In vitro Antimicrobial Activity of Secondary Metabolites of an Endophytic *Aspergillus brunneoviolaceus* Isolated from *Bryophyllum pinnatum* (Lam.) Oken

Nkechi J. Okonkwo-Uzor[1], Ugochukwu M. Okezie[2], Peter M. Eze[3*], Chidozie Ikegbune[1], Festus B.C. Okoye[4], Charles O. Esimone[2]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences. Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Awka Anambra State Nigeria
[3]Department of Environmental Health Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Anambra State Nigeria
[4]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Anambra State Nigeria

Submitted:; Accepted:; Available online: 31st August, 2022

Doi: https://doi.org/10.54117/jcbr.v2i4.1
*Correspondence: ezep2004@hotmail.com

Abstract
The antimicrobial properties of secondary metabolites produced by the endophytic *Aspergillus brunneoviolaceus* associated with the Nigerian plant *Bryophyllum pinnatum* were investigated in this study. Standard procedures were followed for fungal isolation and identification, as well as fermentation and extraction of fungal secondary metabolites. The agar-well diffusion method was used to assess the antimicrobial activity of the fungal extract. At 1 mg/mL, the fungal extract demonstrated broad spectrum antibacterial activity against the test bacteria (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Bacillus subtilis*), with inhibition zone diameters (IZD) ranging from 14 to 16 mm. The extract also exhibited antifungal activity with an IZD of 16 mm produced against *Candida albicans.* The findings of this study indicate that endophytic *A. brunneoviolaceus* associated with *B. pinnatum* has the potential to be a promising source of novel antimicrobial compounds with pharmaceutical importance.

Keywords: *Aspergillus brunneoviolaceus, Bryophyllum pinnatum*, endophytes, antimicrobial activity, secondary metabolites.

INTRODUCTION

Endophytic microorganisms have been demonstrated to be a reliable source of novel lead molecules with a wide range of biological activities (Okezie *et al.*, 2021). This study was conducted as part of our ongoing research into the drug discovery potentials of endophytes. It was designed to evaluate the antimicrobial activity of an endophytic fungus isolated from the leaves of *Bryophyllum pinnatum* growing in Anambra State, South-East Nigeria.

B. pinnatum (known as Odaa opuo in the South-East of Nigeria) is a member of the Crassulaceae family and is found in tropical areas of Africa, America, India, China, and Australia. The entire plant or plant parts are used for ethnomedicinal purposes, and the plant is reported to possess significant antimicrobial, anti-inflammatory, analgesic, antihypertensive, wound healing, hepatoprotective,

neuropharmacological and antidiabetic activities (Umebese and Falana, 2013; Fürer *et al.,* 2016).

Investigations of the endophytic fungal populations of some Nigerian medicinal plants have revealed the enormous potentials possessed by these organisms as sources of compounds of pharmaceutical and industrial importance (Okezie *et al.,* 2021; Akpotu *et al.,* 2017; Eze *et al.,* 2018; Abba *et al.,* 2016; Ebada *et al.,* 2016; Wang *et al.,* 2018; Eze *et al.,* 2019). The search for biotechnologically-important microorganisms based on the ethnobotanical pharmacology of the plants with which they are associated is an alternative method of discovering new microorganisms and bioactive molecules (Conti *et al.,* 2012; Nnanna *et al.,* 2018). In addition, endophytes are a promising environmentally-friendly source of high-valued bioactive phytochemicals produced by their host. These organisms have been found to produce compounds that are similar to those found in their host plants (Ebada et al., 2016). Based on these principles, this study justifies the investigation of endophytes from *B. pinnatum*, a well-known Nigerian medicinal plant.

MATERIALS AND METHODS

Isolation and Identification of Endophytic Fungus

Fresh *B. pinnatum* leaves were collected in September 2020 from a private garden in Okpuno-Awka, Anambra State, Nigeria. The plant was identified by a plant taxonomist and deposited in the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria, under the voucher number: PCG/474/A/073. An endophytic fungus was isolated from the plant leaves using a previously described method (Abba et al., 2016). The fungus was identified through DNA amplification and sequencing of its internal transcribed spacer ribosomal-deoxyribonucleic acid (ITS-rDNA) region (Kjer *et al*., 2010).

Fungal Fermentation and Extraction of Secondary Metabolites

The fungus was subjected to solid state fermentation in sterile rice medium as described by Abba et al. (2016). At the end of fermentation, the fungal secondary metabolites were extracted with ethyl acetate and concentrated under pressure with rotary evaporator.

Antimicrobial Assay

Preliminary antimicrobial evaluation of the fungal extract (1 mg/mL) against laboratory strains of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis,* and *Candida albicans* was carried out using the method described by Akpotu et al. (2017). Ciprofloxacin (5 µg/ml) and miconazole (50 µg/ml) were used as positive controls in antibacterial and antifungal studies respectively, and DMSO (100% v/v) was used as the negative control. The experiment was conducted in triplicate, and the resulting inhibition zone diameter (IZD) was measured in millimeters (mm). The mean IZD ± standard deviation (SD) were calculated and recorded.

RESULTS

Following standard procedures, an endophytic fungus was isolated from fresh leaves of *B. pinnatum* (Figures 1 and 2). The isolated fungus was identified as *Aspergillus brunneoviolaceus* according to molecular identification protocols for DNA extraction, PCR amplification, sequencing of the fungus internal transcribed spacer (ITS) region and comparing the resulting DNA sequence data with existing NCBI GenBank data. The sequence data was deposited in the NCBI GenBank database and ascribed the accession number OM530251.

After fermentation and extraction of the fungal secondary metabolites, the fungal extract was tested for antimicrobial activity against laboratory strains of *S. aureus, B. subtilis, K. pneumoniae, E. coli,* and *C. albicans.* Result of

the antimicrobial assay (Table 1) shows that the fungal extract (1 mg/mL) displayed a broad spectrum antibacterial activity with inhibition zone diameters (IZD) in the range of 14 - 16 mm produced against the test bacteria. The extract also exhibited antifungal activity with an IZD of 16 mm produced against *C. albicans.*



Figure 1: Leaves of *B. pinnatum*

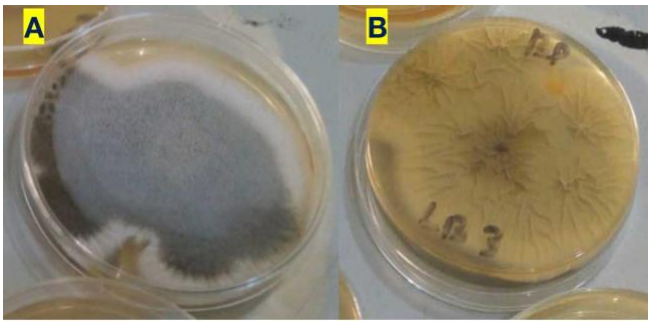


Figure 2: Axenic culture of *A. brunneoviolaceus* in malt extract agar showing the top (A) and bottom (B) views

Table 1: Antimicrobial activity of *A. brunneoviolaceus* extract showing mean IZD ± SD (mm) produced against test isolates

Test Organisms	Fungus Extract (1 mg/mL)	Positive control [Ciprofloxacin (5 µg/ml)]	Negative control [DMSO (100% v/v)]
S. aureus	16 ± 0.00	28 ± 1.40	0 ± 0.00
B. subtilis	14 ± 1.00	19 ± 3.68	0 ± 0.00
K. pneumoniae	15 ± 0.50	25 ± 1.41	0 ± 0.00
E. coli	15 ± 0.00	24 ± 2.45	0 ± 0.00
		Miconazole (50 µg/ml)	**DMSO**
C. albicans	16 ± 0 .00	41 ± 1.70	0 ± 0.00

DISCUSSIONS

B. pinnatum is a well-known tropical plant readily available in South-Eastern Nigeria. The antimicrobial properties of the plant have been reported (Umebese and Falana, 2013; Fürer *et al.*, 2016). A previous study have also investigated the endophytic populations of *Bryophyllum* plants for bioactive secondary metabolites (Sowemimo *et al.*, 2008).

This current study evaluated the antimicrobial potentials of secondary metabolites of an endophytic *A. brunneoviolaceus* associated with the leaves of *B. pinnatum.* The antibacterial and antifungal activities exhibited by the endophytic fungal extract (Table 1) may be attributed to antimicrobial compounds present in the fungal extract. According to Abba *et al.,* (2016), it is reasonable to suggest that the antimicrobial activity of this plant is related to or dependent on its association with the endophytic fungus and other endophytes that express antimicrobial compounds.

Endophytic fungus of the *Aspergillus* genera are well known to produce secondary metabolites with antimicrobial and other pharmacological properties (Varga et al. 2011, Vesth et al. 2018, Ezekiel et al. 2020a; Ezekiel et al. 2020b; Wang *et al.*, 2018).

A. brunneoviolaceus, which is phylogenetically related and considered to be synonymous with *A. fijiensis* (Vo et al., 2021). The fungus belongs to the commercially important *Aspergillus* Section Nigri, which are employed in the industrial production of enzymes and organic acids (Vo et al., 2021; Jurjević et al., 2012; Varga et al., 2011). One of the most widely used enzymes in the food industry, β-fructofuranosidase (invertase), which catalyzes the hydrolysis of sucrose into fructose and glucose, was isolated from *A. brunneoviolaceus* and found to be a safe and suitable source of enzymes for use by the food industry (Vo et al., 2021).

A. brunneoviolaceus thrives in a variety of environments. This fungus has previously been isolated from food, soil, water, plants, animal droppings, industrial material, indoor air environment, corneal scrapings, and human sputum (Rozaliyani et al., 2022; Mistry et al., 2021; Ezekiel et al., 2020a; Ezekiel et al., 2020b; Jurjević et al., 2012; Varga et al. 2011).

A. brunneoviolaceus culture extracts have been found to contain antimicrobial compounds such as paspalin (syn. cytochalasin H), tryprostatin B, brevianamide F, emodin, citreorosein (syn. -hydroxyemodin), nigragillin, meleagrin, and pyrophen (Varga et al. 2011, Vesth et al. 2018, Ezekiel et al. 2020a, Ezekiel et al., 2020b). A wide variety of other compounds with diverse biological properties have been reported from *A. brunneoviolaceus*, including: 16-ketoaspergillimide, aspergillimide (syn. asperparaline A), endocrocin, epoxyagroclavin, secalonic acids B, D, and F, iso-rhodoptilometrin, aurasperon C, dimethylsulochrin, fellutanine A, methylorsellinic acid, iso-rhodoptilometrin, oxaline, and paraherquamide E (Varga et al. 2011, Vesth et al. 2018, Ezekiel et al. 2020a, Ezekiel et al., 2020b). These bioactive compounds have the potential for pharmaceutical and industrial applications.

Endophytic fungi are interesting and being studied because they have the potential to be a source of new drugs and novel bioactive metabolites. As a result, the crude extract of secondary metabolites of endophytic *A. brunneoviolaceus* evaluated in this study is promising enough to warrant further research into the isolation and characterization of its active antimicrobial principles.

CONCLUSION

The findings of this study highlights the potential of endophytic *A. brunneoviolaceus* associated with *B. pinnatum* as a promising source of novel antimicrobial compounds with pharmaceutical significance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abba CC, Nduka I, Eze PM, Ujam TN, Abonyi DO, Okoye FBC. Antimicrobial Activity of Secondary Metabolites of Endophytic *Aspergillus* Species Isolated from *Loranthus micranthus*. Afr J Pharm Res Dev., 2016; 8(2): 136-140.

Akpotu MO, Eze PM, Abba CC, Umeokoli BO, Nwachukwu CU, Okoye FBC, Esimone CO. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. J Health Sci., 2017; 7(1): 15-22. https://doi.org/10.17532/jhsci.2017.421

Conti R, Cunha IGB, Siqueira VM, Souza-Motta CM, Amorim ELC, Araújoc JM. Endophytic microorganisms from leaves of *Spermacoce verticillata* (L.): Diversity and antimicrobial activity. Journal of Applied Pharmaceutical Science, 2012, 2 (12): 017-022.

Ebada SS, Eze P, Okoye FBC, Esimone CO, Proksch P. The Fungal Endophyte *Nigrospora oryzae* Produces Quercetin Monoglycosides Previously Known Only from Plants. Chem

Select, 2016; 1: 2767 – 2771. https://doi.org/10.1002/slct.201600478

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

Eze PM, Ojimba NK, Abonyi DO, Chukwunwejim CR, Abba CC, Okoye FBC, Esimone CO. Antimicrobial Activity of Metabolites of an Endophytic Fungus Isolated from the Leaves of Citrus jambhiri (Rutaceae). Trop J Nat Prod Res., 2018; 2(3): 145-149. http://dx.doi.org/10.26538/tjnpr/v2i3.9

Ezekiel CN, Oyedele OA, Kraak B, Ayeni KI, Sulyok M, Houbraken J, Krska R. Fungal Diversity and Mycotoxins in Low Moisture Content Ready-To-Eat Foods in Nigeria. Front Microbiol., 2020a; 11: 615. https://doi.org/10.3389/fmicb.2020.00615

Ezekiel CN, Kraak B, Sandoval-Denis M, Sulyok M, Oyedele OA, Ayeni KI, Makinde OM, Akinyemi OM, Krska R, Crous PW, Houbraken J. Diversity and toxigenicity of fungi and description of *Fusarium madaense* sp. nov. from cereals, legumes and soils in north-central Nigeria. MycoKeys, 2020b; 67: 95-124. https://doi.org/10.3897/mycokeys.67.52716

Fürer K, Simões-Wüst AP, von Mandach U, Hamburger M, Potterat O. *Bryophyllum pinnatum* and Related Species Used in Anthroposophic Medicine: Constituents, Pharmacological Activities, and Clinical Efficacy. Planta Med., 2016; 82: 930–941. http://dx.doi.org/10.1055/s-0042-106727

Jurjević Z, Peterson SW, Stea G, Solfrizzo M, Varga J, Hubka V, Perrone G. Two novel species of *Aspergillus* section Nigri from indoor air. IMA Fungus, 2012; 3(2): 159-73. https://doi.org/10.5598/imafungus.2012.03.02.08

Mistry H, Thakor R, Patil C, Trivedi J, Bariya H. Biogenically proficient synthesis and characterization of silver nanoparticles employing marine procured fungi *Aspergillus brunneoviolaceus* along with their antibacterial and antioxidative potency. Biotechnol Lett., 2021; 43(1): 307-316. https://doi.org/10.1007/s10529-020-03008-7

Nnanna JC, Eze PM, Anyanwu OO, Ujam TN, Ikegbunam MN, Okoye FBC, Esimone CO. Screening of Metabolites of Endophytic Fungi Isolated from Leaves of *Azadirachta indica* for Antimicrobial and Cytotoxic Activities. The Pharmaceutical and Chemical Journal, 2018; 5(3): 20-27.

Okezie UM, Eze PM, Okoye FBC, Esimone CO. Preliminary investigation of the chemo diversity of bioactive molecules produced by endophytic fungi isolated from *Manihot utilisima* leaf. GSC Biological and Pharmaceutical Sciences, 2021, 17(01): 011–025. https://doi.org/10.30574/gscbps.2021.17.1.0284

Rozaliyani A, Abdullah A, Setianingrum F, Sjamsuridzal W, Wahyuningsih R, et al. Unravelling the Molecular Identification and Antifungal Susceptibility Profiles of *Aspergillus* spp. Isolated from Chronic Pulmonary Aspergillosis Patients in Jakarta, Indonesia: The Emergence of Cryptic Species. Journal of Fungi, 2022; 8(4): 411. https://doi.org/10.3390/jof8040411

Sowemimo AA, Edrada-Ebel R, Ebel R, Proksch P, Omobuwajo OR, Adesanya SA. Major Constituents of the Predominant Endophytic Fungi from the Nigerian Plants *Bryophyllum Pinnatum*, *Morinda Lucida* and *Jathropha Gossypiifolia*. Natural Product Communications, 2008, 3(8): 1217 - 1222. https://doi.org/10.1177/1934578X0800300802

Umebese CE, Falana FD. Growth, phytochemicals and antifungal activity of *Bryophyllum pinnatum* L. subjected to water deficit stress. African Journal of Biotechnology,

2013; 12(47): 6599-6604. https://doi.org/10.5897/AJB2013.12151

Varga J, Frisvad JC, Kocsubé S, Brankovics B, Tóth B, Szigeti G, Samson RA. New and revisited species in *Aspergillus* section Nigri. Stud Mycol., 2011; 69(1): 1-17. https://doi.org/10.3114/sim.2011.69.01

Vesth TC, Nybo JL, Theobald S, Frisvad JC, Larsen TO, Nielsen KF. Investigation of inter- and intraspecies variation through genome sequencing of *Aspergillus* section Nigri. Nat. Genet., 2018; 50: 1688–1695. https://doi.org/10.1038/s41588-018-0246-1

Vo TD, Meetro J, Lynch B, Tafazoli S, Ichihara A, Chikamatsu G. Safety assessment of β-fructofuranosidase from *Aspergillus brunneoviolaceus*. Toxicology Research and Application, 2021; 5: 1–17. https://doi.org/10.1177/23978473211055361

Wang H, Eze PM, Höfert S, Janiak C, Hartmann R, Okoye FBC, Esimone CO, Orfali RS, Dai H, Liu Z, Proksch P. Substituted L-tryptophan-L-phenyllactic acid conjugates produced by an endophytic fungus *Aspergillus aculeatus* using an OSMAC approach. RSC Adv., 2018; 8: 7863–7872. http://dx.doi.org/10.1039/c8ra00200b