

Phytochemical Associates of Fungal Endophytes in Nigeria Riverine Mangrove Ecosystem

Udoukpo, F.C.¹, Bassey, I.N.¹, Yaro, C.A.², Fatunla, O.K.^{3,4,*}, Ita, B.⁵, Inam, E.J.^{4,5} and Essien, J. P.^{3,4}

¹ Department of Botany & Ecological Studies, University of Uyo, Uyo, Nigeria

² Department of Animal & Environmental Biology, University of Uyo, Uyo, Nigeria

³ Department of Microbiology, University of Uyo, Uyo, Nigeria

⁴ International Centre for Energy and Environmental Sustainability Research (ICEESR), University of Uyo, Uyo, Nigeria

⁵ Department of Chemistry, University of Uyo, Uyo, Nigeria

*Corresponding author: Fatunla, O.K (opeyemifatunla@uniuyo.edu.ng)

Received: August 30, 2024; Received in revised form September 16, 2024; Accepted: September 18, 2024; Published: October 28, 2024

© 2024 Centre for Energy and Environmental Sustainability Research, University of Uyo, Uyo, Nigeria

Abstract

The interaction between endophytic fungi and phytochemicals in the dominant mangroves (*Rhizophora mangle*, *Lacugularia racemosa*, and *Avicennia africana*) of the Iko River Estuary in Nigeria was investigated. Standard analytical procedures were employed to measure antioxidant levels, while endophytic fungi were isolated, morphologically characterized, and identified using culture-dependent methods. The study identified various bioactive compounds, including alkaloids, saponins, tannins/phenols, flavonoids, deoxy-sugars, cardiac glycosides, and steroids, with their concentrations varying depending on the mangrove species and plant parts. Alkaloids were present in all mangrove samples, with particularly high concentrations in the leaves of white (5%) and red (4.2%) mangroves. Principal Component Analysis (PCA) was used to analyze the interaction between the endophytic fungi and phytochemicals, revealing a distinct affinity of certain fungal species for specific antioxidants in the mangroves. Fungal species such as *Phoma* sp., *Rhizopus arrhizus*, *Aspergillus niger*, and others exhibited a negative relationship with tannins, flavonoids, deoxysugars, and cardiac glycosides, but a positive affinity for saponins, alkaloids, and steroids. Conversely, *Candida pseudotropicalis*, *Fusarium oxysporum*, *P. italicum*, and others showed a positive affinity for tannins, flavonoids, cardiac glycosides, and deoxysugars, but a negative affinity for alkaloids, saponins, and steroids. Notably, *Absidia* sp. was the only frequently isolated species with a strong correlation (r value ≥ 75 at a 95% confidence limit) with deoxy-sugars, while *Aspergillus niger*, *Aspergillus terreus*, and *Absidia* sp. demonstrated significant relationships with alkaloids, saponins, and steroids. These phytochemicals, which serve as precursors to antioxidants with promising biotechnological applications, likely play a crucial role in determining the association of endophytic fungi with deltaic mangroves in Nigeria.

Keywords: Endophytic fungi; Phytochemical Associates; Mangroves; Antioxidants; Principal Component Analysis (PCA)

DOI: 10.55455/jmesr.2024.009

1. Introduction

Mangrove endophytes are specialized fungal communities that colonize the tissues of mangrove plants, residing intracellularly or intercellularly within healthy plant segments (Deepthi et al., 2018). These endophytic fungi

contribute significantly to plant health by producing a variety of bioactive compounds. This mutualistic relationship helps the host plants defend against biotic stressors such as herbivores and pathogens, as well as abiotic stressors including drought and salinity (Kumaresan & Suryanarayanan, 2002). Consequently, mangrove plants with endophytes often exhibit better health and resilience compared to those without (Waller et al., 2005). Endophytic fungi are known to produce various important plant metabolites, which include nitrogen-containing compounds (alkaloids and amines), terpenoids, and phenolics (Saddhe et al., 2016). Mangroves themselves are recognized for their diverse array of bioactive metabolites, such as essential oils, flavonoids, and alkaloids. These metabolites are produced not only by the mangrove plants but also by their associated endophytes (Hamzah et al., 2020). The concept of "associates" becomes particularly relevant in this context, as it refers to the complex associations or groups of phytochemicals produced by these fungal endophytes. These secondary metabolites, including phenols, terpenes, and flavonoids, are also synthesized by fungi and bacteria, with endophytic fungi contributing significantly to this process (Mousavi & Karami, 2022). The distribution and population structure of endophytic fungi within mangroves are influenced by the host plant's characteristics and its habitat. For example, endophytes are often found in higher diversity within root tissues compared to stems and leaves (Arnold, 2008). Mangrove plants, which have been utilized in traditional medicine, host a wide variety of fungal endophytes, with over 200 species identified to date (Hamzah et al., 2018). These fungi vary in their colonization abilities depending on the host plant system (Gadkar et al., 2001). Despite the significant roles of endophytes, there is a need for more research on their interactions with mangrove plants, particularly concerning phytochemicals and antioxidants. This investigation aims to evaluate the phytochemical properties of dominant mangrove species in the Niger Delta—*Rhizophora mangle*, *Laguncularia racemosa*, and *Avicennia africana*—and analyze the correlation between bioactive compounds and fungal species richness and colony frequency. The study will also assess the response of endophytic species to the presence of antioxidants, enhancing our understanding of their ecological interactions and contributions.

2. Materials and Methods

2.1 Collection of Samples

A collective of 81 healthy mangrove leaf, stem/bark and root samples were collected for analysis. The later comprises prop roots of red and white mangroves (*Rhizophora. mangle* and *Laguncularia racemosa*, respectively) and pneumatophores of black mangrove (*Avicennia africana*) were collected from a riverine mangrove ecosystem of Iko River Estuary located in the Niger Delta region of Nigeria. The samples which were obtained near the fishing settlements of Iso Otoyo (04°30'55.06"N and 007°39'03.5"E), Okoro-Inyang (04°31'27.2" and 007°38'45.5") and Etekun (04°29'49.8" and 007°39'42.5") representing the upper reaches, middle reaches and lower reaches of the estuarine mangrove ecosystem (**Figure 1**). The bio-specimens were picked with sterile scissors, stored in sterile labelled bags separately and then transferred to the research laboratory for analysis.



Figure 1. Sample Locations along Iko River Estuary Mangrove Ecosystem

2.2 Extraction and Analyses of Phytochemical Components of Mangrove Plants

The method previously described by Nurunnabi et al. (2020) was adopted for extraction of phytochemicals. Petroleum extracts of the mangrove plant part (root, stem and leaf) were prepared. Precisely 500g of the fine sample powder of each sample was successively extracted with petroleum ether at room temperature ($28\pm 2^\circ\text{C}$). The extracts were concentrated under pressure to yield 40g of petroleum ether extract used for the phytochemical assays. Quantitative analysis of phytochemicals in the mangrove plant samples was carried out according to methods outlined by Harborne (1988), AOAC (1990) and Trease & Evans (1989). The total alkaloids content was determined by the alkaline precipitation gravimetric method described by Harborne (1988). The saponins analysis was carried out through direct spectrophotometry as reported by Brunner (1984) and Obadoni & Ochuko (2002), and the tannins by the Folin-Denis colorimetric method described by Bos & Jetten (1989). The flavonoids were measured as described by Harbone & Grayer (1988). The cardiac glycosides content was evaluated using Buljet's reagent as described by Al-Azizi et al., (1994), while deoxy-sugars were quantified using the anthrone method as detailed by Sztaricskai & Pelyvás-Ferencsik (2020). The steroids and phlobatannins in mangrove plant parts were quantitatively measured using methods reported by Olayinka et al., (2017).

2.3 Isolation and Identification of Fungal Endophytes

The endophytic fungi were isolated and morpho-taxonomically characterized, and then identified using methods previously described by Udoukpo et al. (2024), Zihad et al., 2022 and Wacira et al. (2024). Pure isolates obtained from the culture plates were grouped based on their morphological features (pigmentation/color, texture). Discrete colonies were subjected to microscopic analysis of stained (using cotton blue in lactophenol) mycelium to evaluate the type of hyphae and spores as well as the characteristics of hyphae and sporangia, features of conidia and arrangement of sporangiophore and conidiophores. References were made to fungal identification guide published by Samson et al. (1984), Pitt & Hocking (2009), Cabral et al., (1993) and Dugan (2017). Prior to identification the colony frequency of the discrete fungal isolates were observed, counted and recorded while the richness of diverse species of endophytic fungi were also recorded after identification.

2.4 Data Analyses

The relationship between colonization effect/species richness of mangroves and antioxidants concentrations were calculated using Paleontological Statistics (PAST) version 4.0.3 (Hammer et al., 2001). Similarly, the Principal Component Analysis (PCA) was performed determine affinity of fungi species to antioxidants presents on mangrove communities. The response of individual species of endophytes to phytochemicals was also evaluated using correlation analysis.

3. Results and Discussion

Wide range of antioxidants were obtained from the mangrove communities (*Rhizophora. mangle*, *Lacugularia racemosa* and *Avicennia africana*) investigated in this study (**Table 1**). The bioactive compounds detected were alkaloids, saponins, tannins/phenols, flavonoids, deoxy-sugars, cardiac and steroids. Their concentrations however varied with the type of mangrove and between plant parts. Alkaloids was detected in all the mangrove plant/parts analyzed but remarkably present in the leaves of white (5%) and red (4.2%) mangroves. Its concentrations in white (3.4%) and black (2.0%) mangrove root were also relatively high. Saponins was highest in white mangrove leaf (1.78%) followed by the red mangrove leaf (1.3 %) and black mangrove pneumatophores (1.13 %). Phlobatannins was not detected in any of the mangrove plants analyzed but tannins were found in all mangroves and plant parts but highest in the root of white mangrove with 7.23 mg/g concentration. The white mangrove leaf also contained remarkably high concentrations of flavonoids (4.48 %) and cardiac glycoside (4.02 mg/g) while steroids was detected more in pneumatophores (1.03 mg/g) and in the red mangrove stem (1.19 mg/g) and root (1.03 mg/g).

Culture dependent species of endophytic fungi with known capacity to elaborate many of the classes of secondary metabolites listed above were isolated from the deltaic or riverine mangroves investigated (**Table 2**). The isolates comprise 56 diverse species and their occurrence or colony frequency (CF), also varied between the mangrove type and plant parts.

Table 1: Quantitative Properties of Phytochemicals in Dominant Mangroves of the Deltaic ecosystem

Bioactive Compounds	White Mangrove (<i>L. racemosa</i>)			Black Mangrove (<i>A. africana</i>)			Red Mangrove (<i>R. mangle</i>)		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Alkaloids (%)	3.37	1.14	5.00	2.02	1.01	1.15	1.933	1.02	4.21
Saponins (%)	1.11	0.17	1.78	1.13	0.03	0.14	0.45	1.03	1.34
Tannins (mg/g)	7.3	3.35	3.36	0.02	1.87	1.12	0.03	1.11	0.10
Flavonoids (%)	1.42	1.34	4.48	1.01	1.21	1.12	0.01	0.02	0.004
Deoxy-sugars (mg/g)	0.12	1.21	1.00	0.004	1.10	1.23	0.004	0.22	0.10
Cardiac glycosides (mg/g)	4.01	3.42	4.02	0.01	3.00	2.93	1.10	2.01	0.00
Steroid	0.02	0.02	0.04	1.03	0.03	0.02	1.03	1.17	0.02
Phlobatannins (mg/g)	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not detected

Table 2: Occurrence/Diversity and Colonization Frequency (CF) of Endophytic Fungi in Deltaic Mangrove Ecosystem

S/N	Isolate	White Mangrove			Black Mangrove			Red Mangrove			Total CF (n=81)
		Root (n=9)	Stem (n=9)	Leaf (n=9)	Root (n=9)	Stem (n=9)	Leaf (n=9)	Root (n=9)	Stem (n=9)	Leaf (n=9)	
1	<i>Absidia</i> sp*	-	+(4)	+(3)	+(1)	-	-	-	+(1)	+(1)	10
2	<i>Aureobasium pullulans</i>	-	-	-	+(1)	-	-	-	-	-	1
3	<i>Alernaria alternata</i>	-	-	-	-	-	+(1)	-	-	-	1
4	<i>Aspergillus allahabadii</i>	+(1)	-	-	-	-	-	-	-	-	1
5	<i>Aspergillus candidus</i>	-	-	+(1)	+(1)	-	+(1)	+(1)	-	-	4
6	<i>A. carbonarius</i>	-	+(1)	+(1)	+(4)	-	-	-	-	+(1)	7
7	<i>A. clavatus</i>	-	-	+(1)	-	-	-	-	-	-	1
8	<i>A. flavipes</i>	-	-	-	+(4)	-	-	-	-	+(1)	5
9	<i>A. flavus</i>	-	-	-	+(1)	-	+(1)	+(1)	-	-	3

10	<i>A. fumigatus</i>	-	-	+(2)	+(1)	-	+(1)	+(1)	-	+(1)	6
11	<i>A. glaucus</i>	+(1)	-	+(2)	+(3)	-	+(2)	+(1)	-	-	9
12	<i>A. nidulans</i>	-	-	-	-	-	-	-	+(1)	-	1
13	<i>A. niger*</i>	-	+(5)	+(5)	+(4)	-	+(2)	+(5)	+(1)	+(3)	25
14	<i>A. ochraceous</i>	-	-	-	+(1)	-	-	-	-	-	1
15	<i>A. parasiticus</i>	-	+(1)	-	-	-	-	-	-	-	1
16	<i>A. penicilloides</i>	-	+(1)	-	-	-	-	-	-	-	1
17	<i>A. restrictus</i>	-	-	+(1)	-	-	-	-	-	-	1
18	<i>A. terreus*</i>	+(1)	+(2)	+(4)	+(2)	+(1)	+(1)	+(3)	+(1)	-	15
19	<i>A. ustus</i>	-	-	+(1)	+(2)	-	+(1)	-	-	-	4
20	<i>A. versicolor</i>	-	-	-	+(2)	-	-	-	-	-	2
21	<i>Candida pseudotropicalis</i>	-	+(2)	-	-	-	-	-	-	-	2
22	<i>Candida utilis</i>	-	-	-	+(2)	-	-	-	-	-	2
23	<i>Cladosporium</i> sp	-	+(1)	-	+(2)	-	+(1)	-	-	+(1)	5
24	<i>Epicoccum</i> sp	+(1)	-	+(1)	+(1)	+(1)	+(1)	-	+(1)	-	6
25	<i>Fusarium culmorum</i>	+(1)	-	-	+(1)	-	-	-	-	-	2
26	<i>F. oxysporum</i>	-	-	+(1)	-	-	-	-	-	-	1
27	<i>F. solani</i>	-	-	+(1)	-	-	-	-	-	-	1
28	<i>F. oxysporum</i>	-	-	+(2)	-	-	-	-	-	-	2
29	<i>F. solani</i>	-	-	-	-	-	+(1)	-	-	-	1
30	<i>F. verticilloides</i>	+(2)	-	-	-	-	+(1)	-	-	-	3
31	<i>Geotrichum</i> sp	-	+(2)	-	-	-	-	-	+(1)	-	4
32	<i>Microsporum</i> sp	-	-	-	+(1)	-	-	-	-	-	1
33	<i>Moniliella suaveolens</i>	-	-	-	+(1)	-	-	-	-	-	1
34	<i>Mucor racemosus</i>	-	-	-	+(1)	-	-	+(1)	-	-	2
35	<i>Nigrospora sphaerica</i>	-	-	-	-	-	-	+(1)	-	-	1
36	<i>Paecilomyces</i> sp	-	+(1)	-	+(1)	-	+(1)	-	-	-	3
37	<i>Penicillium citrinum</i>	-	-	+(1)	-	-	-	-	-	-	1
38	<i>Penicillium coryphylum</i>	-	-	-	+(1)	-	-	-	-	-	1
39	<i>P. expansum</i>	-	-	-	-	-	+(1)	-	-	-	1
40	<i>P. frequentans</i>	-	-	-	+(1)	-	-	+(1)	-	-	2
41	<i>P. italicum</i>	-	+(1)	+(1)	-	-	1	-	-	-	3
42	<i>P. paraherquei</i>	-	-	-	+(2)	+(1)	-	+(1)	-	-	3
43	<i>P. regulosum</i>	-	-	-	-	-	+(1)	-	-	-	1
44	<i>P. restrictum</i>	-	-	-	+(1)	-	-	-	-	-	1
45	<i>P. variable</i>	-	-	-	+(1)	-	-	-	-	-	1
46	<i>P. verrucosum</i>	-	+(1)	-	-	-	-	-	-	-	1
47	<i>Phoma</i> sp	-	-	-	+(1)	-	-	+(1)	-	+(2)	4
48	<i>Rhizopus arrhizus</i>	-	-	-	-	+(1)	-	-	+(2)	+(1)	4
49	<i>Rhizopus oryzae</i>	-	-	-	+(1)	-	+(1)	-	-	-	2
50	<i>Rhizopus stolonifer</i>	-	-	-	+(2)	-	-	-	-	-	2
51	<i>Saccharomyces estuary</i>	-	-	-	+(1)	-	-	-	-	-	1
52	<i>Saccharomyces uterus</i>	-	-	+(1)	+(1)	-	-	-	-	-	2
53	<i>Sclerotium</i> sp	-	-	-	-	-	+(1)	-	-	+(1)	2
54	<i>Torula</i> sp	-	-	+(1)	-	-	-	-	-	-	1
55	<i>Trichoderma</i> sp	-	-	-	+(1)	-	-	+(1)	-	-	2
56	<i>Verticillium</i> sp	-	-	-	+(1)	-	-	-	-	-	1
Colony Frequency (CF)		7	22	30	51	4	20	18	8	12	172
Species Richness		6	12	18	33	4	18	12	7	9	

Note: + = Species incidence/Occurrence/Isolated; - = Not isolated/Not detected. * = most frequently isolated species
Values in parenthesis = are colony frequency (CF) of isolated species

Analysis of the non-climax biotic community (endophytic fungi) interaction and association with phytochemicals using Principal Component Analysis (PCA) was carried out. This was done to measure the affinity of fungi

species to bioactive compounds in mangrove communities, in other words to establish the phytochemical associates of the endophytes. The result presented in **Figure 2** revealed the affinity of fungi species to the following antioxidants: alkaloids, saponins, flavonoids, deoxysugar, cardiac and steroid were measured. *Phoma* sp., *Rhizopus arrhizus*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. candidus*, *A. terreus*, *A. ustus*, *A. glaucus*, *A. carbonarius*, *Paecilomyces* sp., *Epicoccum* sp., *Sclerotium* sp., *Rhizopus oryzae*, *Geotrichum* sp., *A. nidulans*, *Saccharomyces estuarine*, *Nigrospora sphaerica* all had the negative affinity to tannins, flavonoids, deoxysugar and cardiac glycosides but positive affinity to saponins, alkaloids, and steroids. Meanwhile, *Candida pseudotropicalis*, *Fusarium oxysporum*, *F. verticilloides*, *P. italicum*, *P. verrucosum*, *Fusarium almorum*, *Saccharomyces utrus* has positive affinity to tannins, flavonoids, cardiac and deoxysugar but negative affinity to alkaloids, saponins, and steroids. The observation is a pointer to probable relation between bioactive compounds in mangroves and the endophytic fungal associates. It is a clear index of the effect of phytochemical product(s) on the associated mangrove mycoflora or probably the selective influence of bioactive compounds on the existence of endophytes in the stressed of mangroves of the Niger Delta region of Nigeria.

The observations deduced a very high stimulating effect of highest colonization of fungi species on the black mangrove which is high in deoxy sugar and moderate colonization on white and red mangroves which high concentration of tannins, flavonoids, saponins, alkaloids, cardiac and steroids. This observation is similar to the report of Sopalan et al. (2021), who stated that antioxidants such as saponins, tannins, flavonoids, and steroids possess inhibitory effects on the interaction between endophytic fungi and deoxy sugars, thereby affecting the metabolic activities of these fungi. Some studies reported that deoxy sugars may help fungi adapt to environmental stressors such as salinity and temperature fluctuations which can impact community composition (Tan & Zou, 2001; Gostinčar & Gunde-Cimerman, 2023). It has also be reported that deoxy sugar participate in nutrient recycling, influencing the availability of resources for other microorganisms and impacting fungal diversity, as well as a defensive barrier for the plants against pathogens (Ingle et al., 2020).

Plants and their endophytic microbial associates are known to utilize three pathways for secondary metabolite synthesis; the shikimate, polyketide and mevalonate (Burrage & Jeon, 2021, Tsipinana et al., 2023). Shikimate is associated with the synthesis of aromatic amino acid compounds, whereas polyketide and mavalonate pathways are utilized for the synthesis of molecules of medicinal importance (Tsipinana et al., 2023). Studies have revealed three principal kinds of secondary metabolites biosynthesized by plants. These include phenolic compounds, terpenoids/isoprenoids, and alkaloids and glucosinolates (Mohiuddin, 2019).

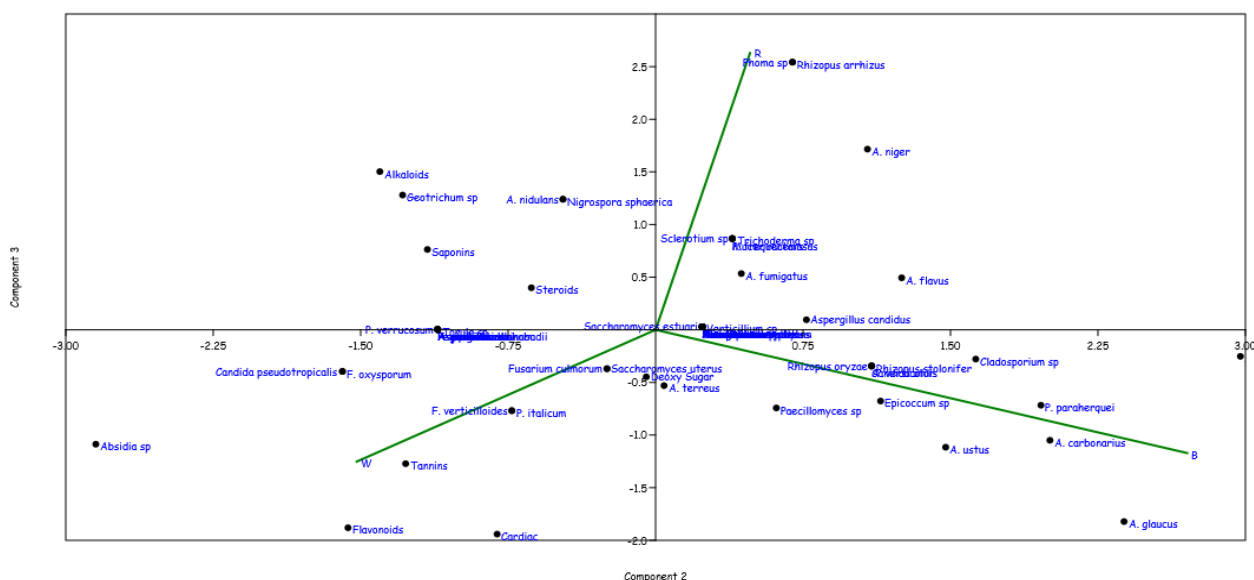


Figure 2. Principal Component Analysis (PCA) of Fungi Species on Mangrove Communities.

Further, secondary metabolites are usually categorized into three major groups: the terpenes comprising volatiles, glycosides, carotenoids and sterols; the phenolics which such as phenolic acids and tannins; and nitrogen containing compounds such as alkaloids and glucosinolates (Agostini-Costa et al., 2012). A study by Edu &

Edwin-Wosu (2015) has shown that Nigeria mangrove plant tissues contained highly polar bioactive compounds such as alkaloids, saponins, tannins, flavonoids and reducing sugars with the highest concentrations of alkaloids and saponins in *A. africana*. Flavonoids and tannins were found to be higher in *R. racemosa*. Our study has shown that although the concentrations varied between mangrove types, antioxidants found most abundantly in Nigeria deltaic mangroves would be alkaloids and tannins. Many of the bioactive compounds especially the phenolic compounds listed above are precursors of products of shikimate and polyketides pathways commonly associated with mangrove plants (Xu, 2015; Chen et al., 2022). These aromatic compounds have important roles, as pigments, antioxidants, signaling agents, the structural element lignin, defense mechanism, and useful as promising biotechnological applications.

Jamwal & Gandhi (2019) have reported that the molecular machinery adopted by endophytes for the production of phytochemicals is likely acquired from the host plant. They are known to influence genes responsible for the generation of secondary metabolites in plant cells through signal transduction pathways. Analysis of the relations between individual species and bioactive compounds in the mangroves (Table 3) revealed varied relationships. Very strong or significant (r-value above 0.75*) positive relationship were established between deoxy-sugars and *Aspergillus carbonarius*, *A. glaucus*, *A. ustus*, *Epicoccum sp*, *Fusarium culmorum*, *F. verticilloides*, *Paecilomyces sp* and *Penicillium italicum*. Similarly, the existence of *Absidia sp*, *Aspergillus niger*, and *Geotrichum sp* were significantly and positively influenced by the concentrations of alkaloids, saponins and steroids in mangroves while *Alternaria sp*, *Aspergillus ochraceous*, *Aspergillus ustus*, *Fusarium solani*, *Moniliella sp* and *Saccharomyces estuari* were negatively affected. None of the frequently isolated species but *Absidia sp* had strong relationship with deoxy-sugars but all of them including *Aspergillus niger*, *Aspergillus terreus* and *Absidia sp* demonstrated a strong and significant relationship with alkaloids, saponins and steroids. These observations are despite the no definite or poor relationship recorded between bioactive compounds concentrations against the counts of colony frequency and species richness of endophytic associates of the riverine mangroves (Table 4). These findings demonstrate the importance of antioxidants in shaping diversity of fungi species on mangrove community in coastal part of Akwa Ibom State, Nigeria. This diversity is also driven by a combination of environmental variables.

Table 3: Relation between individual species of endophytes with bioactive compounds of Mangroves

Species	Alkaloids	Saponins	Tannins	Flavonoids	Deoxy Sugar	Cardiac	Steroids
<i>Absidia sp</i>	0.77*	0.81*	0.89*	0.89*	0.19	0.77*	0.98*
<i>Aureobasium sp</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Alternaria sp</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Aspergillus allahabadii</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>Aspergillus candidus</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>A. carbonarius</i>	-0.86*	-0.83*	0.12	0.12	0.86*	0.33	-0.50*
<i>A. clavatus</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>A. flavipes</i>	-1.00*	-1.00*	-0.44	-0.44	0.44	-0.23	-0.89*
<i>A. flavus</i>	-0.95*	-0.97*	-0.67*	-0.67*	0.18	-0.49	-0.98*
<i>A. fumigatus</i>	NA	NA	NA	NA	NA	NA	NA
<i>A. glaucus</i>	-0.75*	-0.71*	0.31	0.30	0.94*	0.51*	-0.33
<i>A. nidulans</i>	0.32	0.26	-0.74*	-0.74*	-0.98*	-0.87*	-0.19
<i>A. niger</i>	1.00*	1.00*	0.44	0.44	-0.44	0.23	0.89*
<i>A. ochraceous</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>A. parasiticus</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>A. penicilloides</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>A. restrictus</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>A. terreus</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*

<i>A. ustus</i>	-0.86*	-0.83*	0.12	0.12	0.86*	0.33	-0.50*
<i>A. versicolor</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Candida pseudotropicalis</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94
<i>Candida utilis</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Cladosporium sp</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Epicoccum sp</i>	-0.75*	-0.71*	0.31	0.30	0.94*	0.51*	-0.33
<i>Fusarium culmorum</i>	-0.32	-0.26	0.74*	0.74*	0.98*	0.87*	0.19
<i>F. equiseti</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>F. graminearum</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>F. oxysporum</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>F. solani</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>F. verticilloides</i>	0.19	0.26	0.98*	0.98*	0.76*	1.00*	0.65*
<i>Geotrichum sp</i>	0.98*	0.97*	0.21	0.21	-0.65 *	-0.01	0.76*
<i>Microsporium sp</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
Table 3. Cont'd	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Mucor racemosus</i>	-0.66*	-0.71*	-0.95*	-0.95*	-0.34	-0.86*	-0.94*
<i>Nigrospora sp</i>	0.32	0.26	-0.74*	-0.74*	-0.98*	-0.87*	-0.19
<i>Paecilomyces sp</i>	-0.75*	-0.71*	0.31	0.30	0.94*	0.51*	-0.33
<i>Penicillium citrinum</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>Penicillium coryphylum</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>P. expansum</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>P. frequentans</i>	-0.66*	-0.71*	-0.95*	-0.95*	-0.34	-0.86*	-0.94*
<i>P. italicum</i>	0.19	0.26	0.98*	0.98*	0.76*	1.00*	0.65*
<i>P. paraherquei</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>P. regulosum</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>P. restrictum</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>P. variable</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>P. verrucosum</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>Phoma sp</i>	-0.01	-0.07	-0.92*	-0.92*	-0.87*	-0.98*	-0.50*
<i>Rhizopus arrhizus</i>	-0.01	-0.07	-0.92*	-0.92*	-0.87*	-0.98*	-0.50*
<i>Rhizopus oryzae</i>	-0.988	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Rhizopus stolonifer</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Saccharomyces estuari</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*
<i>Saccharomyces uterus</i>	-0.32	-0.26	0.74*	0.74*	0.98*	0.87*	0.19
<i>Sclerotium sp</i>	-0.66*	-0.71*	-0.95*	-0.95*	-0.34	-0.86*	-0.94*
<i>Torula sp</i>	0.66*	0.71*	0.95*	0.95*	0.34	0.86*	0.94*
<i>Trichoderma sp</i>	-0.66*	-0.71*	-0.95*	-0.95*	-0.34	-0.86*	-0.94*
<i>Verticillium sp</i>	-0.98*	-0.97*	-0.21	-0.21	0.65*	0.01	-0.76*

Asterisk (*) indicate either strong negative or positive correlation.

Table 4: Correlation of Phytochemicals against Endophytic Fungi Colony Frequency and Species Richness on Mangrove Communities

	Alkaloids (%)	Saponins (%)	Tannins (mg/g)	Flavonoids (%)	Deoxy-sugars (mg/g)	Cardiac glycosides (mg/g)	Steroid	Colony Frequency (CF)	Species Richness
--	---------------	--------------	----------------	----------------	---------------------	---------------------------	---------	-----------------------	------------------

Alkaloids (%)	1.00								
Saponins (%)	0.83*	1.00							
Tannins (mg/g)	0.27	0.14	1.00						
Flavonoids (%)	0.53*	0.39	0.46	1.00					
Deoxy-sugars (mg/g)	-0.24	-0.47	0.18	0.51*	1.00				
Cardiac glycosides (mg/g)	0.03	-0.14	0.79*	0.64*	0.65*	1.00			
Steroid	-0.33	0.10	-0.51*	-0.45	-0.63*	-0.56*	1.00		
Colony Frequency (CF)	0.01	-0.31	-0.03	0.56*	0.84*	0.49	-0.51*	1.00	
Species Richness	0.07	0.24	-0.35	0.24	-0.09	-0.37	0.31	0.03	1.00

Asterisk (*) – Strong positive or negative correlation (Values greater than -0.50 or +0.50)

4. Conclusion

This study has shown that mangroves in the crude oil impacted region of the Niger Delta region of Nigeria are capable of producing secondary metabolites which potent biotechnological implication and chemotactic-like influence on endophytic fungal species colonization. The endophytic fungi were to be either negatively or positively and strongly influenced, or may have contributed more to concentrations of alkaloids, saponins, flavonoids, deoxy-sugars, cardiac glycosides and steroid in the mangrove plants and plant parts investigated. Our findings have shown that the dominant endophytes, the aspergilli and species of *Epicoccum* sp., *Sclerotium* sp., *Rhizopus oryzae*, *Geotrichum* sp *Saccharomyces estuari*, and *Nigrospora* sp. were negatively influence by the presence of tannins, flavonoids, deoxy-sugar and cardiac glycosides even though they exhibited positive affinity to saponins, alkaloids, and steroids. Analysis of the species- antioxidants specificity has shown that none of the frequently isolated species but *Absidia* sp had strong relationship with deoxy-sugars but all of them including *Aspergillus niger*, *Aspergillus terreus* and *Absidia* sp demonstrated a strong and significant relationship with alkaloids, saponins and steroids. These compounds which are precursor of antioxidants with promising biotechnological applications may have been the major determinants of endophytic fungal association with deltaic mangroves in Nigeria.

Authors' Contributions

The authors hereby acknowledge the Tertiary Education Trust Fund (TETFund) through the Institution Based Research (IBR) Intervention of 2023.

References

- Agostini-Costa, T. D. S., Wondracek, D. C., Rocha, W. D. S., & Silva, D. B. D. (2012). Carotenoids profile and total polyphenols in fruits of *Pereskia aculeata* Miller. *Revista Brasileira de Fruticultura*, 34, 234-238.
- Al-Azizi, M. M., Al-Said, M. S., El-Olemy, M. M., Sattar, E. A., & Khalifa, A. S. (1994). Rhombifoline and 5, 6-dehydrolupanine from *Anagyris foetida* L. *Archives of Pharmacal Research*, 17, 393-397.
- AOAC. (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (Vol. II, 15th ed., Sec. 985.29). Arlington, VA: The Association.
- Arnold, A. E. (2008). Endophytic fungi: Hidden components of tropical community ecology. In *Tropical forest community ecology* (pp. 178-188).
- Bos, K. D., & Jetten, J. (1989). Determination of tannins in faba beans. In J. Huisman, A. F. B. van der Poel, & I. E. Liener (Eds.), *Recent advances of research in antinutritional factors in legume seeds* (pp. 168-171). PUDOC, Wageningen, The Netherlands.

- Brunner, F. K. (1984). *Geodetic refraction*. Berlin: Springer.
- Burrage, S. G., & Jeon, J. (2021). Applications of endophytic microbes in agriculture, biotechnology, medicine, and beyond. *Microbiological Research*, 245, Article 126691.
- Cabral, D., Stone, J. K., & Carroll, G. C. (1993). The internal mycobiota of *Juncus* spp.: Microscopic and cultural observations of infection patterns. *Mycological Research*, 97(3), 367-376.
- Chen, S., Cai, R., Liu, Z., Cui, H., & She, Z. (2022). Secondary metabolites from mangrove-associated fungi: Source, chemistry and bioactivities. *Natural Product Reports*, 39(3), 560-595.
- Deepthi, R., Ravindranath, S., & Raj, K. G. (2018). Extraction of urban footprint of Bengaluru city using microwave remote sensing. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences*, 42, 735-740.
- Dugan, H. A., Bartlett, S. L., Burke, S. M., Doubek, J. P., Krivak-Tetley, F. E., Skaff, N. K., ... & Weathers, K. C. (2017). Salting our freshwater lakes. *Proceedings of the National Academy of Sciences*, 114(17), 4453-4458.
- Edu, E. A. B. and Edwin-Wosu, N. I. (2015). Evaluation of bioactive compounds in mangroves: A panacea towards exploiting and optimizing mangrove resources. *Journal of Natural Sciences Research*, 5 (23): 1 – 9.
- Gadkar, V., David-Schwartz, R., Kunik, T., & Kapulnik, Y. (2001). Arbuscular mycorrhizal fungal colonization: Factors involved in host recognition. *Plant Physiology*, 127(4), 1493-1499.
- Gostinčar, C., & Gunde-Cimerman, N. (2023). Understanding fungi in glacial and hypersaline environments. *Annual Review of Microbiology*, 77(1), 89-109.
- Hammer, Ø., & Harper, D. A. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1.
- Hamzah, H., Pratiwi, S. U. T., & Hertiani, T. (2018). Efficacy of thymol and eugenol against polymicrobial biofilm. *Indonesian Journal of Pharmacy*, 29(4), 214-221.
- Hamzah, T. N. T., Ozturk, M., Altay, V., & Hakeem, K. R. (2020). Insights into the bioactive compounds of endophytic fungi in mangroves. In *Biodiversity and biomedicine* (pp. 277-292). Academic Press.
- Harborne, J. B., & Grayer, R. J. (1988). The anthocyanins. In *The flavonoids: Advances in research since 1980* (pp. 1-20). Boston, MA: Springer US.
- Ingle, A. P., Philippini, R. R., Martiniano, S., Marcelino, P. R. F., Gupta, I., Prasad, S., & da Silva, S. S. (2020). Bioresources and their Significance: Prospects and obstacles. In *Current Developments in Biotechnology and Bioengineering* (pp. 3-40). Elsevier.
- Jamwal, V. L., & Gandhi, S. G. (2019). Endophytes as a source of high-value phytochemicals: Present scenario and future outlook. In S. Jha (Ed.), *Endophytes and secondary metabolites* (pp. 1-20). Springer, Cham.
- Kumaresan, V., & Suryanarayanan, T. S. (2002). Endophyte assemblage in young, mature and senescent leaves of *Rhizophora apiculata*: Evidence for the role of endophytes in mangrove litter degradation. *Fungal Diversity*, 9, 81-91.
- Mohiuddin, A. K. (2019). Chemistry of secondary metabolites. *Ann Clin Toxicol*. 2019; 2 (1), 1014.
- Mousavi, S. S., & Karami, A. (2022). Application of endophyte microbes for production of secondary metabolites. In *Application of microbes in environmental and microbial biotechnology* (pp. 1-37).

- Nurunnabi, T. R., Sabrin, F., Sharif, D. I., Nahar, L., Sohrab, M. H., Sarker, S. D., ... & Billah, M. M. (2020). Antimicrobial activity of endophytic fungi isolated from the mangrove plant *Sonneratia apetala* (Buch.-Ham) from the Sundarbans mangrove forest. *Advances in Traditional Medicine*, 20, 419-425.
- Obadoni, B. O., & Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8(2), 203-208.
- Olayinka, B. U., Abdulkareem, A. K., Lawal, A. R., Akinwunmi, M. A., & Etejere, E. O. (2017). *Ficus ingens* (Miq.) Miq.(Moraceae): phytochemical and proximate composition. *Annales of West University of Timisoara. Series of Biology*, 20(2), 153-158.
- Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (Vol. 519, p. 388). New York: Springer.
- Saddhe, A. A., Jamdade, R. A., & Kumar, K. (2016). Assessment of mangroves from Goa, west coast India using DNA barcode. *SpringerPlus*, 5(1), 1554.
- Samson, R. A., Hockstra, E. S., & Van Orschot, A. N. (1984). *Introduction into foodborne fungi*. The Netherlands Academy of Arts and Science.
- Sopalun, K., Laosripaiboon, W., Wachirachaikarn, A., & Iamtham, S. (2021). Biological potential and chemical composition of bioactive compounds from endophytic fungi associated with Thai mangrove plants. *South African Journal of Botany*, 141, 66-76.
- Sztaricskai, F., & Pelyvás-Ferenczik, I. (2020). Chemistry of carbohydrate components. In *Glycopeptide Antibiotics* (pp. 105-194). CRC Press.
- Tan, R. X., & Zou, W. X. (2001). Endophytes: A rich source of functional metabolites. *Natural Product Reports*, 18(4), 448-459.
- Trease, G. E., & Evans, W. C. (1989). *Pharmacognosy* (13th ed.). ELBS/Bailliere Tindall.
- Tsipinana, S., Husseiny, S., Alayande, K. A., Raslan, M., Amoo, S., & Adeleke, R. (2023). Contribution of endophytes towards improving plant bioactive metabolites: A rescue option against red-taping of medicinal plants. *Frontiers in Plant Science*, 14, Article 1248319.
- Udoukpo, F.C., Bassey, I.N., Yaro, C.A., Fatunla, O.K. & Essien, J. P. (2024). Diversity and Host Structure Affinity of Endophytic Fungi Associated with Deltaic Mangroves in Iko River Estuary, Nigeria. *Recent Advances in Natural Sciences*, In Press.
- Wacira, T. N., Makonde, H. M., Bosire, C. M., & Kibiti, C. M. (2024). Molecular characterization and antibacterial potential of endophytic fungal isolates from selected mangroves along the coastline of Kenya. *Frontiers in Plant Science*.
- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., ... & Kogel, K. H. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences*, 102(38), 13386-13391.
- Xu, J. (2015). Bioactive natural products derived from mangrove-associated microbes. *RSC Advances*, 5(2), 841-892.
- Zihad, S. N. K., Hasan, M. T., Sultana, M. S., Nath, S., Nahar, L., Rashid, M. A., ... & Shilpi, J. A. (2022). Isolation and characterization of antibacterial compounds from *Aspergillus fumigatus*: An endophytic fungus from

a mangrove plant of the Sundarbans. *Evidence-Based Complementary and Alternative Medicine*, 2022(1), Article 9600079.