
Antimicrobial Activities of Crude Extracts from the Stem Bark of *Strombosia Grandifolia* Hook.F. Ex. Benth

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Abstract

The dichloromethane (DCM) and methanol (MeOH) extracts of *Strombosia grandifolia* were screened for their antimicrobial activity using ciprofloxacin and terbinafine as standards. The diameter of zones of inhibition (ZOI), the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the DCM and MeOH extracts of *S. grandifolia* showed remarkable activities against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans*. The DCM extract of *S. grandifolia* at 100 mg/ml inhibited the growth of *S. aureus* with the diameter of zone of inhibition of 25 mm.

Keywords: *Strombosia grandifolia*, antimicrobial activity, minimum inhibitory concentration, minimum bactericidal

1. Introduction

Traditional medicinal practices have formed the basis for most of the early medicines used which were followed by subsequent clinical, pharmacological and chemical studies (Dias *et al.*, 2012). Documented evidences have showed that medicinal plants have the potency to inhibit microbial activities. Consequently, medicinal plant extracts and their secondary metabolites offer a wide range of application in the treatment of various ailments could provide useful sources in the treatment and management of these diseases. Herbal based extracts have been used extensively to treat microbial diseases particularly in developing countries while the bioactive compounds have displayed antimicrobial activities against various microbes. The choice of the stem bark of *Strombosia grandifolia* in this work was based on their previous medicinal uses by the locals in Okpokwu, Benue state of Nigeria. *Strombosia grandifolia* have been used traditionally for the treatment stomach ache, tuberculosis, skin infections, coughs and colds, wounds, gonorrhea, typhoid fever, dysentery, kidneys, diuretics, pulmonary troubles and erectile dysfunction (Kew Royal Botanical Gardens, 2019).

Materials and Methods

2.1 Materials

2.2 Plant Materials and Extraction

The stem bark of *Strombosia grandifolia* were collected in April 2018 from Ugbokolo forest, in Okpokwu Local Government Area (7°03'27.60"N, 8°12'21.60"E) of Benue State-Nigeria. Voucher specimen 03689 has been retained at the Herbarium, Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria-Nigeria. The collected plant materials were air dried for 21 days. The dried plant was pulverised and stored in a well-ventilated environment. The pulverized plant material (1 kg) was extracted with 2.5 litres of dichloromethane (CH₂Cl₂) and methanol for 72 hrs and then filtered. Each extract was concentrated in vacuo using a rotary evaporator at 40 °C to yield a dark brown gum 9g and 10g for the DCM and methanol extracts of *Strombosia grandifolia* respectively.

2.2.1 Thin Layer Chromatography

Prior to evaporation to dryness, thin layer chromatography (TLC) was run using silica gel (ALUGRAM® SIL G/UV₂₅₄ 20 x 20 cm, 60 P_{F254}) precoated on aluminium TLC plate using n-hexane:CH₂Cl₂:ethylacetate (8:2:1) as the solvents.

3. Antimicrobial Screening

3.1 Test Microorganisms

The microorganisms tested were: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. They were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria. All the isolates were checked for purity and maintained in nutrient agarslant.

3.2 Determination of Zone of Inhibition

The standardized inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), 5 appropriately labelled wells were punched into each agar plate. Aliquot of 0.3mL of the appropriate isolate concentration was placed in each well and then allowed to diffuse into the agar. An extra plate was streaked with the isolate and ciprofloxacin (10

μ/disc) was placed on it. The plates were incubated at 37 °C for 24 h. While for the fungi, Sabouraud dextrose broth was used and the incubation period was 30 °C and 48 h. The antimicrobial activities were expressed as diameter (mm) of inhibition zones produced by the plant extracts.

3.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the isolates were carried out on the test microbes using the broth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2017). Mueller Hinton broth was prepared; 10 mL was dispensed into tubes and was sterilized at 121 °C for 15 minutes and allowed to cool. The McFarland turbidity standard scale 0.5 was prepared to give turbidity solution. Normal saline was prepared, 10 mL was dispensed into sterile test tube and the test microbes was inoculated and incubated at 37 °C for 24 hours. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the McFarland turbidity scale by visual comparison at this point the test microbe has a concentration of about 1.5×10^8 cfu/mL. Two-fold serial dilution of the extracts in the sterilized broth was made to obtain the concentrations of 3, 1.5, 0.75, 0.37, 0.18 and 0.09 mg/ml. The initial concentration was obtained by dissolving 6 mg of the isolate in 10 ml of the sterile broth. Having obtained the different concentrations of the extracts in the sterile broth, 0.3 ml of the standard inoculum of the test microbe in the normal saline was then inoculated into the different concentrations. Incubation was made at 37 °C for 24 hrs, after which each test tube of the broth was observed for turbidity (growth). The lowest concentration of the extract in which the broth shows no turbidity was recorded as the Minimum Inhibitory Concentration (MIC).

3.4 Determination of Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

The minimum bactericidal concentration of the extracts was determined as outlined by the (CLSI) on the nutrient agar plates. Minimum bactericidal concentrations were determined by assaying the test tube contents of the MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 °C for 24 hrs for bacterial and 30 °C for 48 h for fungi. After which it was observed for microbial growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration/ minimum fungicidal concentration.

4 Results

The extract from *Strombosia grandifolia* (1.0 kg) each was weighed and the yields calculated. From the results shown in Table 1, the MeOH extract had the highest yield for the *Strombosia grandifolia*. The antimicrobial test results (Table 2-5), shows the diameter of zones of inhibition (mm) and other activities of the crude extracts of *Strombosia grandifolia*. The DCM extract showed the highest activity against *S. aureus* at the concentration of 25 mg/ml. The diameter of zone of inhibition ranged between 12-28 mm against the microorganisms at the various concentrations. The DCM extract at the concentration of 18 mg/ml showed the highest activity against *S. aureus*, *S. typhi* and *C. albican*. The MIC of these crude extracts against *S. aureus*, *E. coli*, *S. pneumoniae*, *S. typhi* and *C. albicans* ranged between 6.25-50.0 mg/ml. The minimum bactericidal/fungicidal concentration (MBC/MFC) of the crude extracts showed that most of the organisms were killed at 50 mg/ml. The MBC of these crude extracts against *S. aureus*, *E. coli*, *S. pneumoniae*, *S. typhi* and *C. albicans*

ranged between 50.0-100.0 mg/ml. The DCM extract of *S. grandifolia* showed MBC/MFC between 50.0-100.0 mg/ml while the MeOH showed MBC/MFC at 100.0 mg/ml.

Table 1: Percentage recovery of the various solvents extracts

Plants	Solvent	Yield of extract (g)	Percentage yield (%)
<i>Strombosia grandifolia</i>	DCM	09	0.9
	MeOH	10	1.0

Table 2: Diameter of Zone of Inhibition (mm) of DCM extract of *Strombosia grandifolia*

Microorganisms	Concentration (mg/ml)				Ciprofloxacin 10×10^{-6}	Terbinafine 30×10^{-6}
	100	50	25	12.5		
<i>Staphylococcus aureus</i>	25	18	15	12	20	
<i>Escherichia coli</i>	19	17	13	0	33	
<i>Streptococcus pneumoniae</i>	17	14	0	0	30	
<i>Salmonella typhi</i>	18	15	12	0	18	
<i>Candida albicans</i>	22	19	16	13		23

Table 3: Diameter of Zone of Inhibition (mm) of MeOH extract of *Strombosia grandifolia*

Microorganisms	Concentration (mg/ml)				Ciprofloxacin 10×10^{-6}	Terbinafine 30×10^{-6}
	100	50	25	12.5		
<i>Staphylococcus aureus</i>	18	16	13	0	20	
<i>Escherichia coli</i>	17	15	12	0	33	
<i>Streptococcus pneumoniae</i>	16	14	12	0	30	
<i>Salmonella typhi</i>	18	15	12	0	18	
<i>Candida albicans</i>	18	15	12	0		23

Table 4: Minimum Inhibitory Concentration (MIC) of *S. grandifolia* crude extracts

Concentration(mg/ml)		
<i>S. grandifolia</i>		
Microorganisms	DCM extract	MeOH extract
<i>Staphylococcus aureus</i>	12.5	25.0
<i>Escherichia coli</i>	25.0	50.0
<i>Streptococcus pneumoniae</i>	50.0	50.0
<i>Salmonella typhi</i>	25.0	25.0
<i>Candida albicans</i>	12.5	25.0

Table 5: Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) of *S. grandifolia* crude extracts

Concentration(mg/ml)		
<i>S. grandifolia</i>		
Microorganisms	DCM extract	MeOH extract
<i>Staphylococcus aureus</i>	50.0	100.0
<i>Escherichia coli</i>	100.0	ND
<i>Streptococcus pneumoniae</i>	100.0	ND
<i>Salmonella typhi</i>	100.0	100.0
<i>Candida albicans</i>	50.0	100.0

Key: ND=not determined

5 Discussion

The methanolextracted the highest mass (1,0 %) of material from the stem bark of *S. grandifolia*, while the dichloromethane extracted the lowest mass (0.9 %).

The results of diameter of zones of inhibition of the extracts ranged between 12-18 mm and 17-25 for the DCM and MeOH extracts of *S. grandifolia* respectively. The DCM extract of *S. grandifolia* at 100 mg/ml inhibited the growth of *S. aureus* with ZOI of 25 mm higher than the ZOI of 20 mm of ciprofloxacin (10 µg/ml) used. The MIC studies showed that the crude DCM and MeOH extracts from this plant inhibited the growth of *S. aureus*, *S. pneumoniae*, *E. coli*, *S. typhi* and *C. albicans*.

The result of the percentage recovery yield of *B.togoense* and *S. grandifolia* when 1.0 kg were extracted with DCM and MeOH gave 3.6 %, 3.0 % and 0.9 %, 1.0 % respectively. These fractions of these extracts exhibited antimicrobial activities against *S. aureus*, *S. pneumoniae*, *E.coli*, *S.typhi* and *C. albicans*. The preliminary antimicrobial studies of the DCM and MeOH extracts of the stem bark of *Strombosia grandifolia* showed broad spectrum antimicrobial activities against the tested microorganisms.

Abbreviations

Dichloromethane: CH₂Cl₂, methanol: MeOH, Minimum inhibitory concentration: MIC, minimum bactericidal concentration: MBC, minimum fungicidal concentration: MFC.

Declarations

Ethics approval and consent to participate

The collection of the stem part of *Strombosia Grandifolia* Hook.F. Ex. Benth used in this study complied with the local guidelines.

Permission to collect plant specimen

Appropriate permission was granted for the collection of the plant specimen.

Consent for publication

Not applicable

Availability of data and material

The search was by using keywords such as, *Strombosia, grandifolia*, antimicrobial activity. Other sources used are “Science direct”, “Google Scholar”, “Scopus”, database and many journal sites.

Competing interests

The authors declare that they have no competing interests.

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None

Author’s contribution

The research was designed and carried out by Ekalu Abiche while Stephany Unekwuajo Ukoje reviewed the manuscript. All authors read and approved the final manuscript.

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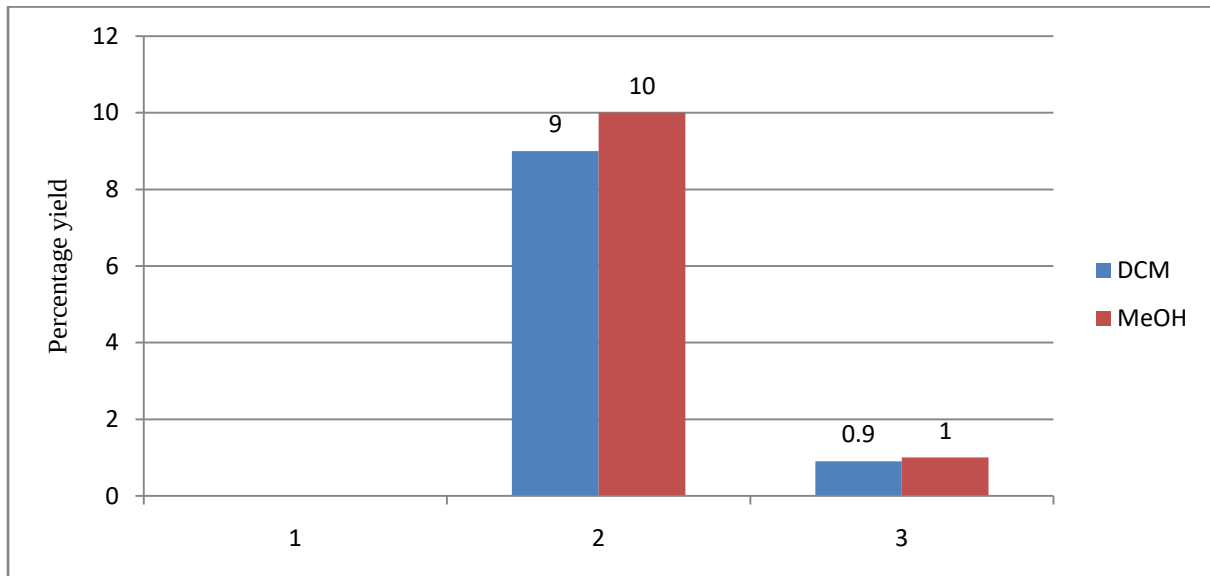
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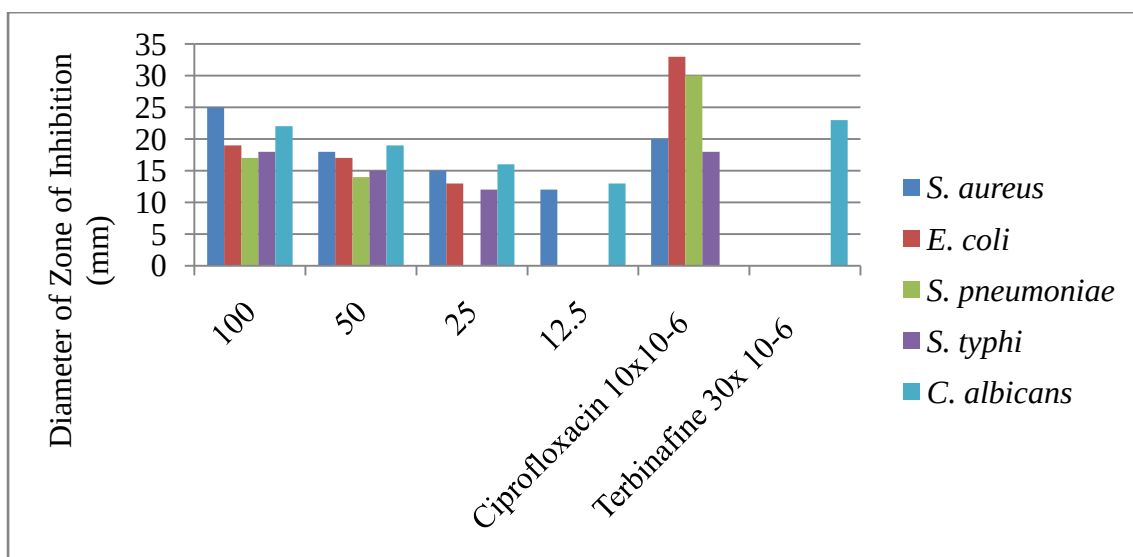
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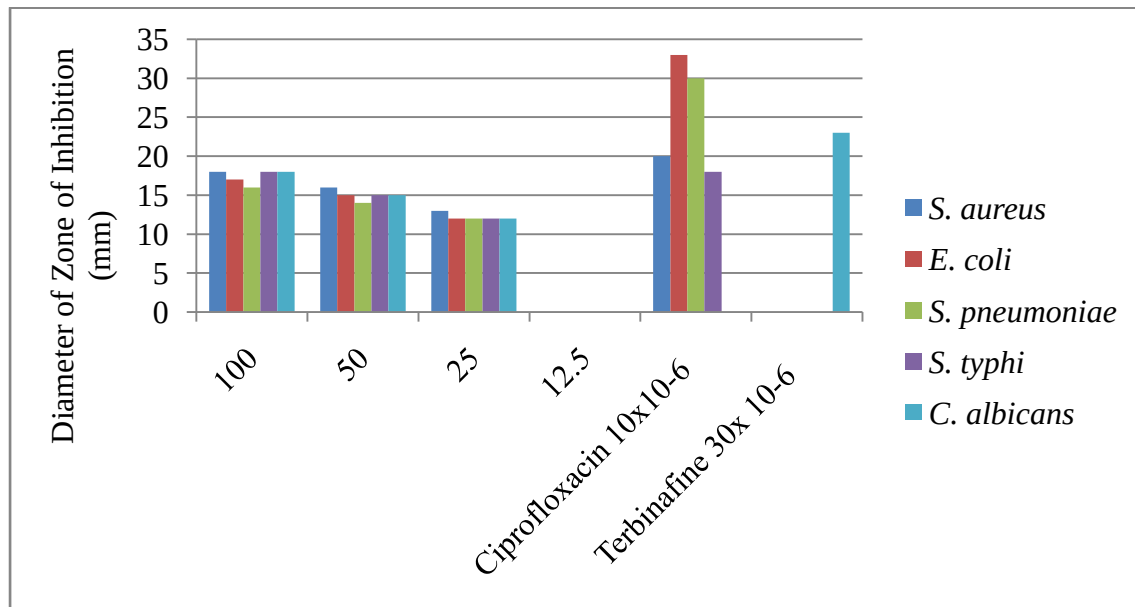
APPENDICES



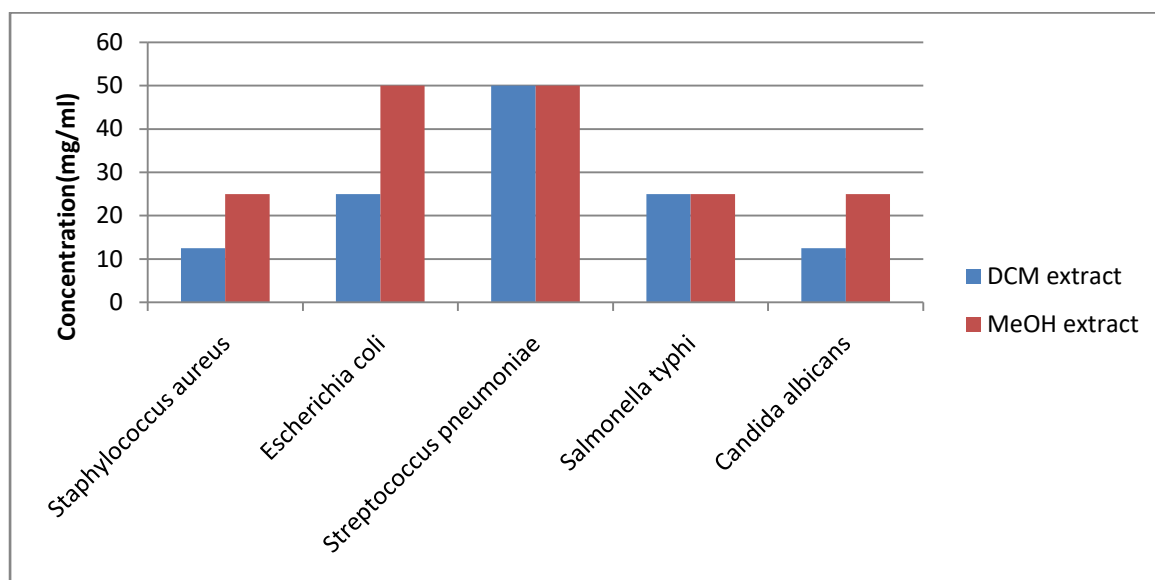
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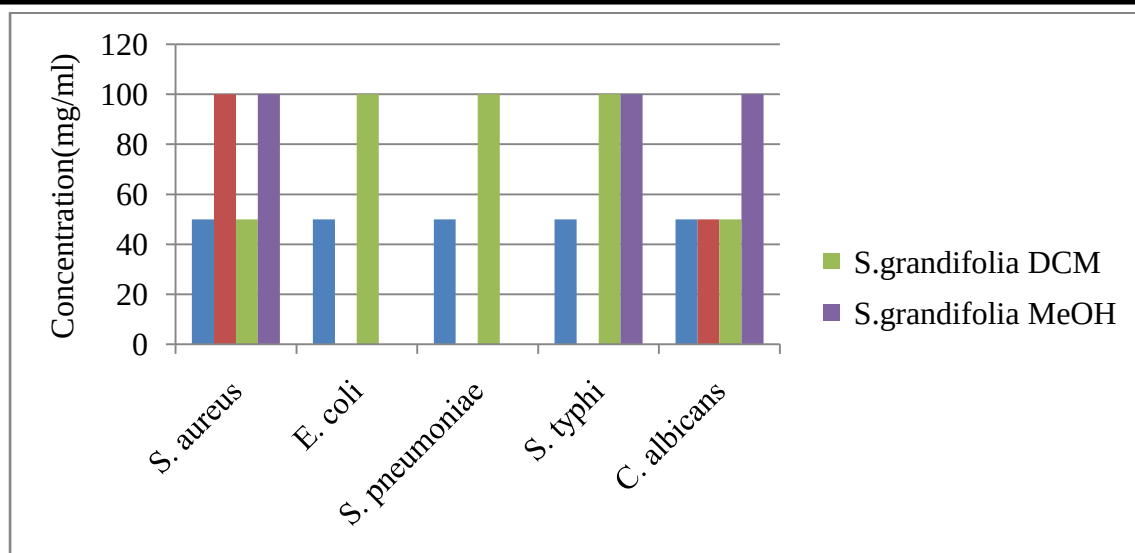
Appendix 2: Diameter of Zone of Inhibition (mm) of DCM extract of *Strombosia grandifolia*



Appendix 3: Diameter of Zone of Inhibition (mm) of MeOH extract of *Strombosia grandifolia*



Appendix 4: Minimum Inhibitory Concentration (MIC) of *S. grandifolia* crude extracts



Appendix 5: Minimum Bactericidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC) of *S. grandifolia* crude extracts