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In vitro antimicrobial activity of lignan from the stem bark of *Strombosia grandifolia* Hook.f. ex Benth

Abiche Ekalu[1*], Rachael Gbekele-Oluwa Ayo[2], James D. Habila[2] and Ibrahim Hamisu[2]

Abstract

Background: The continuous spread of multidrug-resistant and new strains of disease-causing microbe have become a great concern to the world health community. There is therefore the need for the development of new and effective drugs for the treatment of these diseases. Traditional medicinal plants used in our community could be a good source of drugs to fight these problems. This is why this study is focused on the antimicrobial properties of *Strombosia grandifolia* used as traditional medicines in Nigeria.

Results: The first reported investigation into the phytochemical constituents of *S. grandifolia* led to the isolation of (-)-8-hydroxypinoresinol from the CH_2Cl_2 extract of the stem bark of the plant. The compound was identified as (-)-8-hydroxypinoresinol using 1D and 2D NMR spectroscopic methods and by comparison with literature data. The compound was active against tested microorganisms which included *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* using ciprofloxacin and terbinafine as standards. The zones of inhibition (ZOI) ranged between 11 and 17 mm for the compound against the microorganisms. The compound had a minimum inhibitory concentration (MIC) ranging from 0.75 to 3.0 mg/ml, and minimum bactericidal concentration (MBC) of 1.5, and a minimum fungicidal concentration (MFC) of 3.0 mg/ml. The lignan also showed antifungal activity against *C. albicans*. This experiment confirmed the efficacy of the lignan as a natural antimicrobial and suggested the possibility of employing it in drugs for the treatment of infectious diseases caused by the test organisms. This is the first report of the isolation of this compound from *Strombosia grandifolia*.

Conclusion: In this study, antimicrobial activities of (-)-8-hydroxypinoresinol isolated from the stem bark of *Strombosia grandifolia* used in Nigeria for the treatment of various ailments were assessed. The result showed potential antibacterial effects of the phytochemical against bacterial strains tested. The compound also exhibited antifungal activity against *C. albicans*. This justifies the ethnomedicinal uses of the plant in Nigeria.

Keywords: Lignan, (-)-8-hydroxypinoresinol, Antimicrobial activity, *Strombosia grandifolia*

Background

Antimicrobial agents are useful agents in reducing the global menace of infectious diseases. However, the emergence of multidrug-resistant (MDR) pathogenic bacteria and their continuous increase has become a health concern. This is mainly because of the limited antimicrobial agents available to treat the infections caused by these pathogens (Luitel and Dahal 2019). However, many medicinal plants have been identified as alternative natural sources of antimicrobial agents that could be used in the treatment of these infections (Luitel and Dahal 2019). The World Health Organization (WHO) stated that medicinal plants would be the best source to obtain a variety of drugs against the MDR bacteria (Yadav et al. 2009). A number of medicinal plants have been used because of their antimicrobial traits due to the presence of various phytochemicals in them (Altemimi et al. 2017; Luitel and Dahal 2019).

Lignans isolated from five cultivars of flax growing in Egypt have been reported to show various biological activities. These lignans have exhibited numerous antioxidant and antibacterial activities (Gaafar et al. 2013). Pinoresinol for instance is one of the structurally

* Correspondence: ekalumiracle@gmail.com
[1]Department of Chemistry, Nigerian Army School of Education, PMB 1410, Ilorin, Kwara, Nigeria
Full list of author information is available at the end of the article

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simplest lignans, called a dimer of coniferyl alcohol, and found in plants (Schroeder et al. 2006). They have shown antioxidant, antitumor, antiviral, antibacterial, insecticidal, fungistatic and anti-platelet activities as well as protective effects against coronary heart disease (Hwang et al. 2010). Pinoresinol had been reported for its antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella enterica* (Zhou et al. 2017). The compound is therefore generally presumed to be a defensive agent, as is suggested also by its antihelminthic and antifungal activity (Schroeder et al. 2006). Lignans and neolignans represent a large class of pharmacologically active compounds (Teponno et al. 2016). Various lignans have shown anti-tumour, antimitotic and antiviral activity. Toxicity to fungi, insects and vertebrates has also been reported for lignans, and a variety of physiological activities have been documented (MacRae and Towers 1984). Antibacterial, antioxidant, antagonist activities and general toxicity of lignans have been assessed (Kumarasamy et al. 2003). Lignans are known for their antioxidant, apoptotic, anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, and anti-protozoal properties (Marcotullio et al. 2018). The most studies of the lignans have concentrated on the bioactivities to provide the future lead drugs. Based on the effects of numerous biological research, pinoresinol is being reported as a potential protecting agent of human health (Mistrzak et al. 2015).In view of these medicinal uses, *S. grandifolia* is a good candidate for screening for bioactive compounds. To study the plant with a view to justifying the claims by the traditional users and possibly isolating and characterizing the compound(s) responsible for the perceived activity, we now report the isolation and characterization of a bioactive compound from the stem bark of *S. grandifolia* and its antimicrobial properties.

Materials and methods

Sample collection

The stem bark of *S. grandifolia* was collected in April 2018 from Otukpo (7° 12′ 60.00″ N, 8° 08′ 60.00″ E), Benue State, Nigeria. The plant was identified by the plant taxonomist Mallam Sanusi Namadi, and a voucher specimen (03689) is retained in Biological Science Department, Ahmadu Bello University, Zaria, Nigeria

Extraction and isolation

Air-dried stem bark of *S. grandifolia* (1.0 kg) was extracted on a shaker at standard room temperature of 25 °C successively with 100 % CH_2Cl_2 for 72 h in the ratio of 1:4 *w*/*v*. The extract was concentrated using a rotary evaporator at 40 °C to yield extract (10.0 g). The CH_2Cl_2 extract was separated by flash chromatography over silica gel in a Biotage SP1 Flash Chromatography Purification System using three solvent mixtures: first with a hexane/-CH_2Cl_2 step gradient starting with 100 % hexane and gradually increasing the polarity to 100 % CH_2Cl_2, then EtOAc was added gradually until 100 % EtOAc was reached to yield 8 fractions (Fr.1–Fr.8). Fr.4 (4.0 g) was separated successively by silica gel column chromatography (CH_2Cl_2 /EtOAc 2:8) to yield (-) 8hydroxypinoresinol (41.0 mg).

Antimicrobial screening of the isolate

The microorganisms tested against Gram-positive *Staphylococcus aureus*, *Streptococcus pneumoniae* and Gram-negative *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 agar slant. All isolates were checked for purity and maintained in slants of blood agar. A solution of 6.0 mg of the compound was made using 10 mL DMSO (Bello et al. 2011).

Determination of zone of inhibition of the isolate

The standardized inocula of the isolates were uniformly streaked into 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 0.3 mL 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 (Madumelu Mark 2014).

Determination of minimum inhibition concentration (MIC) of the isolate

The antimicrobial activity of the isolates was evaluated using the broth dilution assay as described previously for the bacteria and Mueller Hinton broth assay for the fungus (Niaz et al. 2018). Twofold serial dilutions of the isolate in the sterilized media were made to obtain the concentrations of 3.00 mg/ml, 1.50 mg/ml, 0.75 mg/ml, 0.37 mg/ml, 0.18 mg/ml and 0.09 mg/ml. The initial concentration was obtained by dissolving 6.0 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 microbes in the normal saline was then inoculated into the different concentrations. Incubation was made at 37 °C for 24 h, after which each test tube of the broth was observed for turbidity (growth). The lowest concentration of the isolate in which the media show no turbidity was

Table 1 Correlation table for NMR data of compound 8: (-)-8-hydroxypinoresinol

No.	^{13}C NMR (125 MHz) in $CDCl_3$	^{13}C NMR (125 MHz) in $(CD_3)_2CO$ (Dong et al. 2018)	^{1}H NMR (500 MHz) in CD_3OD (*J* in Hz)	COSY	NOESY
1	132.6 C	132.4	–		
2	109.3 CH	109.1	6.99 d, *J* = 2.0		2′β
3	145.7 C	147.3	–		
4	146.3 C	145.8	–		
5	114.9 CH	114.7	6.95 d, *J* = 8.1		
6	119.9 CH	119.7	6.90 dd, *J* = 2.0, 8.0		
7	86.0 CH	85.7	4.86 d, *J* = 4.7		
8	60.3 CH	60.2	3.12 m	7β, 9β, 9′β	
9α	71.9 CH_2	71.7	4.52 dd, *J* = 8.2, 9.3		
9β			3.85 dd, *J* = 6.3, 9.3		
1′	127.2 C	127.0			
2′	109.6 CH	109.2	6.98 d, *J* = 1.8		2β
3[1]	146.9 C	147.3			
4′	147.2 C	145.8			
5′	114.5 CH	114.7	6.90 d, *J* = 8.0		
6′	119.8 CH	119.7	6.87 dd, *J* = 2.0, 8.0		
7′	88.0 CH	87.8	4.85 s		
8′	91.9 C	91.7			
9′α	74.9 CH_2	74.7	4.01 dd, *J* = 9.4		
9′β			3.92 dd, *J* = 9.4		
OCH_3	56.3	56.0	3.92 s		2′
OCH_3	56.2	56.0	3.90 s		2

recorded as the minimum inhibition concentration (MIC) (Madumelu Mark 2014).

Determination of minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) of the isolate

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 h 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 (Madumelu Mark 2014).

Results

The compound was isolated as a yellow solid from the CH_2Cl_2 extract of the stem bark of *Strombosia grandifolia* and was identified as the known (-)-8-hydroxypinoresinol, which has been previously isolated from the methanol extract of the roots of *Vladimiria muliensis* (Chen et al. 2013; Dong et al. 2018).

The HRESIMS (Additional file 1) showed a molecular $[M\text{-}H]^-$ ion at *m/z* 373.1289 (calcd 373.1287 for $C_{20}H_{21}O_7$) indicating a molecular formula of $C_{20}H_{22}O_7$ and 10 degrees of unsaturation for the compound. The IR spectrum

Table 2 Diameter of zone of inhibition (mm) of the isolate

	Concentration (mg/ml)					
Microorganisms	3	1.5	0.75	0.375	Ciprofloxacin 10×10^{-6}	Terbinafine 30×10^{-6}
S. aureus	13	12	0	0	22	
E. coli	13	12	0	0	33	
S. pneumoniae	17	15	13	11	20	
S. typhi	12	0	0	0	23	
C. albicans	14	13	11	0		26

(Additional file 1) showed absorbance bands for hydroxyl (3406 cm^{-1}) groups.

Although the NMR spectra showed similarities to those of compound (-)-pinoresinol, indicating that the compound was closely related, 20 ^{13}C NMR resonances could be seen, and many looked paired, indicating the molecule was no longer symmetrical. The major difference was a fully substituted oxygenated resonance that was assigned as C-8' (δ_C 91.9). The ^{1}H NMR spectrum (Additional file 1) of compound showed the presence of two methoxy group proton resonances at δ_H 3.92 and δ_H 3.90 (with the integrated value of 3H each) and the presence of two aromatic rings, each showing ABX systems with proton resonances at δ_H 6.99 (H-2, d, J = 2.0 Hz), δ_H 6.95 (H-5, d, J = 8.1 Hz), δ_H 6.90 (H-6, d, J = 2.0, 8.0 Hz), δ_H 6.98 (H-2', d, J = 1.8 Hz), δ_H 6.90 (H-5', d, J = 8.0 Hz) and δ_H 6.87 (H-6', d, J = 2.0, 8.0 Hz). The corresponding carbon signals occurred at δ_C 109.3 (C-2), δ_C 114.9 (C-5), δ_C 119.8 (C-6), δ_C 109.6 (C-2'), δ_C 114.5 (C-5') and δ_C 119.8 (C-6').

A singlet proton resonance at δ_H 4.85 was assigned to H-7' as it showed correlations in the heteronuclear multiple bond correlation (HMBC) spectrum (Additional file 1) with the C-1', C-2', C-6', C-8, C-8' and C-9' resonances. The two non-equivalent H-9' resonances appeared as a pair of doublets at δ_H 4.01 and δ_H 3.92 and showed no further coupling in the correlation spectroscopy (COSY) spectrum (Additional file 1), confirming the presence of the hydroxyl group at C-8' (Table 1).

The COSY spectrum showed coupling between the two H-9 (δ_H 4.52 dd, J = 8.2, 9.3, δ_H 3.85 dd, J = 6.3, 9.3), H-8 (δ_H 3.12 m) and H-7 (δ_H 4.86 d, J = 4.7) resonances, and the H-7 resonances showed correlations in the HMBC spectrum with the C-1, C-2, C-6, C-8, C-8', C-9 and C-9' resonances. The H-2 and H-2' resonances showed correlations in the nuclear Overhauser effect spectroscopy (NOESY) spectrum (Additional file 1) with the methoxy group proton resonances, so methoxy group were placed at C-3 and C-3' and the remaining hydroxyl groups at C-4 and C-4'. The data suggested that the compound was the lignan, (-)-8-hydroxypinoresinol (Chen et al. 2013).

A specific rotation of $[\alpha^\circ]_D^{20} = -46.7$ (c = 1.00 g/ml, CH_2Cl_2) was measured, and the compound was identified as (-)-8-hydroxypinoresinol. The configurations at the chiral centres were confirmed using the NOESY spectrum.

The ^{1}H and ^{13}C NMR resonances were assigned using heteronuclear single quantum correlation (HSQC) and HMBC spectra and are given in Table 1; the structure of the compound is shown in Fig. 1.

The result showed the zone of inhibition ranging from 11 to 17 mm (Table 2). The MIC was observed from 1.50 to 3.00 mg/ml, MBC was 1.50 mg/ml for *S. pneumoniae* and MFC was 3.00 mg/ml (Table 3).

Table 3 Summary of MIC, MBC and MFC of the isolate (mg/ml)

Concentration (mg/ml)			
	MIC	MBC	MFC
Microorganisms			
S. aureus	3.00	ND	
E. coli	3.00	ND	
S. pneumoniae	0.75	1.5	
S. typhi	3.00	ND	
C. albicans	1.50		3.0

ND not determined

Discussion

The antimicrobial activity of the isolates was evaluated using the broth dilution assay as described previously for the bacteria and Mueller Hinton broth assay for the fungus (Niaz et al. 2018). These organisms are the causes of infectious diseases. The study showed that the

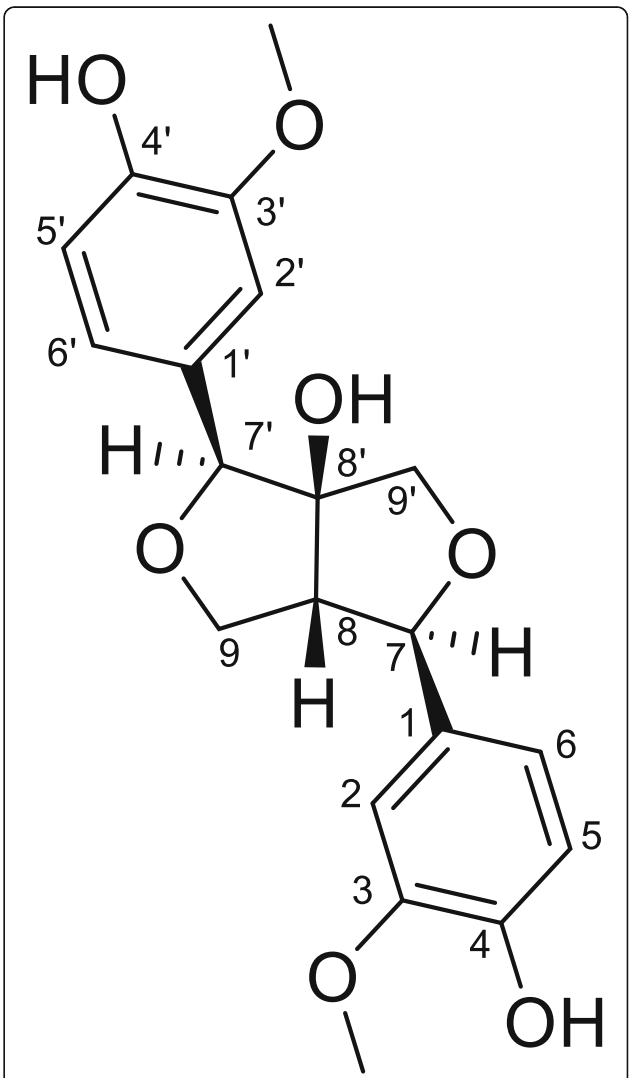

Fig. 1 (-)-8-hydroxypinoresinol

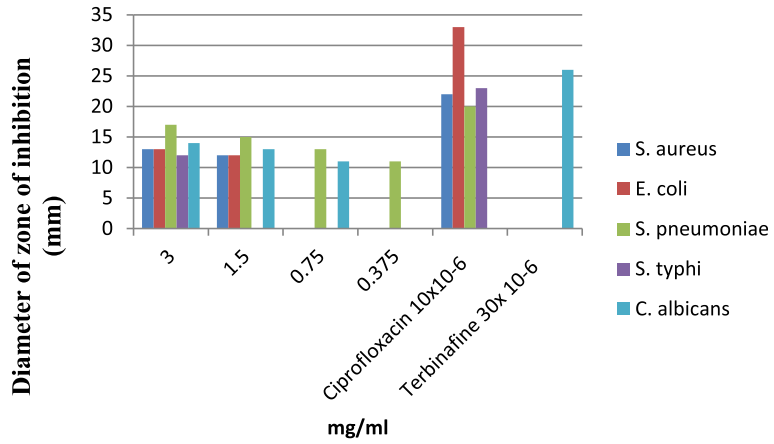

Fig. 2 Diameter of zone of inhibition of the isolate

phytochemical exhibited a varying degree of antimicrobial activity against all microorganisms (Table 2).

The compound was active against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. The zones of inhibition (ZOI) ranged between 11 and 17 mm (Fig. 1) for the compound against the microorganisms. The compound had MIC ranging from 0.75 to 3.0 mg/ml, MBC of 1.5, and MFC of 3.0 mg/ml (Figs. 2 and 3). The lignan also showed antifungal activity against *C. albicans* (Table 3). This experiment confirmed the efficacy of the lignan as natural antimicrobials and suggested the possibility of employing them in drugs for the treatment of infectious diseases caused by the test organisms. This is the first report of the isolation of this compound from *Strombosia grandifolia.*

This study has been conducted to evaluate the antimicrobial activity of lignin isolated from *Strombosia grandifolia* against some human pathogens including two reference strains (Luitel and Dahal 2019).

The compound was very active against *S. pneumonia* and *C. albicans*. It also exhibited significant MIC value against *E. coli*, *S. aureus*, and *S. typhi*. This result was similar to those reported antibacterial activity (Zhou et al. 2017). Similarly, the oxidative effect of 8-hydroxylpinoresinol and (-)-olivil have been reported to protect human high-density lipoprotein (HDL) against lipid peroxidation (Chang et al. 2008). It has been widely observed and accepted that the medicinal value of plants lies in the bioactive constituents present in them (Luitel and Dahal 2019). Multidrug-resistant *S. Typhi* and *S. pneumoniae* possess the greatest threat to mankind. Therefore, the significant activity the compound against them makes us believed that it could be an important alternative to fight these diseases.

Previously, three lignans carissanol, carinol and nortrachelogenin were reported (Kaunda and Zhang, 2017) to exhibit cytotoxicity against breast (MCF7) and lung (A549) cancer cells. Additionally, carinol showed considerable antimicrobial activity against *P. aeruginosa*, *E. coli*, *S. aureus*

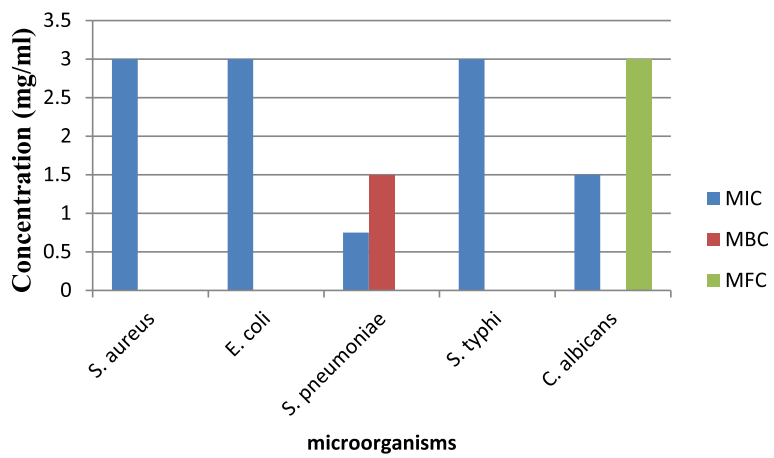

Fig. 3 MIC, MBC and MFC of the isolate

and *B. subtilis*, with a MIC of 1.25 mg/mL by a micro-broth dilution technique (Kaunda and Zhang, 2017). This is similar to our finding that the lignan was active against *E. coli* and *S. aureus.* Also, in vitro antifungal activity of lignans have been reported (Chapa et al. 2007) in agreement with the activity of the isolate against *C. albicans*. Pinoresinol exhibited significant antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Céspedes et al. 2006).

Though the lignan exhibited good antimicrobial activity against the test organisms which is in agreement with the traditional uses of stem bark of *S.grandifolia* in treatment of infectious diseases in Nigeria, further study, however, is required to explore the effectiveness in inhibiting the growth of parasites and viruses.

Conclusion

In this study, antimicrobial activities of (-)-8-hydroxypinoresinol isolated from the stem bark of *Strombosia grandifolia* used in Nigeria for the treatment of various ailments were assessed. The result showed potential antibacterial effects of the phytochemical against bacterial strains tested. The compound also exhibited antifungal activity against *C. albicans*. This justifies the ethnomedicinal uses of the plant in Nigeria. The results from this research have supported the ethnomedicinal uses of this plant in the treatment of skin infections, abdominal disorders, and gonorrhoea and as a cough and cold remedy. Further investigations are necessary to evaluate the antimycobacterial, antiviral and antiparasitic activity of this lignan. Moreover, other parts of the plant need to be studied to evaluate the plant extracts as a potential antimicrobial agent.

Additional file

Additional file 1: Supplementary spectra. (DOCX 193 kb)

Abbreviations

COSY: Correlation spectroscopy; FTIR: Fourier-transform spectroscopy; HMBC: Heteronuclear multiple bond correlation; HRESIMS: High-resolution electrospray ionization mass spectrometry; HSQC: Heteronuclear single quantum correlation; MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration; MIC: Minimum Inhibition Concentration; NOESY: Nuclear Overhauser effect spectroscopy; ZOI: Zone of inhibition

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Authors' contributions

AE designed the research work and performed the experiments for extraction, isolation and characterization of the bioactive lignan. RGA, HDJ and IH guided and supervised the work. All authors read and approved the final manuscript.

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Availability of data and materials

Data used to support the findings of this study are included within the supplementary information file.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participants, human or animal data and animal or human tissue.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Chemistry, Nigerian Army School of Education, PMB 1410, Ilorin, Kwara, Nigeria. [2]Department of Chemistry, Ahmadu Bello University Zaria, PMB 1045, Zaria, Kaduna, Nigeria.

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