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Endophytic Fungi Isolated from *Pteridium acquilinium* and *Newbouldia laevis* Leaves Exhibited Antioxidant Activities and Inhibitory Potential Against Selected Clinical Bacteria Isolates

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Abstract

Endophytic fungi are important components of plant micro-ecosystems and they exist usually inside the healthy tissues of living plants. Endophytes possess encouraging source of biologically active metabolites for pharmaceutical applications. This study isolated endophytic fungi from *Pteridium acquilinium* and *Newbouldia laevis* leaves and evaluated their antioxidant and antibacterial activities. A total of ten endophytic fungal species were isolated from the leaves of the plants, from which six were selected and identified, including; *Epicoccum thailandicum*; *Trichoderma atroviride*; *Lasiodiplodia parva*; *Trichoderma yunnanense*; *Colletotrichum cobbittiense*; and, *Trichoderma crissum*. Among the isolated fungi, *Trichoderma crissum* (266.2956 ± 84.84 µg/ml) and *Lasiodiplodia parva* (293.0755 ± 64.95 µg/ml) showed significant scavenging activity on comparison with ascorbic acid. Furthermore, the isolated endophytes showed potential antibacterial activity against the tested clinical pathogens. This study validates that endophytic fungi dwelling in the inner tissue of medicinal plants studied could be a potential source of biologically active metabolites with free radical scavenging activities and also for treatment of infectious diseases caused by *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Escherichia coli*.

Keywords: Endophytes; antibacterial activity; antioxidant potential; *Pteridium acquilinium Newbouldia laevis*.

Abbreviations: PTALB: *Pteridium acquilinium* leaf blade, PTAMR: *Pteridium acquilinium* midrib, NLLB: *Newbouldia laevis* leaf blade, NLMR: *Newbouldia laevis* midrib.

INTRODUCTION

The search for novel compounds to alleviate or ameliorate some diseases has been on the increasing sides ever-since health challenge became a global threat to man-kind. Recently on-going researches has focused on developing new leads from medicinal plants which are major sources of some secondary metabolites that are of health benefits. Scientists equally are harnessing natural flora for new leads to tackle infectious and chronic diseases. Endophytic fungi has been recognized to be one of the most distinguished producers of medicinal products that are natural (Malhadas et al. 2017).

Endophytes live in some plants where it co-ordinates it activities having influence on the physiological, growth and chemical characteristics of the plant without causing any harm. Endophytic fungi belong to *mitosporic* and *meiosporic ascomycetes* that "asymptomatically reside in the internal tissues of plants beneath the epidermal cell layer, where they colonize healthy and living tissue via quiescent infections (Jia et al. 2016). Nevertheless, the productive result of several medicinal drugs from microbial origin like the antibiotic; penicillin from *Penicillium sp*; immunosuppressant from *Tolypocladium inflatum* and *Cylindrocarpon lucidum*, the antifungal agent griseofulvin from *Penicillium griseofulvum*, the cholesterol biosynthesis inhibitor lovastatin from *Aspergillus terreus*, and ß-lactam antibiotics from

various fungal taxa has broadened our focus to include both plants and microbes and encouraged the focus of drug discovery from not just plants alone but on microorganisms (Adeyemi et al. 2015). Previous researches has given credit to endophytes for their potential benefits in health management.

Infectious diseases including urinary tract disease (UTIs) and wound infection that are caused by multi-resistant organisms such as (*Staphylococcus aureus* and *Escherichia coli*) (Frickmann et al. 2019) and oxidative stress caused by the presence of free radicals resulting in the imbalance within the body system has been a growing concern to scientists as it affects and has significant impact on the economy (Hadadi et al. 2020). Antibiotic resistance has arose as a result of indiscriminate use of antibiotics without strict adherence to prescriptions hence creating more opportunities for development of multi-resistant microbes which is now a public health challenge (Manganyi et al. 2019). On the other hand, in humans, oxidative stress is thought to be involved in the development of atherosclerosis, neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes (Kowalczyk et al. 2021). In view to provide solutions to these problems, researchers have considered obtaining new leads from natural products other than the conventional means and in this study some medicinal plants have been selected.

Bracken ferns (*Pteridium aquilinum*) is utilized by many different cultures around the globe and has been used over a great period of time. *Pteridium aquilinum* is widely known to have many secondary metabolites with diverse bioactivities that could possibly be beneficial in the management of many diseases. Several reports of pharmacological efficacy and the desire for new drugs have encouraged numerous researchers to carry out pharmacological research on ferns. Pharmacological and ethnopharmacological studies have shown that substances in fern exhibit various medicinal properties such as hepatoprotective, cytotoxicity, leishmanicidal and trypanocidal activities to mention but few (Cao et al. 2017).

Newbouldia laevis (P. Beauv.) Seem. (Family, Bignoniaceae), commonly known as tree of life, is a purple-flowering plant that is widely distributed in many parts of Africa. The leaves, flower, stems and roots from *Newbouldia laevis* are prevalently used in African traditional medicine for the management diabetes, hypertension, skin diseases, ulcer, tumors, pains, infectious diseases, inflammation, dysentery, sickle cell disease and impotency (Okagu et al. 2021). Scientific investigations on *Newbouldia laevis* has lend credence to the ethnobotanical uses of this plant. Considering enormous oxidative mediated disease conditions and health challenges associated with pathogenic bacteria, the present study aims at evaluating the antibacterial and antioxidative activity of endophytic fungi isolated from these plants.

MATERIALS AND METHODS

Plant collection

Healthy (showing no visual disease) leaves of medicinal plants; *Pteridium acquilinium* and *Newbouldia laevis* were collected to isolate possible endophytic fungi and test their *in vitro* antioxidant and antibacterial activity. The plant were deposited at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Nigeria, where a voucher specimen with the identification number PSB/HB/122 and PSB/HB/123 for *Pteridium acquilinium* and *Newbouldia laevis* respectively were assigned.

Isolation, fermentation and molecular characterization of endophytic fungi

The collected plant leaves were subjected to surface sterilization procedures as described in the report of Adeyemi *et al.* (2015). Thereafter, the internal tissues of the disinfected samples were exposed using a sterile scissors. A sterile forceps was used to transfer the midrib and leaf-blade segments to potato dextrose agar (PDA) amended with chloramphenicol 500 mg/l (Adeyemi *et al.* 2015). The dishes were sealed with parafilm and incubated at 27°C for 6 days. In other to get axenic cultures, multiple sub-culturing were done on fresh PDA plates. Solid-state fermentation of endophytic fungi was carried out using sterile solid rice (100g of rice + 100ml of distilled water, sterilized by autoclaving at 121℃ at 15 psi for 15mins) in 1L Erlenmeyer flasks. The flasks were inoculated with 3 mm diameter agar blocks containing the endophytic fungi and incubated at 28 ℃ for 21 days. After fermentation, the secondary metabolites were extracted using ethyl acetate and then concentrated under vacuum at 50℃ with a rotary evaporator (Stuart, USA).

Axenic cultures were identified using standard molecular protocols as follows: Genomic DNA was extracted following the protocol described by Govindarajan *et al.* (2007). Genomic DNA was extracted using the Quick-DNATM Fungal/Bacterial Miniprep Kit; Zymo Research), according to recommended protocol by the manufacturer. Amplification of the internal transcribed region rDNA (ITS1-5.8S-ITS2) was achieved using 0.5 µl each of forward and reverse primers. Each 25 µl reaction volume contained One Taq Quick-Load 2X Master (12.5µl) Mix with Standard Buffer (New England Biolabs Inc.); 0.5 µl each of forward and reverse primers; 8.5 µl of Nuclease free water and 3 µl of DNA template. PCR thermocycling was carried under specified amplification conditions which are: Initial denaturation for 30 s at 94 ℃, followed by 35 cycles of denaturation at 94 ℃ for 20 s, primer annealing at 54 ℃ for 45 s and strand extension at 72 ℃ for 1 minute. Final extension at

72 ℃ for 5 minutes on an Eppendorf nexus gradient Mastercycler (Germany).

The PCR products (10 µl) were purified using 2.5 µl of ExoSAP Mix, following the protocols of the manufacturer, ExonSAP kit, (ThermoFisher Scientific, UK). Briefly, reconstitution of 0.6 mL of the Exo/SAP master mix was carried out by adding 50 µl of Exonuclease I (Catalogue No. NEB M0293L); 200 µl of Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371). Amplified PCR Product (10 µl) and ExoSAP Mix (2.5 µl) were mixed and incubated at 37°c for 15 minutes. The reaction was stopped by heating the mixture at 80°C for 15 minutes. Fragments were sequenced using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions. The labelled products are then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053): The cleaned products were injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50cm array, using POP7. Sequence chromatogram analysis was then performed using FinchTV analysis software Geospiza, Inc; (Benson et al. 2009).

2, 2-diphenyl-1-picryl hydrazyl (DPPH) based free radical scavenging activity

DPPH radical scavenging activity was detected for antioxidant activity by thin layer chromatography (TLC) screening through spotting a concentrated ethanolic solution of the extract on silica gel plates. The plates were developed in ethanol: ethyl acetate (2:1) then air-dried and sprayed with 0.2% w/v DPPH spray. The presence of yellow spots was detected. Radical scavenging activity of extracts was measured according to the DPPH spectrophotometric method (Habu and Ibeh 2015) using vitamin C (Emzor Pharmaceutical Industries, Nigeria) as a reference antioxidant. Ethanol (1.0 ml) plus extract solution (2.5 ml) was used as blank while 1 ml of 0.3 mL DPPH plus ethanol (2.5 ml) was used as a negative control. The free radical scavenging properties of the extracts against DPPH radical were measured at 518 nm, as an index of their antioxidant activity. IC50 values (the concentration of extracts required to scavenge 50% of DPPH free radicals) were also calculated. The absorbance (abs) of the resulting mixture measured at 518 nm was converted to percentage antioxidant activity (AA %) and thus calculated by the equation:

% Scavenging Activity

$$= 100 - \left[\left(\frac{ABSsample - ABSblank}{ABScontrol}\right)\right] \times 100$$

Antibacterial activity

The fungal crude extracts were tested for possible antibacterial activities adopting the agar well diffusion technique as described by Adeyemi *et al*. (2015). Three pathogenic bacterial namely *Escherichia coli* and Pseudomonas aeroginosa- Gram-negative bacteria) and *Staphylococcus aureus* - (Gram-positive bacteria) obtained from Federal Medical Centre Owerri, Imo State, Nigeria were used for the assay. Each of the test bacteria previously standardized to 0.5 MacFarland turbidity standard was applied on the surface of sterile Mueller-Hinton agar using sterile swab sticks. Each fungal crude extract (1000 mg) was reconstituted to form our stock concentration of 500 mg/mL and diluted appropriately. A volume of 20 µl of each extract as well as the Ciprofloxacin (500 mg) were transferred into the wells made into the agar using 5 mm sterilized cork borer. The plates were incubated at 37°C for 24 h. Antibacterial activity was expressed by the production of inhibitory halos around the wells. Each extract concentration was tested in triplicate.

RESULTS

DPPH Scavenging activities of Endophytic fungi

The result in table 1 represented the DPPH Scavenging activities of Endophytic fungi isolated from *Pteridium acquilinium* leaf. The midrib (MR) and leaf blade (LB) of the leaf were used. The PTAMR2, PTAMR1A and PTAMR1B showed better antioxidative property. The result in table 2 also represented the DPPH Scavenging activities of Endophytic fungi isolated from *Newbouldia laevis* leaf. The midrib (MR) and leaf blade (LB) of the leaf were also used and the NLLB2, NLMR3 and NLLB3 showed better antioxidative property among other isolates

Table 1. DPPH Scavenging activities of Endophytic fungi isolated from *Pteridium acquilinium* leaf.

Con (µg/ml)	Inhibition (%) PTALB1A	Inhibition (%) PTALB1	Inhibition (%) PTAMR1A	Inhibition (%) PTAMR1B	Inhibition (%) PTAMR2	Ascorbic Acid
7.81	-85.00±20.46	28.88±0.00	11.18±1.47	-22.75±2.14	8.71±1.34	45.94±4.49
15.625	-61.58±34.23	27.88±1.40	-87.65±14.47	-26.78±3.45	11.62±0.87	53.84±3.25
31.25	-192.63±96.76	-11.15±1.42	-2.94±1.15	-12.09±1.37	12.66±2.93	85.42±0.46
62.5	-351.05±174.17	17.33±1.83	3.18±2.49	-13.27±1.04	21.99±2.64	86.18±0.00
125	-178.42±14.15	-184.96±32.26	-67.53±7.99	-2.61±2.67	32.99±1.17	85.75±0.30
250	-192.63±60.28	-30.38±2.12	8.59±3.65	4.39±2.84	50.32±1.02	84.98±0.77
500	-57.37±40.96	48.01±4.65	-41.88±5.65	13.75±2.67	57.88±5.86	85.86±0.45
1000	-111.05±3.72	46.715±5.21	32.71±6.65	81.05±0.67	73.55±14.81	85.97±0.30

The results are ±SD of triplicate determination.

Table 2. DPPH Scavenging activities of Endophytic fungi isolated from *Newbouldia laevis* leaf.

Con (µg/ml)	Inhibition (%) NLLB1	Inhibition (%) NLLB2	Inhibition (%) NLLB3	Inhibition (%) NLMR1	Inhibition (%) NLMR3	Ascorbic Acid
7.81	0.94±1.71	-13.69±1.05	10.08±1.18	-0.59±0.50	-2.805±0.08	45.94±4.49
15.625	-66.58±8.72	-32.54±1.21	-8.01±1.27	-29.08±7.41	-8.675±0.02	53.84±3.25
31.25	-16.44±1.14	8.55±0.56	11.75±1.34	-65.84±4.78	-39.41±5.63	85.42±0.46
62.5	-14.96±1.20	-34.42±1.35	5.19±1.99	-14.31±1.20	-169.39±35.36	86.18±0.00
125	-70.22±1.72	7.67±1.01	17.32±1.95	-97.05±31.60	-69.005±2.34	85.75±0.30
250	4.32±0.81	-22.74±2.51	43.74±2.07	-10.28±1.20	-13.01±1.81	84.98±0.77
500	6.06±2.38	5.655±0.99	77.30±6.08	-52.25±12.03	60.97±1.44	85.86±0.45
1000	14.96±2.09	37.57±2.31	84.35±3.04	43.38±5.85	64.03±2.56	85.97±0.30

The results are ±SD of triplicate determination.

Half maximal inhibitory concentration (IC_{50}) of all Isolates

The results in Table 3 show that the isolates possess antioxidant activities and the order of decreasing activity include; Ascorbic Acid (2.628388 ± 1.248694 µg/ml) > NLLB3 (266.2956 ± 84.84 µg/ml) > PTAMR2 (293.0755 ± 64.95 µg/ml) > PTAMR1B (1607.549 ± 694.3751 µg/ml) > NLMR3 7163.991 ± 5306.376 µg/ml) > NLLB2 (68201.41 ± 47549.36 µg/ml) > PTAMRIA (23446164 ± 29086173 µg/ml)

Table 3. Summary of IC_{50} of all Isolates.

S/NO	Sample	$1C_{50}$ (Mean ± SD) µg/ml
1	PTALB1A	1.38622E+62 ± 1.9604E+62 µg/ml
2	PTALB1	3.69E+11 ± 5.21735E+11 µg/ml
3	NLLB1	1.03E+21 ± 1.45519E+21 µg/ml
4	NLLB2	68201.41 ± 47549.36 µg/ml
5	NLMR3	7163.991 ± 5306.376 µg/ml
6	NLMR1	6.53E+12 ± 9.23872E+12 µg/ml
7	PTAMR2	293.0755 ± 64.95 µg/ml
8	PTAMR1A	23446164 ± 29086173 µg/ml
9	PTAMR1B	1607.549 ± 694.3751 µg/ml
10	NLLB3	266.2956 ± 84.84 µg/ml
11	ASCORBIC ACID	2.628388 ± 1.248694 µg/ml

Antibacterial activities of crude extract of endophytic fungi

Agar well of free actively growing pure culture of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were tested. The zones of inhibition obtained were between 0.4 to 17.60 mm. It was observed that the crude extract of endophytic fungi isolated from *Pteridium acquilinium* and *Newbouldia laevis* including the antibiotic controls, inhibited the human pathogens as shown in Table 4. PTAMR2 had a high zone of inhibitions of 16.90 mm against *S. aureus*, NLLB3 had a zone of inhibition of 17.60 mm against *Escherichia coli* while NLLB2 *had* a zone of inhibition of 14.40 mm against *Pseudomonas aeruginosa.*

Table 4. Antibacterial activities of crude extract of endophytic fungi isolated from *Pteridium acquilinium* and *Newbouldia laevis* leaves.

S/NO	Sample	*S. aureus* Zone of inhibition (mm)		*E. coli* Zone of inhibition (mm)		*P. aeroginosa* Zone of inhibition (mm)	
		250mg/ml	500mg/ml	250mg/ml	500mg/ml	250mg/ml	500mg/ml
1	PTALB1A	1.00±0.90	1.20±0.56	0.70±0.80	1.90±1.04	0.40±0.10	1.00±0.94
2	PTALB1	0.80±0.08	1.60±0.14	0.40±0.09	0.80±0.40	1.40±0.09	2.00±0.20
3	PTAMR1A	8.90±0.95	11.00±0.80	4.50±1.30	10.60±1.70	3.20±0.60	6.60±1.40
4	PTAMR1B	11.80±1.08	14.40±2.20	10.80±0.60	12.70±1.05	8.30±0.90	9.20±1.05
5	PTAMR2	12.00±0.92	16.90±1.70	10.80±2.50	16.40±2.80	8.80±2.50	10.10±1.50
6	NLLB1	1.60±2.08	2.40±1.00	0.90±0.50	1.40±1.08	0.00±0.50	0.40±1.08
7	NLLB2	12.60±0.48	14.40±0.90	12.00±0.70	14.40±2.05	10.40±0.70	14.40±2.05
8	NLLB3	13.20±3.48	16.00±2.80	13.90±1.90	17.60±2.05	9.50±1.90	11.60±2.05
9	NLMR1	0.30±0.85	0.90±0.08	0.40±0.60	1.20±1.04	1.00±0.30	1.50±1.04
10	NLMR3	12.80±1.40	16.00±0.80	11.70±1.40	15.50±0.95	8.20±1.40	13.50±0.95
11	Ciprofloxacin (500mg)	13.40±2.70	17.00±2.30	13.50±1.07	19.00±1.40	11.90±1.00	15.00±1.40

The results are ±SD of triplicate determination.

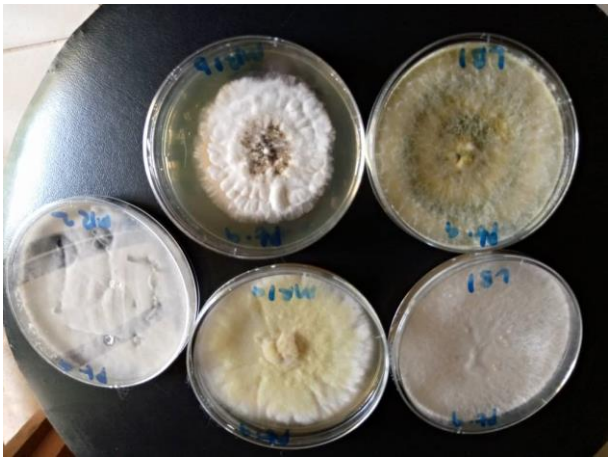

Figure 1. Pictorial of endophytic fungi isolated from *Pteridium acquilinium* leaf.

Figure 2. Pictorial of endophytic fungi isolated from *Newbouldia laevis* leaf.

DISCUSSION

The search for novel pharmacological and bioactive compounds from endophytic fungi has generated much interest. This is because of the diverse array of chemo-diversity of important novel secondary metabolites that are structurally unique and possess beneficially pharmacological and agricultural significance (Okezie et al. 2022; Tiwari and Bae 2022). In this study, a total of ten (PTALB1A, PTALB1, PTAMR2, PTAMR1A, PTAMR1B and NLLB1, NLLB2, NLMR3, NLMR1, NLLB3) endophytic fungi were isolated from healthy leaves of *Pteridium acquilinium* and *Newbouldia laevis* (fig. 1 and 2). Following standard taxanomic identification protocol for DNA amplification accompanied by sequencing of the ITS region, the endophytic fungi with the best results and promising antioxidant and antibacterial agent; NLLB2, NLMR3, PTAMR2, PTAMR1A, PTAMR1B, and NLLB3 were identified to be *Epicoccum thailandicum* (NR_152926.1); *Trichoderma atroviride* (MH862505.1); *Lasiodiplodia parva* (MH861166.1); *Trichoderma yunnanense* (NR_134419.1); *Colletotrichum cobbittiense* (NR_163538.1); and, *Trichoderma crissum* (NR_134370.1) respectively as confirmed by the 16s rRNA sequence after NCBI BLAST.

Antibacterial and antioxidant evaluations of the crude extracts of the fungal endophytes revealed varying potentials for development into antibacterial and antioxidant agents. *Trichoderma crissum* (IC_{50} 266.29µg/lm) and *Lasiodiplodia parva* (IC_{50} 293.07µg/lm) extracts showed significant scavenging activity (84.35±3.04 and 73.55±14.81%) when compared to ascorbic acid (85.97±0.30%). A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value (Habu JB and Ibeh 2015), thus the results presented here indicates a higher DPPH radical– scavenging activities by the fungal extracts (*Trichoderma crissum* and *Lasiodiplodia parva*). Some scientists have reported the antioxidant activities of different species of *Trichoderma*. The free radical scavenging potential *Trichoderma asperellum* isolated from *ascidian Eudistoma* has been reported by Sumilat *et al.* (2022). Kim *et al*. (2020) reported that *T. bissettii* and *T. guizhouense* have DPPH radical scavenging activity and tyrosinase inhibition activity respectively.

Recently, Ababutain *et al.* (2021) detected some secondary metabolites such as: alkaloids, diphenyl ether, monocarboxylic acid, hydroxycinnamic acid, phenalenones, sterols, terpenoids, and xanthones in the crude extract of some endophytic fungi isolated from *Artemisia sieberi*. Also, all the fungal extracts exhibited good antibacterial activities when compared to Ciprofloxacin (Table 4). The activities was observed to be broad spectrum, inhibiting *S. aureus*, *E. coli* and *P. aeruginosa* at the tested concentrations of 250 – 500 mg/mL. At a concentration of 250 mg/mL, the inhibition zone ranged between 0.30 – 13.20 mm, 0.40 – 13.90 mm and 0.00 – 9.50 against *S. aureus*, *E. coli* and *P. aeruginosa* reactively. While, at a maximum concentration of 500 mg/mL, the inhibition zone ranged between 0.90 – 16.90 mm, 0.80 – 17.60 mm and 0.40 – 14.40mm against *S. aureus*, *E. coli* and *P. aeruginosa* respectively. Furthermore, we observed *E. coli* to be the most sensitive organism to the fungal extracts. Similarly, Okezie *et al*. (2020) reported a broad-spectrum antibacterial activities against *B. subtilis*, *P. aeruginosa* and *E. coli* when tested at 1 mg/mL.

Following this research, it's worth noting that, in addition to the microbial and chemical diversity of plants, the endophyte-host plant interaction also gives microbes the ability to produce a variety of novel medicinally useful compounds, because endophytic fungi live in their hosts' inner tissues and inside the plant cell without causing overt symptoms or damage (Ababutain et al. 2021; Dhayanithy et al. 2019). Medicinal plant is known to be a place of residence for endophytes which could be the reason for the pharmacological potentials of

these endophytes as they interact with the plant host freely and as well possess some novel secondary metabolites which is quite different from the original compound possessed by the plant (Dhayanithy et al. 2019).

Studies on the antimicrobial and antioxidant potentials of crude extracts of endophytic fungi isolated from *Newbouldia laevis* and *Pteridium acquilinium* have been reported. These includes antimicrobial (Amaechi et al. 2020; Eze et al. 2019); antioxidant and immunosuppressive (Ujam et al. 2021) activities.

In public health, Antibacterial agents are of great significance, principally in decreasing the growth of infectious bacteria and restraint the transmission of bacterium. These antibacterial compounds can be isolated from endophytic fungi extracts (Chi et al. 2019).

The varying degrees of antimicrobial and antioxidant potentials exhibited by the various fungal extracts could be as a result of the varying number and concentration of the active compounds present (Malhadas et al. 2017). The extracts with high antimicrobial and antioxidant potency could therefore be said to probably contain quite a number of active compounds or contain high concentration of the available active compounds (Tiwari and Bae 2022; Dhayanithy et al. 2019).

Other endophytic fungal extracts, which showed moderate anti-bacterial and antioxidant activity, may have active compounds, but probably in smaller amounts. Therefore, the present study confirmed and has lend credence that endophytic fungi are potential sources for novel bioactive secondary metabolites that can be developed into new antibacterial and antioxidant drugs.

CONCLUSIONS

The study provides insight into the diversity of endophytic fungi associated with *Pteridium acquilinium* and *Newbouldia laevis* leaves growing in the coastal regions of South-Eastern Nigeria as well as the antioxidant and antibacterial potentials of their secondary metabolites. Further studies involving the identification and isolation of the bioactive compounds should be carried out which may be used for the development of antibacterial and antioxidant medicines. It is worthy to know endophytic fungi from *Pteridium acquilinium* and *Newbouldia laevis* leaves are promising potential sources for antibacterial and antioxidant agents, which can be developed as medicines for antibacterial and oxidative stress-related diseases.

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***Availability of Data and Material*: A**ll data generated or analyzed during this study are included in this published article.

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***Contributions*:** UCC and UEN supervised the work and prof read the manuscript, AO carried out the laboratory analysis, statistical analysis, wrote and edited the work, AOO and OUM carried out laboratory analysis and prof read the work, AC carried out plant sampling and JR carried out chemical and plant sampling. All authors have read and approved the manuscript.

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REFERENCES

Ababutain IM, Aldosary SK, Aljuraifani AA, Alghamdi AI, Alabdalall AH, Al-Khaldi EM, Aldakeel SA, Almandil NB, AbdulAzeez S, Borgio JF (2021) Identification and Antibacterial Characterization of Endophytic Fungi from Artemisia sieberi. Inter J Microb e6651020. https://doi.org/10.1155/2021/6651020

Adeyemi A, Erute Ma, Abiodun TS, Tolulope RA (2015) Isolation and screening of endophytic fungi from three plants used in traditional medicine in Nigeria for antimicrobial activity. Inter J Green Pharm 9(1):58. https://doi.org/10.4103/0973-8258.150929

Amaechi AA, Oli AN, Okezie UM, Adejumo SA, Abba CC, Okeke IJ, Okoye FBC (2020) Secondary metabolites of endophytic fungi from *Newbouldia laevis* and *Cassia tora* leaves: prospecting for new antimicrobial agents. Recent Pat Antiinfect Drug Discov DOI: 10.2174/1574891X15999201222152646

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EJ (2009) GenBank. Nucl Acids Res. 37:D3,D26.

Cao H, Chai TT, Wang X, Morais-Braga MFB, Yang JH, Wong FC, Wang R, Yao H, Cao J, Cornara L, Burlando B, Wang Y, Xiao J, Coutinho HDM (2017) Phytochemicals from fern species: potential for medicine applications. Phytochem Rev 16:379–440 https://doi.org/10.1007/s11101-016-9488-7

Chi WC, Pang KL, Chen WL, Wang GJ, Lee TH (2019) Antimicrobial and iNOS Inhibitory Activities of the

Endophytic Fungi Isolated from the Mangrove plant Acanthus ilicifolius var. xiamenensis Botanic Studies 60:4. https://doi.org/10.1186/s40529-019-0252-3

Dhayanithy G, Subban K, Chelliah J (2019) Diversity and biological activities of endophytic fungi associated with Catharanthus roseus. BMC Microbiol 19(1):22. https://doi.org/10.1186/s12866-019-1386-x

Eze PM, Nnanna JC, Okezie U, Busugbe HS, Abba CC, Chukwunwejim CR, Okoye FBC, Esimone CO (2019) Screening of metabolites from endophytic fungi of some Nigerian medicinal plants for antimicrobial activities. The EuroBiotech J 3(1):10–18. DOI: 10.2478/ebtj-2019-0002

Frickmann H, Hahn A, Berlec S, Ulrich J, Jansson M, Schwarz NG, Podbielski PWA (2019) On the Etiological Relevance of *Escherichia coli* and *Staphylococcus aureus* in Superficial and Deep Infections–A Hypothesis-Forming, Retrospective Assessment. Euro J Micro Immunol 9:4. DOI: https://doi.org/10.1556/1886.2019.00021

Govindarajan M, Kwon S, Weon H (2007) Isolation, molecular characterization and growth-promoting activities of endophytic sugarcane diazotroph Klebsiella sp. GR9. World Microb Biotechnol 23:9971006.

Habu JB, Ibeh BO (2015) In vitro antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of *Newbouldia laevis* ethanolic leaf extract. Biol Res 48(1):16. https://doi.org/10.1186/s40659-015-0007-x

Hadadi Z, Nematzadeh GA, Ghahari S (2020) A study on the antioxidant and antimicrobial activities in the chloroformic and methanolic extracts of 6 important medicinal plants collected from North of Iran. BMC Chem 14(1):33 https://doi.org/10.1186/s13065-020-00683-5

Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between Endophytic fungi and medicinal plants: A Systematic Review. Front Microbiol 7. https://www.frontiersin.org/article/10.3389/fmicb.2016.00906

Kim K, Heo YM, Jang S, Lee H, Kwon S, Park MS, Lim YW, Kim J (2020) Diversity of *Trichoderma spp*. in Marine Environments and their biological potential for Sustainable Industrial Applications. Sustainability 12:4327. doi:10.3390/su12104327

Kowalczyk P, Sulejczak D, Kleczkowska P, Bukowska-O´sko I, Kucia M, Popiel M, Wietrak E, Kramkowski K, Wrzosek K, Kaczynska K (2021) Mitochondrial Oxidative Stress—A Causative Factor and Therapeutic Target in Many Diseases. Inter J Mol Sci 384 https://doi.org/10.3390/ ijms222413384

Malhadas C, Malheiro R, Pereira JA, de Pinho PG, Baptista P (2017) Antimicrobial activity of endophytic fungi from olive tree leaves. World J Microb Biotech 33(3):46. https://doi.org/10.1007/s11274-017-2216-7

Manganyi MC, Tchatchouang CDK, Regnier T, Bezuidenhout CC, Ateba CN (2019) Bioactive Compound Produced by Endophytic Fungi Isolated from *Pelargonium sidoides* Against Selected Bacteria of Clinical Importance. Mycobiol 47(3):335–339. https://doi.org/10.1080/12298093.2019.1631121

Okagu IU, Ndefo JC, Agbo MO (2021) Trado-Medical uses, Chemical Constituents and Biological Activities of *Newbouldia laevis* (Bignoniaceae): A Review. Pharm Sci 1 https://doi.org/10.34172/PS.2021.29

Okezie UM, Eze PM, Okoye FBC, Esimone CO (2022) Orthosporin, a major component of the fermentation product of *Lasiodiplodia theobromae*- an endophytic fungus of *Musa paradisiaca* as a potential antimicrobial agent. Not Sci Bio 14:2. DOI:10.15835/nsb14211084

Sumilat DA, Lintang RAJ, Undap SL, Adam AA, Tallei TE (2022) Phytochemical, antioxidant, and antimicrobial analysis of *Trichoderma asperellum* isolated from *Ascidian eudistoma* sp. J Appl Pharm Sci 12(04):090–095.

Tiwari P, Bae H (2022) Endophytic Fungi: Key Insights, Emerging Prospects, and Challenges in Natural Product Drug Discovery. Microorganisms, 10(2):360. https://doi.org/10.3390/microorganisms10020360

Ujam NT, Ajaghaku DL, Okoye FBC, Esimone CO (2021) Antioxidant and immunosuppressive activities of extracts of endophytic fungi isolated from *Psidium guajava* and *Newbouldia laevis*. Phytomedicine Plus. 1(2):100028 https://doi.org/10.1016/j.phyplu.2021.100028

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