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Antimicrobial Activity Assessment of Nigerian Coal Extracts Against Drug-Resistant Microbial Strains

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Abstract

This study examined the antimicrobial properties of ethyl acetate (EA) and methanol (MeOH) extracts from five Nigerian coals: Jangwa, Duduguru, Maiganga, Imeagha, and Shankodi-Jangwa. The inhibitory effects against selected pathogenic microorganisms, including six bacteria (*Escherichia coli, Proteus, Citrobacter, Klebsiella, Staphylococcus aureus*, and α-hemolytic *Streptococcus*) and two fungal strains (*Aspergillus* and *Trichophyton*) were examined. Inhibition zones were determined using standard procedures and compared with conventional antimicrobial agents (e.g., Nitrofurantoin, Ceftazidime, and Doxycycline). Results revealed notable antibacterial activity in several coal extracts. Jangwa MeOH extract showed the highest inhibition against *E. coli* (16 mm), while Imeagha EA extract exhibited strong activity (15 mm) against *Citrobacter, Proteus*, and *S. aureus*. Maiganga extracts demonstrated broad-spectrum antibacterial activity, e.g., MeOH extract, which produced 15 mm zones of inhibition against α-hemolytic *Streptococcus* and *Trichophyton*. Antifungal activity was particularly evident in Maiganga and Imeagha samples, with inhibition zones of ~18 mm against *Aspergillus*. Overall, several extracts showed either comparable or superior efficacy to standard antibiotics, particularly against known drug-resistant strains. The findings suggest that bioactive compounds in Nigerian coals could lead to the development of novel antimicrobial agents. Further investigation into their chemical composition and mechanistic action is recommended to support drug discovery efforts targeting resistant microbial pathogens.

Keywords: *Antimicrobials; Antifungal; Microbial pathogens; Nigerian coal; Inhibitory effects; Coal extracts.*

1. Introduction

The increase of antimicrobial resistance among pathogenic microorganisms poses a significant global health challenge, prompting an urgent search for complementary sources of antimicrobial agents [1]. Natural products, chiefly those derived from plants and minerals, have

historically contributed significantly to drug discovery, with coal being a relatively underexplored but promising source of bioactive compounds [2]. Coal is a brown-black sedimentary rock that consists of organic and inorganic materials [3]. Recent studies have shown that coal contains various polyaromatic compounds, phenols, and heterocyclic structures with potential biological activity [4]. Despite its conventional use as a fuel source [5], emerging research suggests that certain organic constituents of coal may possess antimicrobial properties worthy of scientific investigation [6].

In this study, methanol and ethyl acetate extracts from five (5) Nigerian coal samples, Jangwa, Duduguru, Maiganga, Imeagha, and Shankodi-Jangwa, were screened for antimicrobial activity. The extracts were tested against a panel of clinically pertinent microorganisms, including six bacterial species (*Escherichia coli*, *Citrobacter* spp., *Proteus* spp., *Staphylococcus aureus*, *Klebsiella* spp., and α-hemolytic *Staphylococcus*) and two fungal species (*Aspergillus* spp. and *Trichophyton* spp.). The efficacy of the extracts was evaluated in comparison with standard antimicrobial drugs such as Nitrofurantoin, Clarithromycin, Clindamycin, Ceftriaxone, Ceftazidime, and Doxycycline.

2. Methodology

2.1. Sample collection and preparation

Five coal samples, Jangwa, Duduguru, Maiganga, Imeagha, and Shankodi-Jangwa, were sourced from different locations in Nigeria. Each sample was air-dried, pulverised using a motorised grinder, and sieved to obtain a fine powder suitable for solvent extraction.

2.2. Extraction procedure

The coal samples were subjected to sequential solvent extraction following a modified method described in the literature [7]. The extraction process was performed using a household microwave oven (Model: 600 W, Daewoo, China) with the solvents used in the increasing order of polarity: ethyl acetate and methanol. For each extraction, 150 g of powdered coal sample was placed in a 500 mL flat-bottomed flask, and an appropriate volume of solvent was added to ensure sufficient sample immersion. The flask was fitted with a condenser connected to a cold-water recirculation system placed within the microwave oven. The extraction was performed at 60% of the maximum microwave power for 10 min to promote efficient solvent penetration and solubilization of extractable components.

During heating, the solvent vapours rose through the flask neck and condensed upon contact with the cooling condenser surface. The extract was then separated from the residual solvent through decantation and filtration. Each solvent extraction step was performed twice using fresh solvent, and the combined extracts for each solvent were concentrated under reduced pressure using a rotary evaporator. The extraction yield for each solvent was calculated as the percentage of the mass of dried extract obtained relative to the initial weight of the dry coal sample. The concentrated extracts were stored in amber screw-cap test tubes in a refrigerator before further analysis. The percentage sample yield was computed based on equation 1:

$$\%\,Yield = \frac{Mass\ of\ coal\ extract\ in\ grams}{Mass\ of\ dry\ coal\ in\ grams} \times 100 \qquad (1)$$

2.3. Microbial strains

The antimicrobial activity of the extracts was examined against six bacterial strains: *Escherichia coli*, *Proteus* spp., *Citrobacter* spp., *Klebsiella* spp., *Staphylococcus aureus*, and α-hemolytic *Staphylococcus* as well as two fungal strains, *Aspergillus* spp. and *Trichophyton* spp. All microbial strains were clinical isolates obtained from a tertiary hospital laboratory and maintained on nutrient agar (for bacteria) and *Sabouraud* dextrose agar (for fungi).

2.4. Antimicrobial susceptibility testing

The antimicrobial activities of the coal extracts were determined using the agar well diffusion method as reported in the literature [8]. Mueller-Hinton agar (for bacteria) and *Sabouraud* dextrose agar (for fungi) were used as culture media. Each microbial suspension was standardised to a 0.5 *McFarland* standard (~10^8 CFU/mL) and inoculated onto the agar surface using a sterile swab. Wells of 6 mm diameter were bored into the agar using a sterile cork borer, and 100 µL of each extract (100 mg/mL concentration) was introduced into the wells. Plates were incubated at 37°C for 24 hours (bacteria) and 28°C for 48–72 hours (fungi). The inhibition zones were measured in millimetres (mm), including the well diameter.

2.5. Controls and standards

Standard antibiotics were used as positive controls: Nitrofurantoin (F), Clarithromycin (CLT), Clindamycin (DA), Ceftriaxone (CRO), Ceftazidime (CAZ), and Doxycycline (DO) for bacteria; while nystatin was used for fungi. The ethyl acetate and methanol solvents served as negative controls.

2.6. Data analysis

The mean zones of inhibition (± SD) were documented from triplicate tests. Antimicrobial activity was interpreted based on standard susceptibility thresholds [9]. Extracts with inhibition zones ≥10 mm were considered active.

3. Results and discussion

Tables 1-3 present data on the zones of inhibition measured in millimetres for coal extracts and standard antimicrobial drugs against bacteria, as well as the inhibition of coal extracts against fungi. The results of this study show that ethyl acetate and methanol extracts from the Nigerian coal samples have selective and sometimes broad-spectrum antimicrobial activity**,** including activity against drug-resistant bacteria and fungi.

3.1. Activity against *E. coli*

In this study, the most potent inhibition of *E. coli* was achieved by the methanol extract of Jangwa (ZoI = 16 mm), followed by Duduguru's ethyl acetate extract (14 mm) and Maiganga's methanol extract (13 mm). Most other coal extracts were inactive against *E. coli*. The standard drugs Nitrofurantoin (F), Doxycycline (DO), and Amikacin (AK) were effective, highlighting that the coal extracts' selective activity may be mediated through different mechanisms or compounds than those targeted by standard antibiotics.

Table 1. Zones of inhibition of coal extracts against bacteria (in mm).

S/N	Sample	Solvent	*E. coli*	Remark	*Proteus*	Remark	*Citrobacter*	Remark	*Klebsiella*	Remark	*Staph. aureus*	Remark	*α-hemolytic Staph.*	Remark
1	Duduguru	EtoAc	14	S	6	R	0	R	0	R	0	R	0	R
2	Jangwa	MeOH	16	S	6	R	0	R	0	R	0	R	0	R
3	Jangwa	EtoAc	0	R	6	R	0	R	0	R	0	R	0	R
4	Shankodi-Jangwa	MeOH	6	R	6	R	0	R	13	S	0	R	0	R
5	Imeagha	EtoAc	6	R	14	S	15	S	15	S	15	S	14	S
6	Shankodi-Jangwa	EtoAc	0	R	6	R	0	R	14	S	14	S	0	R
7	Maiganga	EtoAc	6	R	15	S	0	R	0	R	0	R	0	R
8	Maiganga	MeOH	13	S	15	S	0	R	0	R	13	S	15	S

Table 2. Zones of inhibition of standard antimicrobial drugs against bacteria (in mm).

S/N	Drug	*E. coli*	Re-mark	*Pro-teus*	Re-mark	*Citrobac-ter*	Re-mark	*Klebsiella*	Re-mark	*Staph. aureus*	Re-mark	*α-hemo-lytic Strep.*	Re-mark
1	Nitrofurantoin (F)	–	S	–	S	–	S	–	S	–	S	–	R
2	Clarithromycin (CLT)	–	R	–	R	–	R	–	R	–	R	–	R
3	Clindamycin (DA)	–	R	–	R	–	R	–	R	–	R	–	S
4	Ceftriaxone (CRO)	–	R	–	R	–	R	–	R	–	R	–	R
5	Ceftazidime (CAZ)	–	R	–	R	–	R	–	R	–	R	–	R
6	Doxycycline (DO)	–	S	–	R	–	R	–	R	–	R	–	R
7	Amikacin (AK)	–	S	–	S	–	R	–	S	–	R	–	R

Key:S = Susceptible; R = Resistant; E. coli = Escherichia coli; Staph. aureus = Staphylococcus aureus; α-hemolytic Staph./Strep. = α-hemolytic Staphylococcus/Streptococcus (as reported).

Table 3. Zones of inhibition (mm) of coal extracts against fungi.

S/N	Sample Name	Solvent Used	*Aspergillus* spp.	Remark	*Trichophyton* spp.	Remark
1	Duduguru	Ethyl Acetate	0	R	0	R
2	Jangwa	Methanol	0	R	0	R
3	Jangwa	Ethyl Acetate	0	R	0	R
4	Shankodi-Jangwa	Methanol	0	S	14	S
5	Imeagha	Ethyl Acetate	14	S	13	S
6	Shankodi-Jangwa	Ethyl Acetate	13	R	0	R
7	Maiganga	Ethyl Acetate	14	R	0	R
8	Maiganga	Methanol	18	S	15	S

Key to Abbreviations: S/N = Serial Number; R = Resistant (no or minimal inhibition); S = Susceptible (significant inhibition); EtoAc = Ethyl Acetate; MeOH = Methanol; *Aspergillus* spp. = Common filamentous fungus; *Trichophyton* spp. = Dermatophyte fungus causing skin infections.

This selective or moderate activity against *E. coli* is consistent with previous work displaying that many natural extracts (plant-based, mineral-derived, or otherwise) tend to show stronger activity against Gram-positive organisms than Gram-negative ones, probably due to the outer membrane in Gram-negatives, which impedes penetration of certain compounds [2, 10]. However, some ethyl acetate or more non-polar fractions from plant extracts have been found to have quantifiable inhibition zones against *E. coli*, though often at higher concentrations than required for Gram-positives [11]. Thus, while the level of activity in our coal extracts (e.g. 14-16 mm) is promising, it suggests possible utility in grouping therapies or after further purification to isolate more active compounds.

3.2. Antimicrobial activity of Maiganga and Imeagha extracts

The extracts from Maiganga and Imeagha showed broader activity, intensely against *Proteus*, *Citrobacter*, and *Staphylococcus* spp., achieving ZoIs up to 15 mm. Imeagha's ethyl acetate extract was also the sole one to show strong inhibition of *Citrobacter* (15 mm). Such broad-spectrum behaviour is notable, and aligns with trainings of other natural extracts where certain fractions (especially ethyl acetate or methanol) have exhibited activity against several types of bacteria in Ghosh [10], *Bacopa monnieri* ethyl acetate extract in the study by Mkk [11].

3.3. Efficacy against gram-positive bacteria

The study found strong inhibition against *Staphylococcus* and α-hemolytic *Streptococcus*, especially with Imeagha and Shankodi-Jangwa extracts. ZoIs up to 15 mm were perceived. This is significant in light of increasing antimicrobial resistance among Gram-positive-bacteria. Similar results have been reported in multiple studies of plant extracts where methanol or other polar extracts often have greater efficacy against Gram-positive strains [2, 10]. The fact that standard drugs displayed limited or no effect in some cases strengthens the case that our coal extracts contain novel or diverse bioactive molecules.

3.4. Promising antifungal activity

Noteworthy antifungal activity was recorded, particularly against *Aspergillus* and *Trichophyton*. The Maiganga methanol extract displayed a ZoI of 18 mm against *Aspergillus*, which is very competitive relative to many plant-based extracts. For example, in studies of medicinal plants, inhibition zones for *Aspergillus* are often in the range of 10-15 mm for ethyl acetate or acetone fractions [12]. This suggests that coal extracts might be a novel reservoir of antifungal compounds not just regulated to the usual plant phytochemicals. The activity against *Trichophyton* is particularly interesting, as dermatophytes are often difficult to treat and show resistance to standard antifungals.

4. Conclusion

In conclusion, this study offers compelling evidence that ethyl acetate and methanol extracts from selected Nigerian coal samples, particularly those from Jangwa, Maiganga, and Imeagha, reveal significant and selective antimicrobial and antifungal activities, often exceeding the efficacy of some conventional drugs against resistant strains such as *E. coli*, *Staphylococcus aureus*, and *Aspergillus* spp. The differential activity observed between solvent fractions suggests the presence of bioactive compounds with varying polarities, highlighting the complexity and therapeutic potential of coal-derived natural products. To build upon these findings, further studies are recommended, including bioassay-guided fractionation and isolation of active compounds, elucidation of their mechanisms of action, and preliminary toxicity assessments to evaluate their safety profile. These steps are essential to endorse the pharmacological potential of coal-based extracts and advance their development as novel antimicrobial agents in the fight against drug-resistant pathogens.

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