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Bioactive secondary metabolites of endophytic fungi from three medicinal plants in Nigeria

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ABSTRACT

Endophytic fungi are potential sources of bioactive secondary metabolites that can be exploited for therapeutic purposes. Our study was aimed to identify bioactive secondary metabolites from crude extracts of endophytic fungi of three popular medicinal plants (Alstonia boonei- Ahun, Enantia chlorantha- Awopa and Kigelia africana- Pandoro) that have ethnobotanical history in Nigeria. The endophytic fungi were isolated from the stem barks of the plants using standard procedures. They were later fermented in broth culture, and the extract from the cell free broth of each of the fungi was subjected to Gas-Chromatography Mass-Spectrophotometry (GCMS). The results showed that, twenty-one different compounds were characterised from Aspergillus niger, Macrophomina sp, Trichoderma sp. and Penicillium species. The bioactive compounds include; Griseofulvin, Cinnamic acids, Penicillin, Coumarin, Cubenol, Erythritol among others. The presence of these compounds may contribute to the therapeutic properties of the plants.

Keywords: Endophytic fungi, bioactive secondary metabolites, medicinal plant, GC/MS

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INTRODUCTION

It has been estimated that of the about 1.5 million fungal species on the earth, only about 100,000 species are presently known [1] and a few taxa tested for their biological applications including their ability for drug production and biological control. Thus it seems that the discovered percentage of economically valuable fungal metabolites is small. Soil fungi have been the most studied, and typical soil genera such as *Acremonium*, *Aspergillus*, *Fusarium* and *Penicillium* have shown ability to synthesize a diverse range of bioactive compounds. More than 30% of isolated metabolites from fungi are from *Aspergillus* and *Penicillium.* [2] Fungi however were usually obtained from the same ecological niche using the same fungal isolation methods. Therefore the same fungal strains were re-isolated and this lead to the re-discovery of known compounds as the same taxa produce the same metabolites.

Endophytic fungi are potential producers of bioactive products.[3, 4] They produce different bioactive secondary metabolites which include, steroids, alkaloids, xanthones, benzopyranones, terpenoids, chinones, phenolic acids, quinones, tetralones, flavonoids among others. [5] These secondary metabolites can be exploited for curing diseases. [6] They can be used as immunosuppressants, agrochemicals, antioxidants, antibiotics, antiparasitics, and anti- cancer agents. [7]

In addition, some plants generating bioactive natural products have associated endophytes that produce the same natural products. Such is the case with paclitaxel, a highly functionalized diterpenoid and famed anticancer agent that is found in each of the world's yew tree species - Taxus spp. [8] In 1993, a novel anti cancer producing fungus, *Taxomyces andreanae*, from the *Taxus brevifolia* was isolated and characterized. [9]

As endophytic fungi are rich sources for bioactive compounds and a huge demand arises for novel drugs, it is therefore necessary to exploit the endophytic fungi associated with medicinal plants. Hence, this work analyzed fungal endophytes found in stem barks of three popular medicinal plants (*Alstonia boonei*- Ahun, *Enantia chlorantha*- Awopa and *Kigelia africana*- Pandoro) that have ethnobotanical history in Nigeria

MATERIALS AND METHOD

Reagents and Media

Reagents used in the course of this project include: Absolute ethanol, 95% ethanol, 2% sodium hypochlorite, lactophenol cotton blue, immersion oil; which were all of analytical grade. Media used include: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Mueller Hinton Agar, Nutrient Agar.

Sample Collection and Identification

Healthy (showing no visual disease) barks of medicinal plants; *Alstonia boonei* (Ahun), *Enantia chlorantha* (Awopa) and *Kigelia africana* (pandoro) were obtained from the local herbal market, Mushin, Lagos. The plants were identified and authenticated at the Mr Oyebamji of the Herbarium, University of Lagos, Nigeria.

Isolation and Identification of the fungi

The fungi were isolated under sterile conditions from the stem barks of the plants following an isolation protocol, cultured on Sabourand dextrose agar media as described previously. [10]

Fermentation

Thirty (30) ml of potato dextrose broth was distributed in 100ml conical flasks and autoclaved at 121^{o}C for 15minutes. The isolated endophytic fungal strains were inoculated aseptically into all flasks. The flasks were kept on the shaker at 27^{o}C for 9 days for growth. The flasks were examined periodically for any contamination. After 9 days, culture media were centrifuged at 5,000 rpm for 30 minutes.

Detection of class of secondary metabolites by GC/MS analysis

The fungal extracts were subjected to Gas Chromatography Mass Spectrophotometer (GCMS) for qualitative analysis; purification and identification of the class of compounds present.

Gas chromatography was done to determine the class of compounds of the metabolites obtained from the fermentation process (characterization of compounds).

A Hewlett Packard (HP) 6890 series (USA) with a flame ionization detector (FID) was used. Instrument operating conditions were as follows: an SE-30 glass column pack with internal diameter of 2.5µm, and length of 30m and packed with porapak N, 60/100, a column temperature of 250^{o}C, an injector temperature of 220^{o}C, a detector temperature of 280^{o}C, Nitrogen carrier gas flow rate of 22ml/min and hydrogen carrier gas at a flow rate of 45ml/min and temperature/ramping rate of 10^{o}C/min. In carrying out the GC, a standard profile was first obtained by injecting 1 ml of standard antibiotics into the GC and a chromatogram was generated to serve as a calibration window with which the test sample was analyzed. After generating the standard profile, 20 ml of the sample was extracted with ethyl acetate/ methanol and raced through chloroform, then concentrated to 1ml (test sample) from which 1 µl was injected into the GC and an equivalent chromatogram was generated. The peak areas of the standard and test sample chromatogram were compared with respect to the concentration of the sample. This is given by:

$$\text{Concentration of test sample} = \frac{\text{Total peak of sample} \times \text{concentration of standard}}{\text{Peak area of standard}}$$

RESULTS AND DISCUSSION

Gas chromatography and mass spectrophotometer assay indicated the presence of twenty one (21) metabolites, some of which were common to all the fungi studied. The most common volatile compounds which were detected consistently in all the endophytes were 5, 8 dimethylquinoline, Homosyringic acid and 8, 11 Octadecadienoic acid. Others incude; Cinnamic acids, alpha-Amorphene, 4a methyl-trans-2 decali, Penicillin, alpha-Cadinol, Griseofulvin, n-Tridecanoic acid, Palmitaldehyde, n-Tetradecanoic acid, Olealdehyde, 2-Pentadeconone, Cis-9-hexadecenal, Cis- 2-methoxy cinnamic, Coumarin, Cinnamaldehyde, 2-Furaldehyde 5- hydroxym, Cubenol and Erythritol (table 1)

Our study showed that extracts of fermentation broths from *Aspergillus niger*, *Macrophomina sp*, *Trichoderma sp*. and *Penicillium species* produced a variety of 21 bioactive compounds, ranging from 7-16 different compounds per endophyte (table 1). The endophyte *Penicillium sp* 2 produced the highest number of bioactive compounds (16) while the fungal endophyte Penicillium sp 1 produced the lowest bioactive compounds (7).

Most of these compounds belong to the groups of antimicrobial compounds found in plants [11] and also possess antifungal properties. [12] Cinnamic acid and Cinnamaldehyde are phenolic compounds which are bioactive phytochemical that possess single substituted phenolic ring. Phenolic toxicity to microgranisms is related to the site and number of hydroxyl groups present in the phenolic compounds. [13, 14] Cinnamic acid secreted has been found to be active against bacteria, fungal and viruses. [15] The 5, 8 dimethyl quinoline secreted by all the endophytes studied is a di-ketone, which is known to exhibit activity against microorganisms by forming irreversible complex in nucleophilic aminoacids in their protein structures. Penicillin and Griseofulvin are known antibacterial and antifungal agents which are widely used for therapeutic purposes world over. Penicillin, which was first discovered by Flewin in 1928, has been produced industrially since early forties, [16] and made possible the control of many infectious diseases that had earlier affected mankind, [17] although its efficacy had diminshed due to the emergence of several bacteria that are now resistant to it. Griseofulvin however still remain one of the drugs of choice for treating fungal infection [18] and its efficacy remains largely uncompromised. Coumains are phenolic compounds made of fused benzene and α-pyridine rings. They possess characteristic odour and have been shown to have antimicrobial activities against bacteria, fungi and viruses.[19] Erythritol, a sweetener polyol that can be used as a sugar substitute for people with diabetes and obesity, it is also useful in pharmaceutical as well as food industries. [20]

In general, the secondary metabolites secreted by these endophytes exhibit antimicrobial effects and may contribute in no small measure to the therapeutic value of the medicinal plants harbouring them. They can also serve as potential sources of novel drugs.

Table 1: Bioactive Compounds characterized from extracts of fermentation broths from endophytic fungi

S/N	Pure compounds	Endophytic fungi producing bioactive metabolites				
		Macrophomina	***A.niger***	***P.citrinum***	**Penicillium sp1**	**Penicillium sp2**
1	Cinnamic acids	+ve	-ve	-ve	+ve	-ve
2	alpha-Amorphen	-ve	-ve	-ve	-ve	+ve
3	5,8-dimethylquinoline	+ve	+ve	+ve	+ve	+ve
4	4a-methyl-trans-2-decali	-ve	-ve	-ve	-ve	-ve
5	Penicillin	-ve	-ve	-ve	-ve	+ve
6	alpha-Cadinol	+ve	+ve	+ve	-ve	+ve
7	Griseofulvin	-ve	-ve	-ve	-ve	+ve
8	n-Tridecanoic acid	+ve	-ve	-ve	+ve	+ve
9	Palmitaldehyde	+ve	+ve	+ve	-ve	+ve
10	n-Tetradecanoic acid	-ve	+ve	+ve	-ve	+ve
11	Homosyringic acid	+ve	+ve	+ve	+ve	+ve
12	8,11-Octadecadienoic acid	+ve	+ve	+ve	+ve	+ve
13	Olealdehyde	-ve	+ve	-ve	-ve	+ve
14	2-Pentadeconone	+ve	-ve	+ve	+ve	+ve
15	Cis-9-hexadecenal	-ve	-ve	-ve	-ve	+ve
16	Cis-2-methoxy cinnamic	-ve	-ve	-ve	-ve	+ve
17	Coumarin	-ve	+ve	+ve	-ve	+ve
18	Cinnamaldehyde	-ve	+ve	+ve	-ve	-ve
19	2-Furaldehyde-5-hydroxym	+ve	-ve	-ve	+ve	-ve
20	Cubenol	+ve	-ve	-ve	-ve	-ve
21	Erythritol	-ve	-ve	+ve	-ve	+ve

'+ve' bioactive compounds found '-ve' bioactive compounds not found

CONCLUSION

Our study has shown that fungal endophytes have the ability to produce several types of volatile compounds and these secondary metabolites produced by endophytes associated with medicinal plants can be exploited for therapeutic purposes.

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REFERENCES

1. Hawksworth DL. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 2001; 105: 1422-1432.

2. Bérdy J. Bioactive Microbial Metabolites - A Personal View. Review. The J. Anti. 2005; 58:1–26.
3. Schutz, B. Endophytic fungi: A source of novel biologically active secondary metabolites. Swansea: Proceedings of International Symposium on Bioactive Fungal Metabolites Impact and Exploitation, British Mycological Society, University of Wales 2001.
4. Strobel, GA and Daisy B. Bioprospecting for Microbial Endophytes and their Natural Products. Microbiol and Mol Biol Rev. 2003; 67: 491-502.
5. Tan RX and Zou WX "Endophytes: a rich source of functional metabolites," Natural Product Reports 2001; 18 (4): 448–459
6. Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS. Genetic diversity and antifungal activity of species of Pestalotiopsis isolated as endophytes from medicinal plants. Fungal Diver. 2007; 24:37-54
7. Gunatilaka AAL, "Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence," J. Nat Pro,2006; 69 Suppl 3: 509–526
8. Suffness, M. Discovery and development of taxol. In Taxol*:* Science and Applications. Suffness, M. (ed.). *CRC Press, Boca Raton, Florida*. Arbuck, S.G.1995.P.1-25
9. Strobel GA, Stierle A, Stierle D and Hess WM. *Taxomyces andreanae* a proposed new taxon for a bulbilliferous hyphomycete associated with Pacific yew. Mycotaxon 1993; 47**:**71-78.
10. Abass TR, Adeleye IA, Adongbede EM and Seriki AT. Isolation and screening of endophytic fungi from three plants used in traditional medicine in Nigeria for antimicrobial activity. Int J green Pharm. 2015; 9:58-62
11. Donghai L, Shihong Y, Peter P, Zhenyi L, Qiang L and Jing X. Volatile metabolites profiling of a Chinese mangrove endophytic Pestalotiopsis sp. Strain. Afri.. J. Biotechnol. 2013; 12 Suppl 24:3802-3806.
12. Berger R G., "Biotechnology of flavours the next generation," Biotechnol Lett. 2009; 31 Suppl 11:1651–1659.
13. Sealbert A. Antimicrobial properties of tannins. Photochemistry 1991; 30:3875 – 3883
14. Abbas BA , Al -Saeed MH and Othman RM. Evaluation of antimicrobial activity of phenolic Extrac from *Haloxylon salicornicum* Bas.J.Vet.Res.,2008; 7 Suppl 1:58
15. Sova M. Antioxidant and antimicrobial activities of cinnamic acid derivatives. Mini Rev Med Chem 2012 Jul; 12 Suppl 8:749-67.

16. Wainwright Milton. The History of the Therapeutic use of crude Penicillin. Med His.1987; 31 Suppl 1:41-50
17. Kardos N and Demain AL.Penicillin: the medicine with the greatest impact on therapeutic outcomes. Appl. Microbiol Biotechnol. 2011; 92 Suppl 4:677-87
18. Park JH, Choi GJ, Lee HB, Kim KM, Jung HS, Lee SW, Jang KS, Cho KY, Kim JC, Griseofulvin from Xylaria sp. strain F0010, an endophytic fungus of Abies holophylla and its antifungal activity against plant pathogenic fungi, J Microbiol Biotechnol. 2005; 15 Suppl 1:112 –117.
19. O'Kennedy R, Thorne RD. Coumarins: Biology, Applications and Mode of Action. Wiley; Chichester: 1997. pp. 23–66.
20. Moon HJ, Marimuthu J, Kim I, Lee J. Biotechnological production of erythritol and its applications. Appl. Microbiol & biotechnol. 2010; 86 Suppl 4:1017-1025.