



## ANTIMICROBIAL ACTIVITY OF SECONDARY METABOLITES OF ENDOPHYTIC *ASPERGILLUS SPECIES* ISOLATED FROM *LORANTHUS MICRANTHUS*

Abba CC<sup>1</sup>, Nduka I<sup>1</sup>, Eze PM<sup>2\*</sup>, Ujam TN<sup>2</sup>, Abonyi DO<sup>2</sup>, Okoye FBC<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria

<sup>2</sup>Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria

---

### ABSTRACT

This study was carried out to evaluate the antimicrobial activity of secondary metabolites from an endophytic fungus isolated from the Nigerian mistletoe, *Loranthus micranthus*. *Aspergillus sp.* was isolated from leaves of *L. micranthus* and subjected to a solid state fermentation process. After fermentation, the secondary metabolite was extracted using ethyl acetate. The antimicrobial activity of the crude extract against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus fumigatus* was determined using the agar well diffusion method. At the concentrations analyzed (5 - 0.625 mg/mL), the extract recorded only antibacterial and no antifungal activity against the test isolates. Antibacterial activity was observed against all test bacteria at 5 and 2.5 mg/mL; while at 1.25 mg/mL inhibition zone diameters (IZD) of 2, 2, 0 and 4 mm were recorded against *S. aureus*, *S. typhi*, *K. pneumoniae* and *B. subtilis* respectively. At 0.625 mg/mL, antibacterial activity was recorded only against *B. subtilis* with an IZD of 3 mm. No IZD was recorded against *C. albicans* and *A. fumigatus* at the various concentrations analyzed. The assessment of the antimicrobial potentials of this endophytic fungus may serve as the baseline for the chemical identification of active molecules which may be used as antimicrobial compounds; or lead compounds which could be chemically manipulated into effective drugs.

**KEYWORDS:** *Endophyte, Antimicrobial, Aspergillus sp., Nigerian mistletoe, Loranthus micranthus*

---

### INTRODUCTION

Endophytes have been shown to possess the capacity to synthesize bioactive compounds that have found great use for novel drug discovery [1,2]. Recently, studies have been carried out to investigate Nigerian medicinal plants for their endophytic fungal population. Results of these studies have shown that endophytes associated with Nigerian plants possess the potentials as sources of novel bioactive molecules [1, 3-8].

The Nigerian mistletoe, *L. micranthus* is the Nigerian species of the African mistletoe [9]. The plant is a member of the Loranthaceae family. It is an obligate semi-parasitic evergreen tropical plant normally found growing on a variety of trees, including palm fruit, mahogany and other tropical plants [10]. It has been shown to possess some medicinal properties including antimicrobial [11], antimotility [12], and antioxidant properties [10].

Ebada *et al.* [8] investigated the bioactive metabolites from an endophytic *Nigrospora oryzae* which was isolated from the leaves of the Nigerian mistletoe. Several bioactive compounds guaijaverin, isoquercetrin, hyperin and luteolin monoglycoside were isolated from the fungus. These compounds have been reported to either possess antibacterial, antiviral, antioxidant, anti-inflammatory, anticancer, antidiabetic activities [13-17].

The assessment of the antimicrobial potentials of endophytic fungi from plants may serve as the baseline for the chemical identification of active molecules which may be used as antimicrobial compounds; or lead compounds which could be chemically manipulated into effective drugs. Thus the aim of the current research is to investigate the antimicrobial potentials of endophytic *Aspergillus sp.* isolated from the Nigerian mistletoe, *L. micranthus*.

## MATERIALS AND METHODS

### Plant Collection, Isolation and Identification of Endophytic Fungi

Fresh Leaves of *Loranthus micranthus* were collected from Ifitedunu, Anambra State, Nigeria. Isolation of endophytic fungi from plant leaves was carried out using the method described by Arnold *et al.* [18]. Harvested healthy plant leaves were washed in running tap water and processed as follows: about 1-2 mm segments were cut from the lamina and surface-sterilized by washing them for 2 min in 2% sodium hypochlorite, 2 min in 70% ethanol and then rinsed in sterile water for 5 min. The segments were selected and placed on Petri dishes containing malt extract agar (MEA) supplemented with chloramphenicol. The plates

were then incubated on laboratory benches at room temperature with ambient light. Periodically, fungal growth from the leaf segments were monitored and hyphal tips from distinct colonies emerging from leaf segments were sub-cultured onto fresh MEA plates to obtain pure colonies.

Identification of fungal isolates was carried out based on their cultural, morphological and microscopic characteristics as described by Barnett and Hunter [19] and Ainsworth *et al.* [20]. Morphological identification, according to the standard taxonomic key, included colony diameter, texture, colour and the dimensions and morphology of hyphae and conidia.

### Fermentation and Extraction of Secondary Metabolites

Solid state fermentation was carried out in 1000 mL conical flasks containing 100 g of rice (100 mL of water was added to the rice, which was then autoclaved at 121°C at 15 psi for 1 hr and allowed to cool). The flasks were inoculated with 3 mm diameter agar blocks containing fungal growth and incubated at 27-28°C for 30 days and extracted with ethyl acetate. The organic phase was then vacuum-concentrated at 40°C using a rotary evaporator to obtain the extracts.

### Antibacterial and Antifungal Assay

The agar plate diffusion assay method described by Onyegbule *et al.* [21] was used to evaluate the antibacterial and antifungal activities of the endophytic fungal extracts against the test microorganisms. Several dilutions of the crude extracts of the fungal metabolites were prepared by dissolving the extracts in Dimethyl sulphoxide (DMSO).

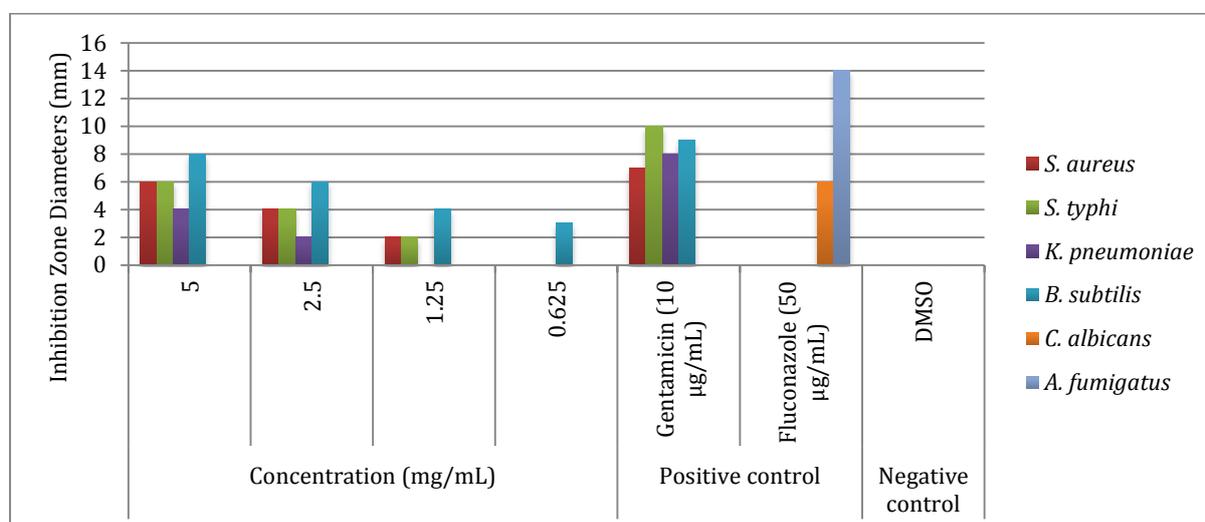


Figure 1: (A) Leaves of the Nigerian mistletoe, *Loranthus micranthus*; (B) Endophytic *Aspergillus sp.* isolated from leaves of *L. micranthus*

Standardized broth cultures of test bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi*) and fungal isolates (*Aspergillus fumigatus* and *Candida albicans*) were spread aseptically onto the surface of Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates respectively by using sterile cotton swabs. All culture plates were allowed to dry for about 5 min and agar wells were made using a sterile cork-borer (8 mm in diameter). These wells were respectively filled with 50  $\mu$ L of the various dilutions of the extracts and controls. The plates were then kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium

and incubated accordingly. Gentamicin (10  $\mu$ g/mL) and fluconazole (50  $\mu$ g/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO was used as the negative control. The MHA plates were then incubated at 37°C for 24 hours, and the SDA plates were incubated at room temperature (25-27°C) for 2-3 days. The inhibition zones diameters (IZD) were measured and recorded. The size of the cork borer (8 mm) was deducted from the values recorded for the IZD to get the actual diameter. This procedure was conducted in triplicate and the mean IZD calculated and recorded.

## RESULT



**Figure 2: Antimicrobial Activity of Crude Ethyl Acetate Extract of Secondary Metabolites Isolated from an Endophytic *Aspergillus sp.***

## DISCUSSION

Previous studies have shown that several extracts from endophytic fungi exhibit antimicrobial activity [22-24]. In this study, the crude ethyl acetate extract of secondary metabolites from an endophytic *Aspergillus sp.* isolated from the leaves of *L. micranthus* was evaluated for antimicrobial activity. In the antimicrobial screening, the extract recorded only antibacterial and no antifungal activity against the test isolates as seen in Figure 2. Antibacterial activity was observed against all test bacteria at 5 and 2.5 mg/mL; while at 1.25 mg/mL inhibition zone diameters (IZD) of 2, 2, 0 and 4 mm were recorded against *S. aureus*, *S. typhi*, *K. pneumoniae* and *B. subtilis* respectively. At 0.625 mg/mL, antibacterial activity was recorded only against *B. subtilis* with an IZD of 3 mm.

*L. micranthus* has been shown to possess some medicinal properties including antimicrobial [11] and antioxidant properties [10].

It may be reasonable to infer that the antimicrobial activity exhibited by this plant may be related or dependent upon its association with the endophytic fungus, *Aspergillus sp.* (whose secondary metabolite also recorded antimicrobial activity.

According to Sutjaritvorakul *et al.* [22], further investigation of endophytic fungi is considered to be important since they hold promise as a source of new drugs and novel bioactive metabolites with a high activity against pathogenic microorganisms. Therefore, the crude extract of secondary metabolites isolated from an endophytic *Aspergillus sp.* is promising enough to deserve further purification and characterization, as this will help in the identification of any novel compounds of medicinal importance.

## CONCLUSION

The results of this study suggest that endophytic *Aspergillus* sp. associated with the Nigerian mistletoe, *L. micranthus* could be a potential source of novel antimicrobial compounds for pharmaceutical applications.

## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

## REFERENCE

1. Strobel G, Daisy B. Bioprospecting for Microbial Endophytes and Their Natural Products. *Microbiology and Molecular Biology Reviews*. 67(4), 2003:491–502.
2. Okoye FBC, Nworu CS, Debbab A, Esimone CO, Proksch P. Two new Cytochalasins from an endophytic fungus, KL-1.1 isolated from *Psidium guajava* leaves. *Phytochemistry Letters*. 14, 2015: 51-55.
3. Okoye FBC, Nworu CS, Akah PA, Esimone CO, Debbab A, Proksch P. Inhibition of inflammatory mediators and reactive oxygen and nitrogen species by some depsidones and diaryl ether derivatives isolated from *Corynespora cassiicola*, an endophytic fungus of *Gongronema latifolium* leaves. *Immunopharmacology and Immunotoxicology*. 35(6), 2013: 662-668.
4. Okoye FBC, Lu S, Nworu CS, Esimone CO, Proksch P, Chaldi A, Debbab A. Depsidone and Diaryl Ether Derivatives from the Fungus *Corynespora cassiicola*, an Endophyte of *Gongronema latifolium*. *Tetrahedron Letters*. 54, 2013: 4210–4214.
5. Abba CC, Umeokoli BO, Eze PM, Onyegbule AF. Antioxidant and Antimicrobial Potentials of Some Metabolites from an Endophytic Fungi Isolated from *Loranthus micranthus* from South-Eastern Nigeria. *Planta Medica*. 80 (P1N29), 2014: 1398. DOI: 10.1055/s-0034-1394619.
6. Ojimba NK, Eze PM, Abba CC, Okoye FBC, and Esimone CO. Investigation of Secondary Metabolites of Endophytic Fungi from *Loranthus micranthus* Linn and *Citrus jambhiri* Lush for their Antioxidant Activity. *Planta Medica*. 81 (PM\_190): 2015. DOI: 10.1055/s-0035-1565567.
7. Chen H, Daletous G, Okoye FBC, Lai D, Dai H, Proksch P. A new cytotoxic cytochalasin from the endophytic fungus *Trichodama harzianum*. *Natural Products Communications*. 10(4), 2015: 585-587.
8. Ebada SS, Eze P, Okoye FBC, Esimone CO, Proksch P. The Fungal Endophyte *Nigrospora oryzae* Produces Quercetin Monoglycosides Previously Known Only from Plants. *Chemistry Select*. 1(11), 2016: 2767-2771.
9. Moghadamtousi SZ, Hajrezaei M, Kadir HA, Zandi K. *Loranthus micranthus* Linn.: Biological Activities and Phytochemistry. *Evidence-Based Complementary and Alternative Medicine*. 2013(Article ID 273712), 2013:1-9. Doi:10.1155/2013/273712.
10. Adebisi F, Oluwaseye A, Fausat A, Michael A, Adedayo A, Ikokoh P, Salisu A, Chukwu M. Chemical characterization and antimicrobial activity of *Loranthus micranthus* Linn leaves. *Journal of Medicinal Plants Research*. 7(45), 2013:3332-3336.
11. Orji FA, Nwachukwu NC, Onyia AU, Nkwocha M. Phytochemical and antimicrobial properties of leaves of African mistletoe (*Loranthus micranthus*) on some selected microbial pathogens in Abia State, Nigeria. *Global Advanced Research Journal of Microbiology*. 2(1), 2013:11-16.
12. Osadebe PO, Abba CC, Agbo MO. Antimotility effects of extracts and fractions of mistletoe (*Loranthus micranthus* Linn) from Eastern Nigeria. *Asian Pac. J Tropical Med*. 5(7), 2012: 556-560.
13. Rogerio AP, Kanashiro A, Fontanari C, da Silva EV, Lucisano-Valim YM, Soares EG, Faccioli LH. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. *Inflamm Res*. 56(10), 2007:402-408.
14. Valentová K, Vrba J, Banciřová M, Ulrichová J, Křen V. Isoquercitrin: pharmacology, toxicology, and metabolism. *Food Chem Toxicol*. 68, 2014:267-82.
15. Caruso IP, Vilegas W, Fossey MA, Cornélio ML. Exploring the binding mechanism of Guajaverin to human serum albumin: Fluorescence spectroscopy and computational approach. *Biomolecular Spectroscopy*. 97, 2012:449–455.
16. Kim SJ, Um JY, Lee JY. Anti-inflammatory activity of hyperoside through the suppression of nuclear factor-κB activation in mouse peritoneal macrophages. *Am J Chin Med*. 39(1), 2011:171-81.
17. López-Lázaro M. Distribution and Biological Activities of the Flavonoid Luteolin. *Mini-Reviews in Medicinal Chemistry*. 9, 2009:31-59.

18. Arnold AE, Maynard Z, Gilbert G, Coley PD, Kursar TA. Are tropical fungal endophytes hyperdiverse? *Ecol Lett.* 3, 2000:267–274.
19. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 3<sup>rd</sup> ed. Burgess Publishing Company. New York. 1972.
20. Ainsworth, G.C., Sparrow, F.K and Sussman, A.S. 1973. The fungi, an advanced treatise. Edition Vol. IV(A), a taxonomic review with keys ascomycetes and fungi imperfecti. Academic Press, New York.
21. Onyegbule FA, Ilouno IO, Eze PM, Abba CC, Chigozie VU. Evaluation of the Analgesic, Anti-Inflammatory and Antimicrobial Activities of Leaf Extracts of *Breynia nivosa*. *Chem Sci Rev Lett.* 3(12), 2014:1126-1134.
22. Sutjaritvorakul T, Whalley AJS, Sihanonth P, Roengsumran S. Antimicrobial activity from endophytic fungi isolated from plant leaves in Dipterocarpaceae forest at Viengsa district Nan province, Thailand. *Journal of Agricultural Technology.* 6(2), 2010: 309-315.
23. Raviraja NS, Maria GL, Sridhar KR. Antimicrobial Evaluation of Endophytic Fungi Inhabiting Medicinal Plants of the Western Ghats of India. *Eng. Life Sci.* 6(5), 2006:515–520.
24. Subbulakshmi GK, Thalavaipandian A, Bagyalakshmi VR, Rajendra A. Bioactive endophytic fungal isolates of *Biota orientalis* (L) Endl., *Pinus excelsa* Wall. and *Thuja occidentalis* L. *International Journal of Advanced Life Sciences.* 2012. 4:1-7.