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Bioprospective Screening and Characterization of Isolates from *Pterocarpus osun* Leaf and Stem Bark Extracts

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Abstract

Antimicrobial resistance (AMR) ranks among the top 10 world health challenges of the 21st century, predicted that it will cause approximately 10 million deaths in 2050. Therefore, more investigation into identifying new antibiotics from natural sources is inevitable. Compounds from the leaf and stem bark of *Pterocarpus osun* were isolated using column chromatography, screened for their bioactivities, and characterized using the FTIR spectroscopy method in the ongoing search for novel antimicrobial therapies. The isolates were tested for antibacterial and antifungal activities against eleven pathogenic organisms. The zones of inhibition ranged in diameter from 22 to 30 mm. The minimum inhibitory concentrations were determined. Thin-layer chromatography was used to determine the purity of the compounds, and their retention factor was calculated. This ranged between 0.2424 and 0.8151. The melting points of the compounds were also recorded, as are FTIR spectra ranging from 4000 to 400 cm^{-1}. Some of the peaks recorded are typical of single bond (4000-25000 cm^{-1}), double bond (2000-1500 cm^{-1}) with confirmation in the fingerprint (1500-400 cm^{-1}) region but none in the triple bond (2500-2000 cm^{-1}) region of the spectra. The antimicrobial screening results show that the identified compounds can potentially lead to the discovery of novel antibiotic medications.

Keywords: Biosprospective, antimicrobial, FTIR spectroscopy, isolates, Pterocarpus osun

INTRODUCTION

Generally speaking, bacteria have the genetic ability to develop and spread antibiotic resistance (Cohen, 1992). The proportion of hospital patients with compromised immune systems should raise some concern, as should the rapid emergence and spread of multi-resistant bacterial strains. New diseases might consequently become widespread in hospitals, increasing the death rate. Bacterial resistance is a growing problem, and the usage of antibiotics moving forward is still up for debate (Nascimento et al., 2000). Antimicrobial resistance (AMR), one of the most significant risks to world health, requires the development of new anti-infective drugs (Thabit et al., 2015). AMR occurs when bacteria can survive in the presence of medications that would usually limit their growth (Founou et al., 2017). By 2050, it's expected that 10 million people will have died due to AMR infections (O'neill, 2016). It is more important than ever to resurrect the pipeline of anti-infectives being researched as novel, difficult-to-treat bacteria emerge.

Furthermore required are novel anti-infectives with unique modes of action (Schroeder et al., 2017). Plants are a promising source of natural chemicals in the search for bioactive molecules (Rossiter et al., 2017). Plants used in traditional medicine to treat illnesses can limit the growth and toxicity of various bacteria (Bibi et al., 2011; Fadeyi et al., 2022). Up to 80% of the population in some areas uses traditional medicine to meet their primary healthcare needs, making it one of the most accessible treatment alternatives in developing nations (Maroyi, 2013). Plants produce a wide range of chemicals known as secondary metabolites as an adaptation for defense and communication with other animals in their habitats (Harbone et al., 1999). These secondary metabolites have the potential for synergy with other secondary metabolites as part of a plant's multi-component defense mechanism, which is one of their many benefits for developing anti-infective drugs (Harvey et al., 2015). They are also typically bioactive, drug-like, and metabolite-like substances. One of the most significant risks to global health has

been highlighted time and time again as infectious illnesses.

According to the World Health Organization (WHO), contagious diseases were to blame for 61.7% (5.9 million) of the 9.6 million fatalities in Sub-Saharan Africa in 2013. Since ancient times, plants with healing properties have been used in medicine. Around the world, studies have been conducted to provide evidence supporting the value of medicinal plants. Some of these slivers of evidence have also shed light on creating plant-based molecules with therapeutic uses (Dhama et al., 2014). The annual value of the global market for medicinal plant products has surpassed $100 billion (Sofowora et al., 2013). Phytochemical screening of medicinal plants is typically performed against a broad spectrum of bacteria to determine their antimicrobial activity, which is based on the active ingredients of the plants, which are generally secondary metabolites.

The current incidence of antimicrobial treatment resistance in most bacteria has created an enormous challenge (Kpadonou et al., 2019), necessitating continual research for newer and safer therapeutic medicines. Most plants that have found use in ethnomedicine have been recorded based on their potential antimicrobial activity against various disease-causing bacteria (Chikezie et al., 2015; Ogbole et al., 2018). Research efforts are accelerating for a better functional knowledge of medicinal plants, offering a model for 25–50% of the commercialized medications (Segun et al., 2021). Nearly 70% of the representative classes of antibiotics of the present age are naturally occurring compounds, including tetracycline, glycopeptides, aminoglycosides, macrolides, and lincosamides.

The present study is a preliminary investigation of the potential role of the secondary metabolites against the antibiotic-resistant pathogens of *Pterocarpus osun*, a plant obtained from the medicinal garden of Sheda Science and Technology Complex, Federal Capital Territory, Abuja, Nigeria.

METHODOLOGY

Column chromatography (CC): Flash column chromatography using gradient elution technique (Nalini & Anuradha, 2017), with hexane and ethyl acetate as elution solvents, was employed to separate the compounds in the crude extracts of the leaf and stem bark of *P. osun* using glass column 4cm diameter x 60cm length. A dry packing method was used. The column was packed with silica gel as adsorbent and tapped to ensure even packing and avoid air bubbles. The column was eluted with n-hexane, and 8 g of the dried powdered extract was introduced. Then solvent mixture (eluent) in the proper ratio was added to the column. Several fractions were obtained and concentrated.

Thin layer chromatography (TLC)

TLC was used to analyze the fractions obtained from column chromatography and verify the isolated compounds' identities and purity. Merck applied spots manually using a capillary tube on commercially prepared glass TLC plates. It was developed using a mixture of n-hexane and ethyl acetate in varying ratios. The spotted plates were air dried, visualized using a UV lamp, sprayed with 5% sulphuric acid in methanol, and then heated at 105 ^{o}C for about 5 minutes (Kafelau et al., 2022). The developed plates were viewed under UV lamps at 255 nm and 366 nm wavelengths. The movement of each separated spot of the extract was expressed by its retention factor (Rf). Values were calculated for each spot using the following formula in Equation 1.

$$R_f = \frac{\text{distance traveled by the solute}}{\text{distance traveled by the solvent front}} \quad (1)$$

Relative retardation factor (rR_f): The rRf was calculated from the Rf value of each extract fraction's most prominent spot of the TLC (Equation 2).

$$R_f = \frac{\text{Distance traveled by component}}{\text{Rf value of the reference}} \quad (2)$$

The In-vitro bioactive screening

Eleven pathogenic microbes were used to test the antimicrobial activities of isolated compounds. The microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University teaching hospital Zaria, Nigeria. Each column fraction was screened for antimicrobial activity using the agar well diffusion method and a fraction concentration of 100 µg/mL. After 24 hours of incubation at 37 ^{o}C, the media plates were examined for the zone of inhibition of growth, and the result was recorded in mm.

Minimum Inhibitory Concentration (MIC)

The broth dilution method determined each compound's minimum inhibitory concentration (MIC). The solution of each compound was prepared *in vitro* at increasing concentrations. Each solution was incubated with a separate aliquot of cultured bacteria, and the results were measured using broth micro-dilution. A Mueller-Hinton broth was made, and 10 mL was dispensed into each test tube before sterilizing at 121°C for 15 minutes and allowing the

broth to cool. In purpose to provide a solution, MC-Farland's turbidity standard scale number 0.5 was prepared. Normal saline was produced; 10 mL was poured into a sterile test tube, and the test microorganism (1.5 x 10^8 cells/mL) was injected and incubated at 37 °C for 6 h. The microorganism was diluted in normal saline by visual comparison until the turbidity matched the MC-Farland's scale.

The working solution was serially diluted twice in sterile broth to generate concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. The test agents (0.001 mg) were separately dissolved in 10 mL sterile broth to obtain the starting concentration. After receiving various concentrations of each of the compounds in the sterile broth, 0.1mLof the test microbe in normal saline was inoculated into the multiple concentrations and incubated at 37 °C for 24 h, after which the test tubes of the broth were examined for turbidity (growth). The lowest compound concentration in the sterile broth that showed no turbidity was recorded as the minimum inhibitory concentration.

Minimum Bacterial/Fungal Concentration (MBC/MFC)

The MBC/MFC test was used to determine whether the test microorganisms were destroyed or slowed in their growth. Mueller Hinton agar was sterilized at 121°C for 15 minutes before being placed into sterile Petri dishes and allowed to solidify. The contents of the MIC in serial dilutions were then subcultured onto the prepared medium, incubated at 37 °C for 24 h, and colony growth was monitored on the medium plates. MBC/MFC was the plate with the lowest compound concentration without colony growth.

Melting point of crystals

The melting points of the individual isolated crystals were determined using the Bristoscope Bristoline melting point apparatus to test for their purity.

RESULTS AND DISCUSSION

After repeated column chromatography of the fractions, Compounds C1 and C2 were isolated from the hexane fraction of the leaves. In contrast, the ethyl acetate fraction of the leaves yielded compounds C4 and C5, and compounds C3 and C6 were obtained from the ethyl acetate fraction of the stem bark.

The Rf values from TLC and melting points of the isolates are presented in Tables 1 and 2, respectively. TLC chromatogram of the extracts and isolates are shown in Figures 1-4, and TLC properties of the Extracts and Compounds are in Table 3.

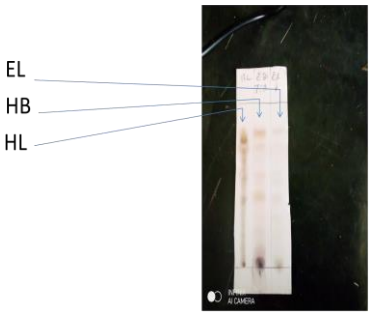

Figure 1. TLC of extracts EL, HB and HL

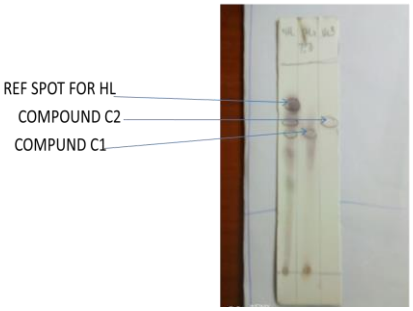

Figure 2. TLC of hexane leaves fraction (HL) and compounds C1 and C2

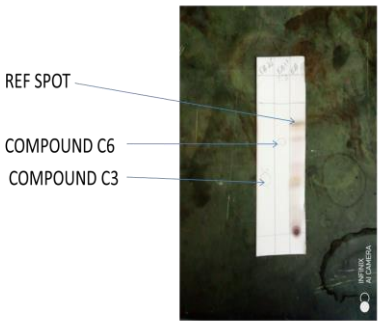

Figure 3. TLC of EtOAC bark fraction (EB) and Compounds C6 AND C7

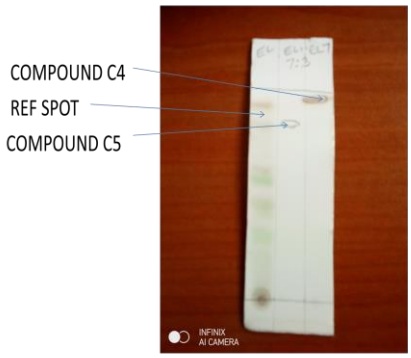

Figure 4. TLC of EtOAC leaf fraction (EL) and Compounds C4 AND C5

Table 1. Thin Layer Chromatography of isolates

Isolates	R_f values	Developing solvent ratio	
C1	0.7183	49:1	(Hex:EtOAc)
C2	0.8125	7:3	(Hex: EtOAc)
C3	0.2424	2:3	(Hex:EtOAc)
C4	0.8182	4:1	(Hex:EtOAc)
C5	0.2535	24:1	(Hex:EtOAc)
C6	0.6901	3:2	(Hex:EtOAc)

TLC is a basic tool for purity testing of organic compounds (Ferenczi-Fodor et al., 2006), hence the single spots obtained for the isolates in the Thin Layer Chromatography (TLC) attest to the purity of the compounds. Table 1 gives the compounds' identity, development solvent ratio, and calculated retardation factor (R_f) values from their TLC analysis. Generally, the stronger a compound binds to the stationary phase adsorbent, the slower it migrates up the TLC plate. As TLC adsorbents are typically polar, non-polar compounds tend to travel more rapidly up the plate, resulting in higher R_f values, whereas polar compounds tend to move slowly and have lower R_f values (Bele & Khale, 2011).

This result implies that compounds C1, C3, C4 and C6 are likely non-polar than the others since they have higher R_f values than the others. The TLC pictures of both the extracts and the compounds are shown in Figures 1-4, while Table 3, reports the Rf and rRf values of the compounds with the colour changes after spraying with 5% sulphuric acid in methanol. Compounds C6 and C4's colour change from non-visible to blue may indicate that they are terpenoids or steroids, while compound C3 changed from non-visible to purple, suggesting that it is probably a steroid (Reich & Schibli, 2007). This condition is in tandem with earlier research on phytochemical constituents of the leaves and stems of *P. osun,* which reported the presence of terpenoids and steroids in the plant's leaves and stemmed (Fadeyi et al., 2022). The melting points of the compounds are shown in Table 2.

Table 2. Melting Points of Isolates

Isolates	Mpt (°C)
C1	40
C2	46
C3	60.5
C4	51
C5	>320
C6	42

Note: Mpt = melting point

The sharpness of the melting temperatures is an indication of the purity of the compounds, especially crystals (Singh et al., 2019). Therefore, the isolates are quite pure, as seen in their melting points' sharp and narrow range.

Table 3. TLC properties of the Extracts and Compounds

Extracts /isolates	Rf	rRf	λ UV	Colour b/4 spray	colour after spray
EL	0.91	1.00			orange
C5	0.74	0.87	366	NV	blue
C4	0.96	1.19	366	orange	orange
EB	0.82	1.00			
C6	0.71	0.87	366	blue	orange
C3	0.42	0.52	366	blue	orange
HL	0.78	1.00	nv		
C1	0.64	0.82	366	NV	purple
C2	0.69	0.88	366	NV	blue

Note: The spray reagent used is 5% sulphuric acid in methanol. Most of the compounds were sensitive to UV at 366 nm. NV = not visible

Antimicrobial activities, Zone of inhibition, Minimum inhibitory concentration and MBC/MBF are presented in Tables 4 and 5, respectively. The IR spectra of the compounds isolated are in Figure 5.

Table 4. Zones of inhibition of the compounds against the test microorganisms (mm)

Test Organism	C1	C2	C3	C4	C5	C6	SPX	CPX	FCZ
MRSA	28	24	25	26	0	0	30	0	0
VRE	0	0	0	0	25	28	32	0	0
S. aureus	25	27	0	24	0	0	0	29	0
E. coli	26	23	0	0	27	29	0	37	0
H. pylori	0	0	24	25	26	27	0	30	0
V.cholerea	29	26	23	22	0	25	0	29	0
S. typhi	24	28	0	0	24	27	0	39	0
C.jejuni	0	25	25	26	0	0	31	0	0
C. albicans	27	24	0	0	25	30	0	0	32
C. krusei	23	27	24	23	0	0	0	0	34
C. tropicalis	0	0	22	0	26	26	0	0	32

The in-vitro qualitative antibacterial activities are reported in Table 4. Of the eleven pathogenic organisms tested, *E.coli, C.albicans, H. pylori and V.cholerea* were sensitive to 5 of the 6 compounds screened. *MRSA, C. krusei, S. typhi,, C. albicans and C.jejuni* were sensitive to 4 of the 6 compounds. *VRE* was sensitive to 2 compounds, while *S.aureus and C. tropicalis* only were sensitive to 3 compounds. The compounds possess a wide spectrum of antimicrobial applications. The zones of inhibition is presented in Table 4. The isolates' inhibitory capacities compare favourably with the standard control drugs analyzed as the positive control (Table 4). The observed

impressive antimicrobial properties of the isolates agree with earlier reports of antimicrobial studies of the extracts of the plants (Adewuyi et al., 2014; Fadeyi et al., 2022; Shobayo et al., 2015).

Table 5. The qualitative antimicrobial activities of compounds

Test Organism	C1	C2	C3	C4	C5	C6	SPX	CPX	FCZ
MRSA	S	S	S	S	R	R	S	R	R
VRE	R	R	R	R	S	S	S	R	R
S. aureus	S	S	S	R	R	R	R	S	R
E. coli	S	S	R	R	S	S	R	S	R
H. pylori	R	R	S	S	S	S	R	S	R
Vibrocholerea	S	S	S	S	R	S	R	S	R
S. typhi	S	S	R	R	S	S	R	S	R
C. jejuni	R	S	S	S	R	S	S	R	R
C. albicans	S	S	R	R	S	S	R	R	S
C. krusei	S	S	S	S	R	R	R	R	S
C. tropicalis	R	R	R	S	S	S	R	R	S

Note: S = Sensitive, R = Resistance

FTIR spectra of the compounds are presented in Figure 5. Some of the peaks recorded are in the single bond (4000-25000 cm^{-1}), double bond (2000-1500 cm^{-1}), and fingerprint (1500-400 cm^{-1}) regions but none in the triple bond regions for compounds C1 to C4. However, for compounds C5 and C6, a weak band at about 2359 cm^{-1} was found in the spectra's triple bond region (2500-2000 cm^{-1}).

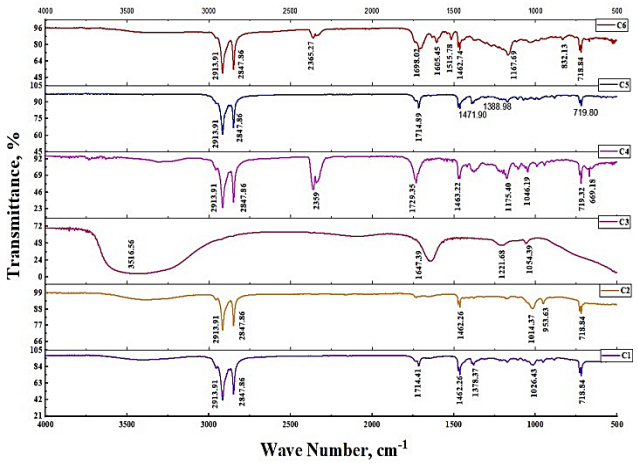

Figure 5. Plots of FTIR spectra of isolates

The alkanes CH stretch at about 2914 and 2847 cm^{-1} are Peculiar bands to all the spectra. The FT IR spectra of Compounds C2, C5, and C6 show OH absorption bands, which implies they are likely phenolics or flavonoids (Stuart, 2005). This condition might contribute to the observed antioxidant properties in an earlier by Odekanyin & Akande, 2019 and Adesegun et al., 2013. Phenols and flavonoids are naturally occurring or dietary antioxidants (Afolayan et al., 2014). Compounds C3 and C4 have absorption bands characteristics of amines suggesting that alkaloids are nitrogen-containing principles (Stuart, 2005). Alkaloids are phytochemicals with known impressive medicinal properties; these may be contributors to the observed antimicrobial properties

CONCLUSION

The isolates from the plant *Pterocarpus osun* are potential drugs for a wide range of human pathogenic organisms and if fully exploited, the compounds from this plant can be a good lead to the development of novel anti-infective and Antimicrobial resistance (AMR) therapy for existing and emerging diseases threatening humanity. Further characterization is required for structure elucidation.

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