have activity against different microorganisms. The phytochemical screening of the extracts revealed the presence of flavonoids, saponins, total phenol, tannins, cardiac glycoside and alkaloid in trace amount. The result of the FT-IR analysis shows the presence of different functional groups for the fractions. The absorption bands for n-hexane fraction have wave numbers of 3339 cm^{-1} (O-H $_{stretch}$), 2854-2921 cm^{-1} (C-H $_{stretch}$), 1611 cm^{-1} (C=C $_{stretch}$) and 1447 cm-1 (C-H $_{bend}$) and the Ethyl Acetate fraction with wave numbers 3204 cm^{-1} (O-H $_{stretch}$), 2921 cm^{-1} (C-H $_{Stretch}$), 1201-1287 cm^{-1} (C-O $_{stretch}$). These functional group from the Spectral shows that there are active compounds present in the fractions.

Keywords: Phytochemicals, Antimicrobial, FT-IR Ciprofloxacin, Cotrimoxazole, Fluconazole, and *Daniellia oliveri*

Introduction

Infectious diseases are the number one causes of death accounting for approximately one half of all death in tropical countries[1]. Traditional medicine like orthodox medicine has its own methods and techniques which aim at healing diseases[2]. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines because of their effectiveness, affordability, availability, low toxicity and acceptability[3], [4] to cure infections. Thus, plants remain the most abundant natural primary source of active drugs and are valuable in the ethno medical treatment of diverse ailments [5].

Daniellia oliveri plant can be effective in the treatment of dysentery, typhoid fever, headache and series of infections caused by microorganisms[6]. Applied externally, the gum resin is used to treat abscesses, itchy skin, skin diseases[7]. An aqueous extract of the powdered bark has been shown to have effective pain reduction properties and also to be antioxidant[8].

The study aimed to carry out extraction, fractionation, antimicrobial screening, phytochemical analysis and Fourier Transform Infrared (FTIR) spectroscopic characterization of *Daniella oliveri* leaf.

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Materials and Methodology

Sample Collection

The leaf of *Daniellia oliveri* also called '*Agba*' by the Igalas was collected from Agbeji, in Anyigba Dekina Local Government of Kogi State, Nigeria. The plant was identified in the Department of Plant and Environmental Biology, Kogi State University, Anyigba, Kogi State, Nigeria. The specimen Voucher number is PT-014.

Preparation and extraction of the plant

The sample was washed to remove sand particle and *was* allowed to dry at room temperature. The dried leaves were pounded to increase the surface area. A 150 g of *Daniellia oliveri* leaves was weighed into 2000 mL conical flask containing 950 mL of methanol. The flask was shaken vigorously and tightly sealed with foil paper and masking tape and left in a cool dry place for 3 days (72 hours). After 72 hrs, the mixture was filtered using cotton wool. The filtrate was concentrated using a rotary evaporator.

Qualitative Phytochemical Screening of the crude Extract of the Plant

A 0.5 g amount of *Daniellia oliveri* methanolic crude extract was weighed using an electronic weighing balance and dissolved in 50 ml of absolute ethanol in a conical flask. The solution was filtered using a filter paper. The filtrate was used for the phytochemical screening for alkaloids, tannins, flavonoids, saponins, total phenols and glycosides.

Test for Alkaloids: 2 mL of the plant extracts and 2 mL of 10% hydrochloric acid was added. To the acidic medium, 1 mL of dragendroff's reagent was added. An orange precipitate indicates a positive result[9] .

Test for Tannins: 5 drops of 0.1% ferric chloride was added to 2 mL of the plant extracts, and a brownish green or blue-black coloration indicates a positive result[10] .

Test for Flavonoids: 2 mL of 10% sodium hydroxide was added to 2 mL of the plant extracts in a test tube. An intense yellow color was formed which turned colorless upon addition of 2 mL of dilute hydrochloric acid indicating a positive result[9].

Test for Saponins: A 2 mL of the plant extracts was diluted with 2 mL distilled water. It was then agitated in a test tube for 5min. About 0.1 cm layer of foam indicates a positive result[9]

Test for Phenols: 2 mL of the plant extracts was mixed with 2 mL of 1% ferric chloride. The formation of deep blue or blue-black coloration is an indication of a positive result[11] .

Test for Glycosides: 2 mL of acetic acid was added to 2 mL of the plant extracts. The mixture was cooled in cold water bath. The 2 mL of concentrated H_2SO_4 was added. Color development from blue to bluish green indicates the presence of glycosides[10].

Quantitative Phytochemical Screening of the Crude Extract of the plant

Determination of Tannins

Tannins were quantified using the Folin-Ciocalteu method[12]. About 0.1 ml of the sample extract was added to volumetric flasks containing 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, and 1 ml of 35 % sodium trioxocarbonate (IV) solution and diluted to 10 ml with distilled water. Each mixture was shaken, kept at room temperature for 30 minutes, and then the absorbance was measured at 725 nm using a spectrophotometer. A blank solution was prepared similarly without the sample extract. The tannin concentration was calculated using the calibration curve of a pure quercetin standard equation: $y=0.057x-0.071$

Determination of Saponins

Total saponin content was determined using the method[13]. In different volumetric flasks, 50 ml of the sample extract was added to 250 ml of distilled water, followed by 250 ml of vanillin reagent, and then 2.5 ml of 72 % sulfuric acid. Each mixture was shaken well, kept in a water bath at 60 ℃ for 10 minutes, then cooled in an ice-water bath. The absorbance was measured at 544 nm. Vanillin was prepared by dissolving 800 mg of vanillin in 10 ml of 99.5 % ethanol solution.

Determination of Total Phenol

Total phenol content was determined using the Folin-Ciocalteu method[12]. In different volumetric flasks, 20 ml of sample extract was added to 6 ml of distilled water, 100 ml of Folin-Ciocalteu

reagent, and 300 ml of 10 % sodium carbonate solution. Each mixture was incubated in a shaking water bath at 40 °C for 30 minutes and the absorbance was measured at 760 nm. A blank solution was prepared similarly without the sample extract. The total phenol concentration was calculated using the calibration curve of a pure quercetin standard equation:

$A=0.00098c+9.925\times10^{-3}$

Determination of Flavonoids

Flavonoid content was determined using the aluminum chloride colorimetric assay[14]. In different volumetric flasks, 1 ml of sample extract was added to 4 ml of distilled water, followed by 0.3 ml of 5 % sodium nitrite. After 5 minutes, 0.3 ml of aluminum chloride was added and left for another 5 minutes, then 2 ml of 1 M sodium hydroxide was added. Each volumetric flask was diluted to 10 ml with distilled water and the absorbance was measured at 510 nm. A blank solution was prepared similarly without the sample extract. The flavonoid concentration was calculated using the calibration curve of a pure quercetin standard equation: $y=0.0096x - 0.0066$

Determination of Cardiac Glycoside

Cardiac glycoside content was determined according to [15]. A 10 % extract of the sample was mixed with 10 ml of Baljet reagent in different volumetric flasks. After an hour, each mixture was diluted with 2 ml of distilled water and the absorbance was measured at 495 nm. A blank solution was prepared similarly without the sample extract. The cardiac glycoside concentration was calculated using the calibration curve of a pure quercetin standard equation: $y=2.285x-0.012$

Determination of Alkaloids

Alkaloids were determined by the method[12]. In different 250 ml beakers, 50 ml of 20 % acetic acid was added to 5 g of the sample extract and covered to stand for four hours (Mayer's test). Each mixture was filtered and the volume was reduced to one quarter using a water bath. Ammonium hydroxide was added dropwise to each extract until the precipitate was completed. Each solution was allowed to settle, and the precipitate was collected by filtration. The filter paper was weighed before and after collecting the precipitate.

$$\% \text{ of Alkaloid} = \frac{W_2-W_1 \times 100}{\text{Weight of Sample}}$$

W_2=Weight of filter paper +Alkaloid, W_1 = Weight of filter paper

Fractionation of the methanolic extract

Solvent-solvent partitioning was done by using the protocol designed by[16],[17] .The prepared solution was then fractionated successively using solvents of increasing polarity from N-Hexane, Ethylacetate, dichloromethane, n-butanol and methanol.

All the fractions were evaporated to dryness using a rotary evaporator at low temperature of 39ºC and kept in air tight containers for further analysis.The methanolic crude extract 50 g was soaked in 50 mL distilled water to make a suspension and it was then transferred into the separatory funnel and about 250

mL of n-hexane was added and shaken carefully, then allowed to stand until clear layer is formed. The bottom of the separating funnel is opened to remove the aqueous layer. The remaining contents in the separating funnel are poured into a clean beaker to get n-hexane fraction.

The addition continued until after adding n-hexane of the same volume and shaken and no reasonable quantity of extract appeared to move into the n-hexane portion. Similar cycle was performed for ethyl acetate, dichloromethane, n-butanol and methanol.

Collection of Microorganism

The test organisms were obtained from the Microbiology Laboratory, Kogi State University, Anyigba, Nigeria. The organisms include bacteria (*Staphylococcus aureus, Escherichia coli, Bacillus Subtillis, Salmonella typhi*) and fungi (*Aspergillus niger, Microsporum Canis, Candida Albicans*). The test organisms were stored in peptone water prior to the bioassay of the extract.

Preparation of standard solution and dilution of the fraction

Different concentration of the crude extract and fractions (N-Hexane, Ethylcaetate, Dichloromethane and N-Butanol) of *Daniellia Oliveri* leaves were prepared by weighing 2.5 mg, 5.0 mg, 7.5 mg and 10.0 mg using electronic balance and dissolving in 1 mL of Dimethyl sulphoxide (DMSO) to make 2.5 mg/ml 5.0 mg/ml, 7.5 mg/ml and 10.0 mg/ml of the extract and fractions.

Antimicrobial Screening

Culture plates of Muller-Hinton Agar were prepared. The nutrient agar was prepared using standard method and sterilized at 120 ^{o}C. In determining the antimicrobial activity of the crude and fractions of *Hymnenocardia acida*, the agar well diffusion method[18] was used for each of the respective Muller Hintun Agar plates, a sterile cork borer of 6 mm in diameter was used to create wells out of the solidified agar. Each of the wells were filled with 0.2 ml of the various concentration of the crude and fractions of the plant i.e 2.5 mg/ml 5.0 mg/ml, 7.5 mg/ml and 10.0 mg/ml of the crude extract, N-Hexane fraction, Ethylacetate fraction, Dichloromethane and N-butanol fractions. One of the wells was filled with a Standard solution of ciprofloxacin, Cotrimoxazole and other well was filled with standard solution of fluconazole. They were allowed to diffuse into the agar at room temperature for one hour, then incubated at 37 ^{0}c for 24 hours. The zones of inhibition were measured to the nearest millimeter using a metric rule in triplicate and the mean values recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The estimation of minimum inhibitory concentration for the crude methanolic extract was carried out using the method of[19]. 0.5 mL of varying concentrations of the extract and fractions (2.5mg/ml, 5.0 mg/ml, 7.5 mg/ml 10.0 mg/ml) were dispensed into each test tubes containing nutrient broth, inoculated with loopful of each test

organism. 0.5 Mcfarland turbidity standard was adopted. A tube containing nutrient broth with test organism, but no extract serve as control. The MIC was taken from the tube with least concentration, showing no visible turbidity after 24 hours incubation at 37^{o}C. Therefore, growth was examined by observing turbidity.

FT-IR Analysis

Fourier transform infrared (FTIR) spectroscopy was used to reveal all the possible functional groups present in the fractions (hexane and ethyl acetate). IR absorption spectra were recorded in the 4000-650 cm^{-1}range on a Perkin-Elmer FT-IR spectrometer model 2000 using KBr pellets. The fractions (n-hexane and ethyl acetate) were placed in contact with KBr disc and FT-IR spectra were collected at the frequency of 4000-650 cm^{-1} by coadding 32 scans at a resolution of 8 cm^{-1}The analysis was carried out by Agilent Technologies, Kastina, Nigeria.

Results

Table 1: Qualitative Phytochemical Screening of *the crude extract* of *Daniellia Oliveri*

Phytochemicals	**Inference**
Alkaloids	+
Tannins	+
Flavonoids	++
Saponins	+++
Total Phenols	++
Cardiac Glycosides	+

Keys: + = Trace, ++ = Abundant, +++ = Very abundant

Table 2: Quantitative Phytochemical Screening of the Crude Extract of *Daniellia Oliveri*

Phytochemicals	**Maximum Wavelength of Absorption (nm)**	**Sample Absorbance**	**Concentration (mg/g)**
Tannins	725	1.1605	21.60
Flavoniods	510	1.0375	115.94
Saponins	544	0.8915	793.40
Total phenol	760	1.3905	106.95
Cardiac glycosides	495	0.3855	0.17

% Alkaloid

W_2-W_1/Weight of sample x 100

= 4.60%.

Note: W_2 =Weight of filter paper +Alkaloid,

W_1 = Weight of filter paper

Table 3: Antimicrobial Activity of Methanol Crude Extract of *Daniellia oliveri* on the Test Organisms

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0 mg/ml	7.5 mg/ml	10 mg/ml	Positive Control	Negative Control
Bacteria						0 mm
Escheridia coli	6 mm	9 mm	14 mm	17 mm	18 mm	
Salmonella typhi	7 mm	8 mm	13 mm	16 mm	17 mm	0 mm
Staphylococcus Aureus	6 mm	8 mm	13 mm	17 mm	18 mm	0 mm
Bacillus Substillis	5 mm	7 mm	12 mm	16 mm	16 mm	0 mm
Fungi						
Candida albicans	8 mm	8 mm	14 mm	15 mm	17 mm	0 mm
Microsporum canis	6 mm	9 mm	15 mm	16 mm	17 mm	0 mm

Table 4: Antimicrobial Activity Of The N-Hexane Fraction of *Daniellia oliveri* On The Test Organism

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0	7.5	10	Positive Control	Negative Control
Bacteria						
Escherichia coli	2 mm	5 mm	9 mm	12 mm	17 mm	0 mm
Salmonella typhi	2 mm	4 mm	6 mm	10 mm	16 mm	0 mm
Staphylococcus aureus	2 mm	3 mm	3 mm	6 mm	15 mm	0 mm
Fungi						
Aspergillous Niger	0 mm	0 mm	0 mm	0 mm	17 mm	0 mm
Candida albicans	0 mm	0 mm	0 mm	0 mm	15 mm	0 mm

Table 5: Antimicrobial activity of the Ethyl acetate fraction of *Daniellia oliveri* on the test organism

Test Organism/Zone of Inhibition	2.5 mg/ml	5.0 mg/ml	7.5 mg/ml	10 mg/ml	Positive control	Negative control
Bacteria						
Staphylococus Aureus	15 mm	17 mm	19 mm	22 mm	28 mm	0 mm
Salmonella Typhi	6 mm	15 mm	18 mm	20 mm	25 mm	0 mm
Bacillus subtilis	10 mm	13 mm	19 mm	21 mm	24 mm	0 mm
Fungi						
Aspergilous Niger	5 mm	8 mm	10 mm	14 mm	19 mm	0 mm
Candida Albicans	10 mm	19 mm	21 mm	23 mm	26 mm	0 mm

Table 6: Antimicrobial Activity of the Dichloromethane Fraction of *Daniellia oliveri* on the Test Organisms

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0	7.5	10	Positive Control	Negative Control
Bacteria						
Escheridia coli	4 mm	5 mm	8 mm	16 mm	16 mm	0 mm
Salmonella typhi	4 mm	5 mm	9 mm	15 mm	15 mm	0 mm
Staphylococcus Aureus	3 mm	6 mm	10 mm	15 mm	16 mm	0 mm
Bacillus Substillis	4 mm	6 mm	10 mm	15 mm	14 mm	0 mm
Fungi						
Candida albicans	5 mm	7 mm	10 mm	16 mm	17 mm	0 mm
Microsporum canis	5 mm	7 mm	8 mm	15 mm	17 mm	0 mm

Table 7: Antimicrobial Activity of the N-butanol fraction of *Daniellia oliveri* on the Test Organisms

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0 mg/ml	7.5 mg/ml	10 mg/ml	Positive Control	Negative Control
Bacteria						
Escheridia coli	5 mm	7 mm	10 mm	16 mm	18 mm	0 mm
Salmonella typhi	6 mm	5 mm	8 mm	16 mm	15 mm	0 mm
Staphylococcus Aureus	5 mm	5 mm	10 mm	17 mm	16 mm	0 mm
Bacillus Substillis	6 mm	5 mm	10 mm	16 mm	14 mm	0 mm
Fungi						
Candida albicans	6 mm	8 mm	11 mm	15 mm	17 mm	0 mm
Microsporum canis	4 mm	5 mm	7 mm	15 mm	17 mm	0 mm

Table 8: Minimum Inhibitory concentration (MIC) of the methanol crude extract of ***Daniellia oliveri*** **on test organism**

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0	7.5	10	Positive Control	Negative Control
Escherichia coli	-	-	+	+	+	-
Salmonella typhi	-	-	+	+	+	-
Staphylococcus aureus	-	-	+	+	+	-
Bacillus subtilis	-	-	-	+	+	-
Candida albicans	-	-	+	+	+	-
Microsporum canis	-	-	+	+	+	-

Key - = Not Active , + = Active

Table 9: Minimum Inhibitory concentration (MIC) of the N-Hexane fraction of ***Daniellia oliveri*** **on the test organism**

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0	7.5	10	Positive Control	Negative Control
Escherichia coli	-	-	-	+	+	-
Salmonella typhi	-	-	-	+	+	-
Staphylococcus aureus	-	-	-	-	+	-
Aspergillous Niger	-	-	-	-	+	-
Candida albicans	-	-	-	-	+	-

Key - = Not Active , + = Active

Table 10: Minimum Inhibitory concentration (MIC) of the ethylacetate fraction of *Daniellia oliveri* on the test organism

Test Organism	2.5 mg/ml	5.0 mg/ml	7.5 mg/ml	10 mg/ml	Positive control	Negative control
Staphylococcus Aureus	-	+	+	+	+	-
Salmonella Typhi	-	-	+	+	+	-
Bcillus subtilis	-	-	+	+	+	-
Aspergilous Niger	-	-	-	-	+	-
Candida Albicans	-	+	+	+	+	-

Key - = Not Active , + = Active

Table 11: Minimum Inhibitory concentration (MIC) of the Dichloromethane fraction of *Daniellia oliveri* on test organism

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0	7.5	10	Positive Control	Negative Control
Escherichia coli	-	-	-	+	+	-
Salmonella typhi	-	-	-	+	+	-
Staphylococcus aureus	-	-	-	+	+	-
Bacillus subtilis	-	-	-	+	+	-
Candida albicans	-	-	-	+	+	-
Microsporum canis	-	-	-	+	+	-

Key - = Not Active , + = Active

Table 12: Minimum Inhibitory concentration (MIC) of the N-Butanol fraction of *Daniellia oliveri* on test organism

Test Organism/Zone of Inhibition(mm)	Conc (mg/mL) 2.5	5.0	7.5	10	Positive Control	Negative Control
Escherichia coli	-	-	-	+	+	-
Salmonella typhi	-	-	-	+	+	-
Staphylococcus aureus	-	-	-	+	+	-
Bacillus subtilis	-	-	-	-	+	-
Candida albicans	-	-	-	+	+	-
Microsporum canis	-	-	-	+	+	-

Key - = Not Active , + = Active

Table 13: FTIR Absorption bands for n- Hexane Fraction

Wavenumber (Cm-1)	Functional Group
3339	O-H $_{Stretch}$ (Carboxylic Acid)
2921, 2955, 2854	C-H $_{Stretch}$ (Aliphatic)
1611,1514	C=C $_{Stretch}$(Aromatic)
1447, 1074	C-H $_{Bend}$(Aliphatic)
1287, 1208	C-O $_{Stertch}$ (Acyl)

Table 14: FTIR absorption bands for Ethyl acetate fraction

Wavenumber (Cm -1)	Functional Group
3202	O-H $_{Stretch}$ (Aromatic)
2921	C-H $_{Stretch}$ (Aliphatic)
1603,1514	C=C $_{Stretch}$ (Aromatic)
1443	C-H $_{Bend}$
1354	C-O $_{Bend}$
1287	C-O $_{Stretch}$ (Acyl)

Table 15: FT-IR Bands of Absorption for Dichloromethane Fraction

Wavenumber (Cm-1)	Functional Group
3317	O-H $_{Stretch}$ (Carboxylic acid)
3026	C-H $_{stretch}$ in alkene
2926	C-H $_{stretch}$ (Aliphatic
1654	C=O $_{Stretch}$ (Carboxylic acid)
1602	C=C $_{stretch}$
1371, 1446	C-H $_{bend}$ (Aliphatic)
1177	C-O $_{Stretch}$ (Acyl)
693, 752	C-H $_{bend}$

Table 16: FT-IR bands of absorption for N-Butanol fraction

Wavenumber (cm-1)	Functional Group
3324	O-H $_{stretch}$ (carboxylic acid)
2870, 2929	C-H $_{Stretch}$ (Aliphatic)
1654	C=O $_{stretch}$ (Carboxylic acid)
1043, 1461	C-H $_{bend}$ (Aliphatic)
738, 849	C-H $_{bend}$ (Aromatic

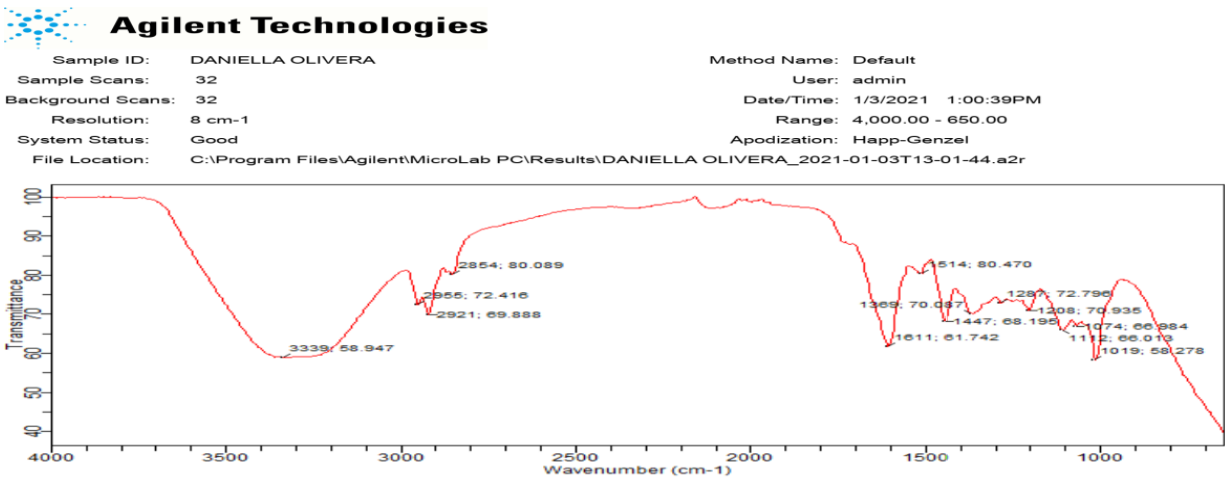

Figure 1: FTIR Spectra of the n- Hexane fraction

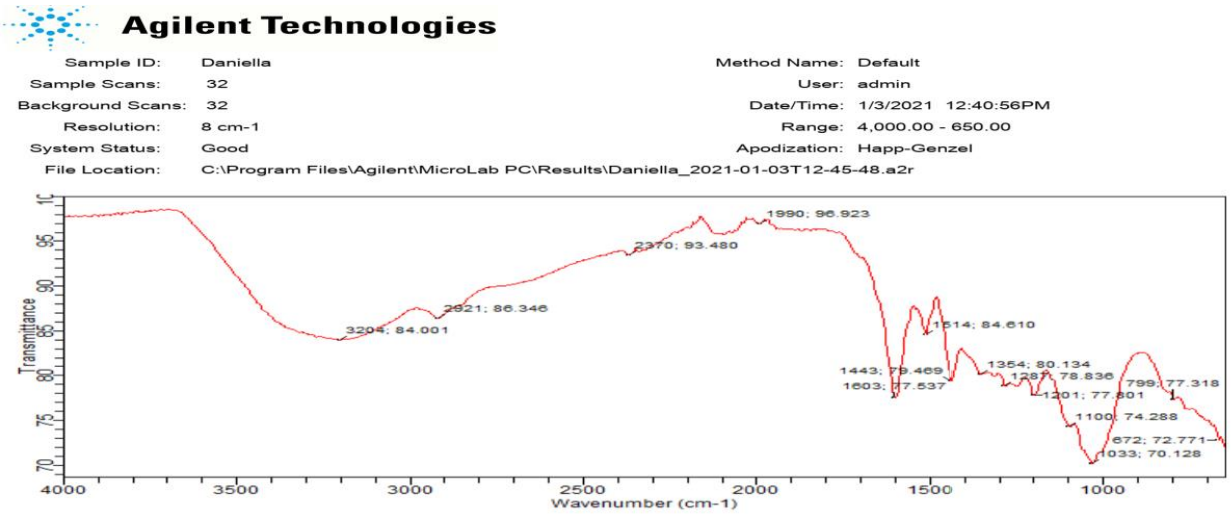

Figure 2: FTIR Spectra of Ethyl acetate fraction

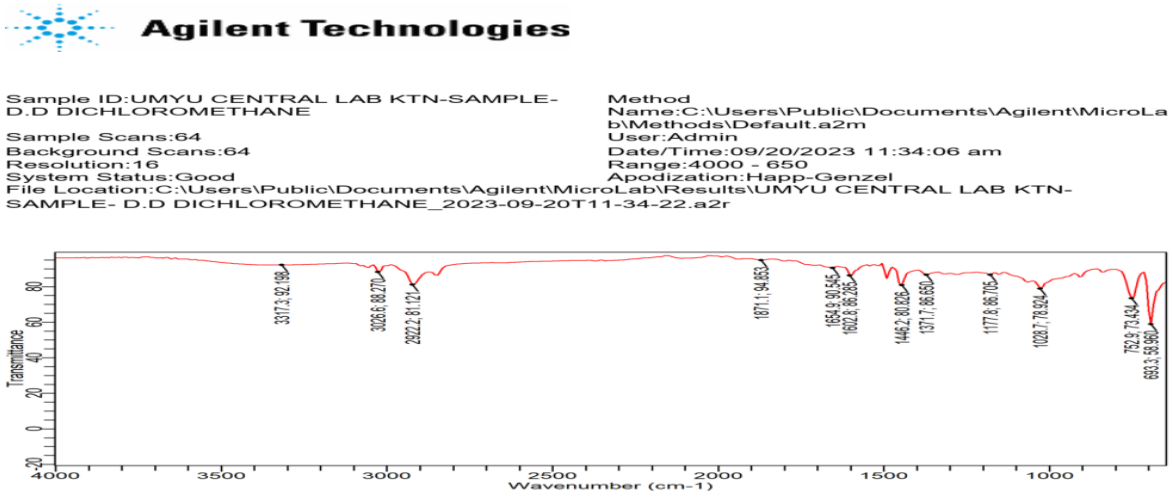

Figure 3: FTIR Spectra of Dichlormethane Fraction

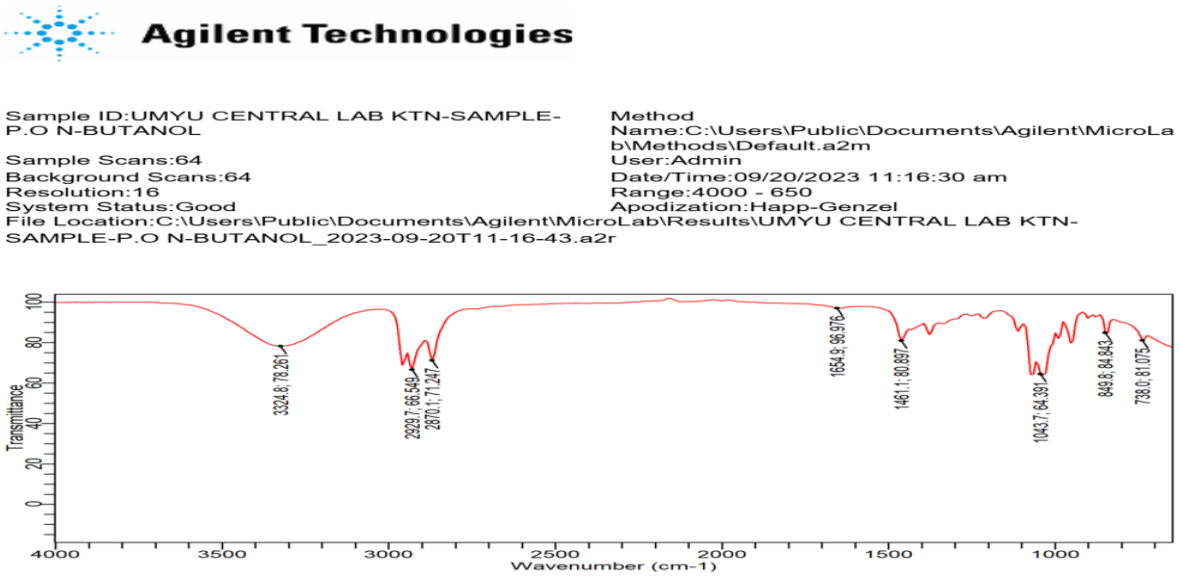

Figure 4: FTIR Spectral of N-butanol fraction

Figure 5: Methanol Antimicrobial Plate

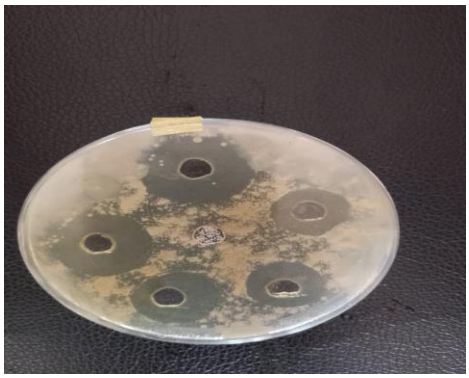

Figure 6: N-Hexane Antimicrobial Plate

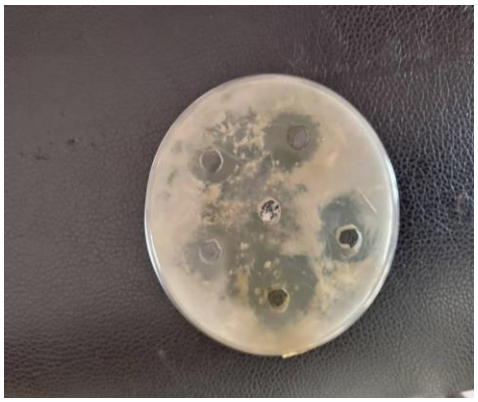

Figure 7:Ethylacetate Antimicrobial Plate

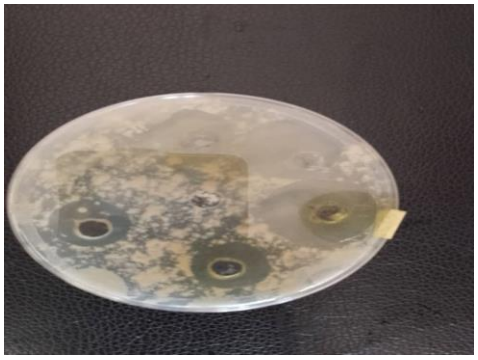

Figure 8:DichloromethaneAntimicrobial plate


Figure 9: N-Butanol Antimicrobial plate

Discussion

This study investigated the phytochemical constituents of the leaves extract, the antimicrobial properties and the Fourier Transform Infra Red Spectroscopy of the fractions of *Daniellia oliveri*.

Phytochemical Analysis

Table 1 -2 shows the phytochemical constituents of the extracts from *Daniella oliveri*.

The qualitative phytochemical screening of the extracts revealed the presence of tannins, alkaloids, flavonoids, saponins, total phenol and cardiac glycosides. The presence of tannin in extracts accounts for its antimicrobial properties[20]. Tannin has antibacterial, antiviral, and astringent properties[21],[22]. Saponins have an antioxidant effect[23] and may be directly toxic to cancer cells[24]. Literature reveals that saponins exhibit a biological role and medicinal properties such as hemolytic factor[25], anti-inflammatory[26], Antibacterial[27], Antifungal[28] and Antiviral[29] action. Phenolic compounds possesses high levels of antimicrobial activity[30].

Antimicrobial Activity of the crude and fractions of the plant

Table 3-7 shows the antimicrobial activity of the crude and fractions (N-hexane, ethylacetate, Dichloromethane, N-butanol and methanol) of *Daniella oliveri* leaves on the test organisms (*Staphylococcus aureus, bacillus subtillis, Escherichia coli, microsporum canis, aspergillus niger, candida albicans and salmonella typhi***.** Figure 5-9 shows the antimicrobial slide of the plate for the crude and fractions of the plant.

The result of the Antimicrobial screening shows that at 10 mg/mL the crude extract was active against *salmonella typhi* with Zone of inhibition of (±16 mm) which compares favourably with the control (±17 mm), *Escherichia coli* (±17 mm) compares favourably with the control ciprofloxacin (+18 mm) .The result of the antimicrobial screening shows that the fractions have activity against different microorganisms. The inhibitory activity was potent at high concentration (10 mg/mL) against *E.coli* and *S.typhi*. *Aspergillus Niger* and *Candida Albicans* shows resistance on both crude extract and the control. The antimicrobial activity demonstrated by the extracts may be due to the presence of the phytoconstituents present in the extracts.

FTIR Spectral Data Interpretation

Table 13-16 and Figure 1-4 shows the interpretation and FTIR spectra of the fractions (N-hexane, ethylacetate, Dichloromethane and N-butanol)**.** For n- hexane fraction, the

characteristics absorption band were exhibited at 3339cm^{-1} (O-H stretching for carboxylic acid) 2858, 2921 cm^{-1} (C-H stretching for Aliphatic), 1611 and 1514 cm^{-1} (C=C stretching), 1447cm^{-1},1033cm^{-1}(C-H bending for Aliphatic) 672 cm^{-1}.

EthylAcetate fraction with wave numbers 3204cm^{-1} (O-H $_{stretch}$), 2921 cm $^{-1}$ (C-H $_{Stretch}$), 1201-1287cm^{-1}(C-O $_{stretch}$). The presence of these functional group in the extract give credence to the antimicrobial property of the plant extract.

For Dichloromethane Fraction, The characteristic absorption bands are O-H $_{Stretch}$ (Carboxylic acid) at 3317cm^{-1}, C-H $_{stretch}$ in alkene at 3026 cm^{-1}, C-H $_{stretch}$ (Aliphatic) at 2926 cm^{-1}, C=O $_{Stretch}$ (Carboxylic acid) at 1654 cm^{-1}, C=C $_{stretch}$, C-H $_{bend}$ (Aliphatic) at 1371, 1446 cm-1.

For N-Butanol Fraction, the characteristic absorption bands are O-H $_{stretch}$ (carboxylic acid) at 3324 cm-1, C-H $_{Stretch}$ (Aliphatic) at 2870, 2929 cm^{-1}, C=O $_{stretch}$ (Carboxylic acid) at 1654 cm^{-1} and C-H $_{bend}$ (Aromatic) at 738, 849 cm^{-1}.

Minimum inhibitory concentration (MIC) of the crude extract and fractions

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (antibiotic, bacteriostatic or antifungal) drugs that will inhibit the visible growth of a microorganism after overnight incubation[31] (Tripathi 2013) The crude extract inhibited the growth of both bacteria and fungi that is the microorganisms at 10 mg/ml (0.1µg/ ml. therefore 10 mg/ ml was taken as the minimum inhibitory concentration.

Conclusion

The result of the antimicrobial screening shows that the fractions have activity against different microorganisms. At concentration of 10 mg/mL, the crude extract was active against *Salmonella typhi* with Zone of inhibition of (±16 mm) which compares favourably with the control (±17 mm), *Escherichia coli* (±17 mm) compares favourably with the control ciprofloxacin (+18 mm).

The phytochemical screening of the extracts revealed the presence of different secondary metabolites with concentration 115.94 mg/g of flavonoids, 793.40 mg/g of saponins, 106.95 mg/g of total phenol, 21.60 mg/g of tannins, 0.17 mg/g of cardiac glycoside and alkaloid in trace amount.

The result of the FT-IR analysis shows the presence of different functional groups for the fractions. The absorption bands for n-hexane fraction have wave numbers of 3339 cm^{-1} (O-H $_{stretch}$), 2854-2921 cm^{-1} (C-H $_{stretch}$), 1611 cm^{-1} (C=C $_{stretch}$) and 1447 cm-1 (C-H $_{bend}$) and the Ethyl Acetate fraction with wave numbers 3204 cm^{-1} (O-H $_{stretch}$), 2921 cm $^{-1}$ (C-H $_{Stretch}$), 1201-1287 cm^{-1} (C-O $_{stretch}$). These functional group from the Spectral shows that there are active compounds present in the fractions

Conflict of Interest

The Authors declare no conflict of interest

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