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Biodiversity, bioactivity, and chemical profile of endophytic fungi isolated from cashew (*Anacardium occidentale*)

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Abstract. *Novianti D, Elfita, Widjajanti H, Salni, Eliza, Oktiansyah R. 2025. Biodiversity, bioactivity, and chemical profile of endophytic fungi isolated from cashew* (Anacardium occidentale). *Biodiversitas 26: 2959-2969. Anacardium occidentale*, called cashew, is used extensively in agriculture and medicine. Traditional medicine has extensively used the plant's root barks, leaves, fruits, and seeds to cure various illnesses, including gastrointestinal issues, infections, and inflammation. Numerous reports have identified secondary metabolites that contribute to the biological activity of *A. occidentale* plants. The symbiotic endophytic fungi that inhabit the plant parts of *A. occidentale* may also generate bioactive substances that could be used as a source of medicine. This study examines the endophytic fungi associated with *A. occidentale* root barks, fruits, and seeds and their chemical profiles and bioactivity. Fresh tissue from root barks, fruits, and seeds was used to isolate endophytic fungi and identify them morphologically. The disk diffusion method was used to evaluate endophytic fungal extracts for antibacterial activity, while the DPPH method was used to test for antioxidant activity. Each endophytic fungal isolate's antibacterial and antioxidant activity was compared to the activity of the host plant. Endophytic fungal extracts with the same morphological characteristics in each plant part were subjected to GC-MS analysis to determine their chemical profiles. Sixteen endophytic fungal isolates were successfully isolated, including 8 isolates from root barks (RM1-RM8), 2 isolates from fruits (FM1-FM2), and 4 isolates from seeds (SM1-SM4). It shows that the highest number of endophytic fungi was found in the root bark, followed by the seed and fruit. The bioactivity of endophytic fungi in each organ is the same as that of its plant part. The results of morphological identification show that *Aspergillus niger* was found in all plant parts, namely in the root bark (RM7), fruit (FM1), and seeds (SM4). The antibacterial and antioxidant activities of *A. niger* isolates from the three different plant parts were varied. The results of the GC-MS analysis show that the endophytic fungal extract of *A. niger* in each plant part also has varying chemical profiles. The findings of this study can be used as information for drug development.

Keywords: *Anacardium occidentale*, antibacterial, antioxidant, chemical profile, endophytic fungi

INTRODUCTION

Endophytic fungi, living within plant tissues without harming the host, are of great interest for their potential to produce bioactive compounds with medicinal properties, including antimicrobial, anticancer, antioxidant, and anti-inflammatory effects (Renteria et al. 2022; Tripathi et al. 2022). As plants are natural sources of beneficial compounds, studying their endophytic fungi provides valuable insights for drug discovery and sustainable product development. This paper explores the role of endophytic fungi from *Anacardium occidentale* L., focusing on their biodiversity, bioactivity, and chemical profile.

Infectious diseases are caused by microorganisms, such as bacteria, viruses, fungi, or parasites, that infect the body. Symptoms can vary from fever and fatigue to specific symptoms, depending on the type of pathogen and the part of the body infected (Ristori et al. 2024; Shukla et al. 2024; Soni et al. 2024). Preventing infectious diseases involves vaccination, maintaining good hygiene, and using antibiotics or antivirals. Advances in medical technology play a significant role in diagnosing and treating infections, allowing for faster identification of pathogens and more effective therapy (Laupèze et al. 2021). In the treatment of infections, antibiotic resistance remains a major issue, so it is important to use drugs carefully, and research is needed for the development of new remedies (Nwobodo et al. 2022; Muteeb et al. 2023; Oliveira et al. 2024). Therefore, searching for natural ingredients with antioxidant and antibacterial properties is necessary.

Antioxidants and antibacterials protect cells from oxidative stress, preventing aging and chronic illnesses (Makarewicz et al. 2021; Mucha et al. 2021; Komariah et al. 2023; Tapias et al. 2023; Wijesekara and Xu 2023; Asiminicesei et al. 2024). Fruits, vegetables, and whole grains are natural sources of antioxidants containing flavonoids, vitamin C, and vitamin E (Zdrojewicz et al. 2018; Al-Ishaq et al. 2019; Pasini et al. 2021; Rudiana et al.

2021; Petrucci et al. 2022; Platzer et al. 2022; Pruteanu et al. 2023). Conversely, antibacterials are compounds that eradicate germs and infections and hasten the healing of wounds (Cock and Cheesman 2019; Almanaa et al. 2022; Nguyen and Bhattacharya 2022; Kaczmarek-Szczepańska et al. 2023). Antibacterials could be antibiotics, as well as natural ingredients, such as honey, garlic, and essential oils (Saini and Keum 2018; Dougoud et al. 2019; Kurek-Górecka et al. 2020; Khalil et al. 2021; Tomas et al. 2022; Elbestawy et al. 2023). The presence of antioxidants and antibacterials provides double protection, improves the immune system, and maintains overall health (Girirajan et al. 2011; Ebrahimi et al. 2023; Falakdin et al. 2023; Song et al. 2023). One plant with antioxidant and antibacterial activity is cashew (*A. occidentale*).

Traditional uses of *A. occidentale* are very diverse, particularly in native and cultivated areas. Its root barks treat hypertension and malaria, while its leaves heal wounds and infections (de Sousa Leite et al. 2016; Salehi et al. 2019; Gutiérrez-Paz et al. 2024). Cashew apple has anti-inflammatory, antimicrobial properties; cashew nuts contain antioxidants, polyphenols, and beneficial compounds (Schultz et al. 2018; dos Santos et al. 2020; da Silva et al. 2024). However, excessive harvesting disrupts ecosystems. Sustainable production and conservation, including endophytic fungi, are essential to maintaining ecological balance while utilizing their medicinal properties (Chaachouay and Zidane 2024; Ekor 2014).

Endophytic fungi reside in plant tissues without harming their hosts, benefiting plants by enhancing growth, stress resilience, and disease resistance (García-Latorre et al. 2023; Shen et al. 2024; Vimal et al. 2024; Elfita et al. 2024). The most significant potential of endophytic fungi lies in producing bioactive compounds with antimicrobial, anticancer, antioxidant, and anti-inflammatory properties, making them valuable for drug discovery (Adeleke and Babalola 2021; Singh et al. 2021; Renteria et al. 2022; Tripathi et al. 2022; Singh and Kumar 2023; Vimal et al. 2024). In addition to its bioactivity, most studies also found that in one plant part, there are many types of endophytic fungi. It indicates that the biodiversity of endophytic fungi in one part of the plant, such as leaves, stems, or roots, is very high (Oktiansyah et al. 2018; Elfita et al. 2023; Oktiansyah et al. 2024; Shen et al. 2024). This diversity aids ecosystem health and enables unique therapeutic discoveries. Additionally, utilizing endophytic fungi for drug development supports plant conservation by reducing the direct exploitation of rare species, promoting sustainability and ecosystem balance.

MATERIALS AND METHODS

Sample preparation and isolation of endophytic fungi

Fresh *A. occidentale* samples were collected from the Ilir Barat I area, South Sumatra, Indonesia (Lat -2.991734° Long 104.706552°). The samples were then identified at Generasi Biologi Indonesia (08.115/Genbinesia/IX/2023). The parts used were the root barks, fruits, and seeds of *A. occidentale*. The samples were washed under running water until clean. The samples were surface sterilized in Laminar Air Flow by soaking them in 70% alcohol and then rinsing for about a minute in sterile distilled water. After soaking in sodium hypochlorite (NaOCl) for approximately 30 seconds, the samples were washed once more with 70% alcohol and sterile distilled water. The sterile samples were cut aseptically. The samples were cultured on PDA in a petri dish at room temperature for three to seven days. Observations were made every day until the fungus appeared to grow. The colony was purified by re-culturing on a fresh PDA medium and incubating at room temperature for 48 hours. Purified fungal colonies were transferred to culture media to observe macroscopic and microscopic characters (Oktiansyah et al. 2023).

Cultivation and extraction of endophytic fungi

Pure colonies of endophytic fungi isolated from root barks, fruits, and seeds grown on Potato Dextrose Agar (PDA) were cut into plugs measuring 5 mm in diameter. The agar plugs were aseptically inoculated into fifteen culture bottles containing 300 mL of Potato Dextrose Broth (PDB). The culture was kept in a static environment at room temperature for 30 days. After incubation, the filter paper separated the mycelia from the media. The medium (1:1) was macerated with ethyl acetate. The ethyl acetate extract was evaporated using a rotary evaporator and concentrated using an oven at 45°C. The concentrated extract was then weighed (Hapida et al. 2021).

Characterization and identification of endophytic fungi

Endophytic fungal colonies were characterized at 3-7 days, including color, texture (floury, cotton, granular, slimy), radial lines, concentric circles, and exudate droplets. Microscopic characteristics were carried out by making microscope preparations using Henrici's slide culture method. The spores' form and the existence or absence of septa in the hyphae were among the microscopic observations (Walsh et al. 2018). Identification was done by comparing the macroscopic and microscopic features with the literature (Watanabe 2010; Walsh et al. 2018).

Antioxidant activity test

Antioxidant activity is measured using the DPPH technique. Methanol was used as a solvent to dissolve endophytic fungal extract at 1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL. The analysis was carried out in triplicate. 0.2 mL of extract in each concentration was added to 3.8 mL of 0.5 mM DPPH solution. After homogenizing, it was placed in a dark tube and left for half an hour. The absorbance was measured using a UV Vis spectrophotometer at λmax 517 nm (Oktiansyah et al. 2023). Ascorbic acid was employed as an antioxidant standard in this test. Antioxidant activity was determined using the IC_{50} value and the percentage of inhibition of DPPH absorption (Abbas et al. 2021).

$$\%\ \text{Inhibition} = \frac{A_k - A_s}{A_s}$$

Where:
A_k : Absorbance of control
A_s : Absorbance of samples

Antibacterial activity test

The antibacterial activity test was conducted using the Kirby-Bauer disk diffusion method. The test bacteria included *Salmonella typhi* (IPCCCB.11.669), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), and *Escherichia coli* (ATCC 25922). Endophytic fungal extracts were applied at a concentration of 400 µg/disk. Negative control disks contained 10 µL of DMSO, while positive control disks were treated with 30 µg of tetracycline. Paper disks were placed on Petri dishes inoculated with the test bacteria, followed by incubation at 37°C for 24 h. The inhibition zones were measured in diameter using a caliper. The inhibition zone diameter was evaluated based on established criteria (Hapida et al. 2021):

Weak : $\frac{A}{B}\times100\%<50\%$; Medium: $50\%<\frac{A}{B}\times100\%<70\%$;
Strong : $\frac{A}{B}\times100\%>70\%$

Where :
A : Sample inhibition zone (mm)
B : Antibiotic inhibition zone (mm)

Chemical profile analysis of identical endophytic fungal extracts in different organs

Using GC-MS, morphologically identical endophytic fungi isolated from root bark organs, fruits, and seeds were analyzed for their chemical profiles. Chemical profile identification techniques for the three identical endophytic fungal extracts by comparing retention time, % area, tentative identification of compounds based on the library's database, and similarity index.

RESULTS AND DISCUSSION

Isolation and identification of endophytic fungi associated with the root bark of cashew (*Anacardium occidentale*)

Eight isolates (RM1 to RM8) of endophytic fungi were successfully isolated from the root barks of cashews. The colony of eight endophytic fungal isolates displayed a variety of macroscopic traits (such as color and shape) and unique microscopic traits (Figure 1). Endophytic fungal colonies associated with cashew root bark samples were primarily white, gray, black, and yellow. Tables 1 and 2 show the macroscopic and microscopic characteristics of fungal endophytes from root bark.

The colony of each endophytic fungus from cashew root barks is described morphologically in Tables 1 and 2. There are 8 genera of endophytic fungus, including *Aspergillus* (3 isolates: RM1, RM6, RM8), *Trichoderma* (1 isolate: RM5), *Fusarium* (1 isolate: RM4), *Sirococcus* (1 isolate: RM3), *Phomopsis* (1 isolate: RM2), and *Lasiodiplodia* (1 isolate: RM7). Eight isolates of endophytic fungi from cashew root barks were identified based on their macroscopic and microscopic morphological characters.

Isolation and identification of endophytic fungi from cashew (*Anacardium occidentale*) fruit

There were 2 isolates were isolated from cashew fruit (FM1 to FM2). The colonies of the 2 endophytic fungal isolates displayed a variety of macroscopic traits (color and shape) and unique microscopic traits (Figure 2). The endophytic fungi from cashew fruit were primarily green and black. Tables 3 and 4 present the macroscopic and microscopic characters of endophytic fungal isolates from cashew fruit.

The physical features of the endophytic fungal colonies on cashew fruit in each isolate are presented in Tables 3 and 4. There are 2 genera of endophytic fungi isolated from cashew fruit, namely *Aspergillus* (1 isolate: FM1) and *Trichoderma* (1 isolate: FM2). Two endophytic fungal isolates from cashew fruit were identified based on their macroscopic and microscopic morphological characters.

Isolation and identification of endophytic fungi from cashew (*Anacardium occidentale*) seeds

Four isolates (SM1 through SM4) were isolated from cashew seeds. The four endophytic fungal isolates have unique microscopic and various macroscopic characteristics (color and form) (Figure 3). Black, gray, and white were the colors of the colonies that were isolated from the cashew seed samples. The macroscopic and microscopic characteristics of fungal endophytes from cashew seeds are presented in Tables 5 and 6.

The morphological characters of the endophytic fungal colonies from cashew seeds are in Tables 5 and 6. *Aspergillus* (1 isolate: SM4), *Collectrotrichum* (1 isolate: SM1), *Penicillium* (1 isolate: SM2), and *Nigrospora* (1 isolate: SM3) are the four genera of endophytic fungi that are present in cashew seeds. Four endophytic fungal isolates from cashew seed were identified based on their macroscopic and microscopic morphological characteristics.

Biodiversity of endophytic fungi isolated from *Anacardium occidentale*

The plant parts used for endophytic fungal isolation were the root barks, fruits, and seeds of *A. occidentale*. Nine genera were obtained from 16 fungal isolates. Table 7 displays the endophytic fungal genera.

Analysis of fungal diversity in different parts of *A. occidentale* showed distinct fungal colonization patterns (Table 7). A total of 16 isolates from 9 genera were identified, with *Aspergillus* as the most dominant genus, present in all parts (root barks, fruits, and seeds), while other genera only appeared in specific tissues. The differences highlight the tissue-specific association of endophytes, suggesting that different plant tissues influence the colonization of fungal species. Further diversity indices support this observation, with the Simpson Index of diversity (1-D) showing that root barks (0.7813) had the highest fungal diversity, followed by seeds (0.75) and fruits (0.5). Shannon Diversity Index (H') showed the highest diversity in root barks (1.667) and the lowest in fruits (0.6931), confirming that root barks provide a more complex habitat for fungal colonization.

Table 1. Macroscopic characteristics of endophytic fungi colonies from the cashew (*Anacardium occidentale*) root bark

Code	Surface colony	Reverse colony	Structure	Elevation	Pattern	Exudate drops	Radial line	Concentric circle
RM1	Black	White	Powder	Umbonate	Spread	-	-	-
RM2	White	White	Velvety	Umbonate	Plat	-	√	√
RM3	White	White	Cottony	Verrucose	Plat	-	-	√
RM4	Grey	Grey to dark	Cottony	Umbonate	Zonate	-	-	√
RM5	White	White to brown	Cottony	Rugose	Flowery	-	√	√
RM6	Black	White	Powder	Umbonate	Spread	-	-	-
RM7	White	White	Cottony	Rugose	Zonate	-	√	-
RM8	White to yellow	White	Powder	Umbonate	Spread	-	-	-

Table 2. Microscopic characteristics of endophytic fungi colonies from the cashew (*Anacardium occidentale*) root bark

Isolate	Spore	Shape	Hyphae	Characteristic	Species of identification
RM1	Conidia	Subglobose	Septate	Phialides are biseriate, radiating around the entire vesicle, and have metulae twice as long as phialides.	*Aspergillus niger*
RM2	Conidia	Globose	Septate	These hyphae form pycnidia structures.	*Phomopsis* sp.
RM3	Conidia	Fusiform	Septate	These conidiophores are usually unbranched or sparsely branched, short to medium-sized, and hyaline	*Sirococcus piceicola*
RM4	RM3	Conidia	Septate	Phialides are usually bottle-shaped with a wide base and narrow tip.	*Fusarium* sp.
RM5	Sporangia	Subglobose	Septate	In the early stages of growth, the hyphae are white and fluffy.	*Trichoderma* sp.
RM6	Conidia	Subglobose	Septate	Phialides, which are biseriate, radiate around the entire vesicle.	*Aspergillus* sp.
RM7	Conidia	Subglubose	Septate	Hyaline, simple, globosely inflated conidiophores.	*Lasiodiplodia theobromae*
RM8	Conidia	Subglubose	Septate	Phialides radiate around the entire vesicle.	*Aspergillus* sp.

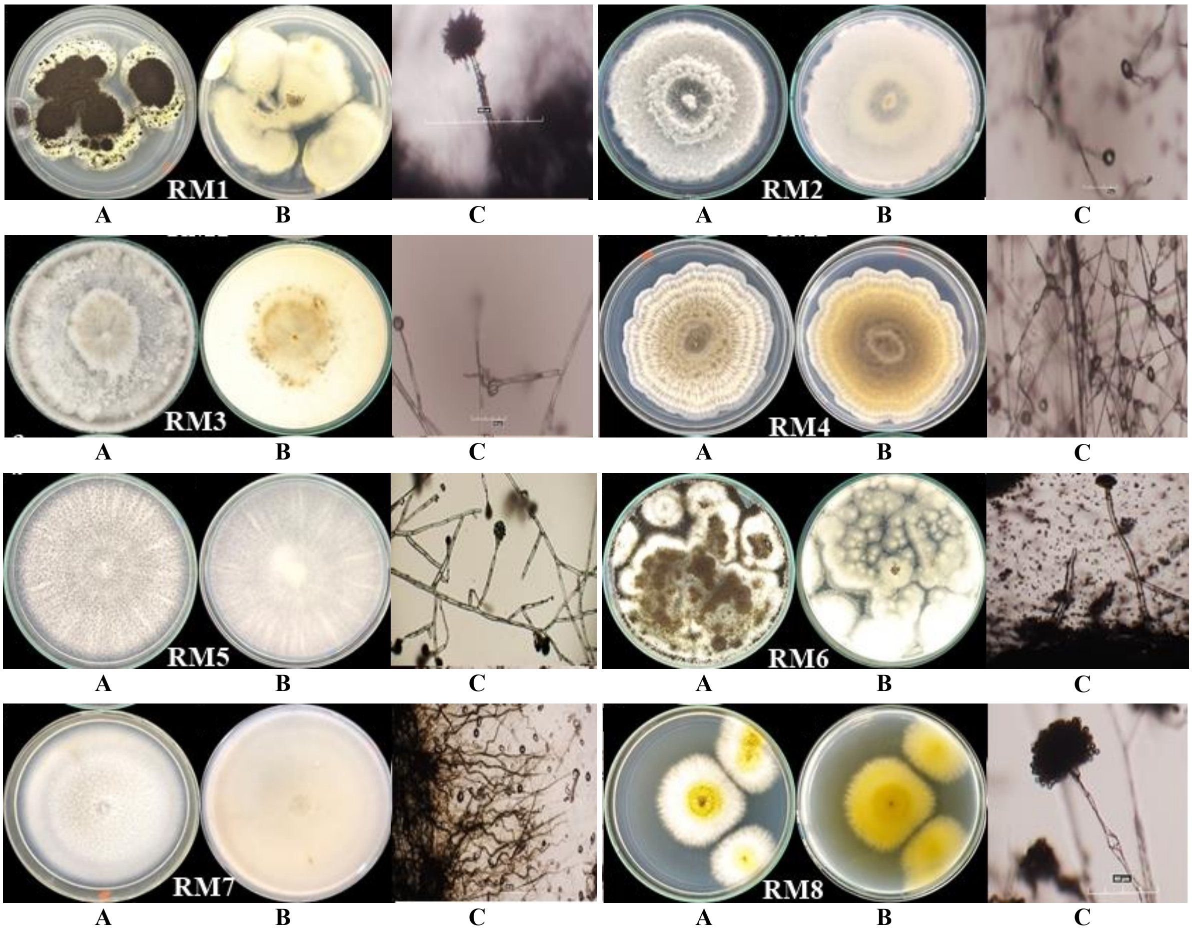

Figure 1. Characteristics of endophytic fungi colonies from cashew root barks. A. Front view, B. Reverse view, C. Microscopic

Table 3. Characteristics of endophytic fungi colonies from cashew fruit

Code	Surface colony	Reverse colony	Structure	Elevation	Pattern	Exudate drops	Radial line	Concentric circle
FM1	Black	White	Powder	Umbonate	Spread	-	-	-
FM2	Green	Green	Powder	Umbonate	Spread	-	-	-

Table 4. Microscopic characteristics of endophytic fungi from cashew fruit

Isolate	Spore	Shape	Hyphae	Characteristic	Species of identification
FM1	Conidia	Subglobose	Septate	With metulae twice as long as phialides, phialides are biseriate and radiate around the entire vesicle	*Aspergillus niger*
FM2	Sporangia	Subglobose	Septate	The hyphae are only beginning to grow	*Trichoderma* sp.

Table 5. Characteristics of endophytic fungi colonies isolated from cashew seeds

Code	Surface colony	Reverse colony	Structure	Elevation	Pattern	Exudate drops	Radial line	Concentric circle
SM1	White to grey	White to grey	Cottony	Umbonate	Zonate	-	-	-
SM2	Grey	Grey	Cottony	Umbonate	Spread	-	-	-
SM3	Grey	White	Cottony	Umbonate	Zonate	-	-	√
SM4	Black	Black	Velvety	Umbonate	Spread	-	-	-

Table 6. Microscopic characteristics of endophytic fungi from cashew seed

Isolate	Spore	Shape	Hyphae	Characteristic	Species of identification
SM1	Conidia	Subglubose	Septate	Conidiophores hyaline, branched, phialides short	*Colletotrichum* sp.
SM2	Conidia	Subglubose	Septate	Erect Conidiophores, slightly rough	*Penicillium* sp.
SM3	Conidia	Glubose	Septate	Simple Conidiospores, hyaline, bearing single conidia apically	*Nigrospora* sp.
SM4	Conidia	Subglobose	Septate	With metulae twice as long as phialides, phialides are biseriate and radiate around the entire vesicle	*Aspergillus niger*

Table 7. Diversity of endophytic fungi isolated from root bark, fruit, and seed of *Anacardium occidentale*

Genera	Part of *A. occidentale*			Total
	Root bark	Fruit	Seed	
Aspergillus	3	1	1	5
Colletotrichum	0	0	1	1
Fusarium	1	0	0	1
Lasiodiplodia	1	0	0	1
Nigrospora	0	0	1	2
Penicillium	0	0	1	1
Phomopsis	1	0	0	1
Sirococcus	1	0	0	2
Trichoderma	1	1	0	2
Simpson Index (D)	0.2188	0.5	0.25	-
Simpson Index of diversity (1-D)	0.7813	0.5	0.75	-
Shannon Index of diversity (H')	1.667	0.6931	1.386	-

Antibacterial and antioxidant activities of endophytic fungal extracts

Endophytic fungal extracts isolated from the root barks, fruits, and seeds of *A. occidentale* were analyzed for their antibacterial and antioxidant properties (Table 8). The extracts showed different antibacterial and antioxidant activities.

The antibacterial and antioxidant properties of the methanol extract of cashew plant parts and its endophytic fungal extracts are shown in Table 8. The extracts of RM2, RM3, RM4, RM5, RM7, FM2, SM1, SM2, and SM4 showed potent antibacterial activity. RM3, RM4, RM7, RM8, SM1, SM2, and SM4 extracts were included in the strong category of antioxidant activity (<100 μg/mL). Based on these data, the extract of RM7 showed the most potential antibacterial and antioxidant activities compared to other endophytic fungal isolate extracts. However, compared to the methanol extract of the host plant, the extract of isolate RM7 had better antibacterial activity because it could inhibit the growth of the four test bacteria. The antioxidant activity of the host plant extract was better, and it was categorized as a very strong antioxidant. Therefore, the RM7 isolate extract is a promising source of new drug ingredients.

Table 8. Antibacterial and antioxidant activity of endophytic fungal extract from cashew (*Anacardium occidentale*)

Sample	**Methanol extract**	**Weight (g)**	**% Antibacterial activity**				**Antioxidant activity IC_{50} (µg/mL)**
			E. coli	***S. aureus***	***S. thypi***	***B. subtilis***	
Host plant	Root bark	1.2	65,9±0,49**	68,3±0,64**	66,7±0,36**	65,2±1,89**	15,619****
	Fruit	1.2	60,4±0,54**	60,9± 0,37**	59,6±0,14**	60,1±0,16**	18,074****
	Seed	0.9	61,9±0,52**	63,2±0,64**	62,6±0,73**	61,6±0,89**	19,024****
Endophytic fungi	RM1	3.8	51,9±1,16**	51,7±0,36**	52,4± 0,60**	52,1±0,49**	106,168**
	RM2	1.2	83,4±0,60***	82,8±0,16***	80,3±0,37***	80,1±1,26***	117,545**
	RM3	1.4	80,3±0,18***	80,6±0,36***	82,2±0,60***	82,6±0,37***	55,017***
	RM4	1.0	71,6±0,37***	70,4±0,54***	70,8±0,16***	71,2±0,28***	62,862***
	RM5	0.8	82,8±0,90***	81,3±0,37***	78,9±0,54***	78,2±1,64***	509,806*
	RM6	3.6	65,3±1,18**	62,6± 0,51**	65,1± 1,79**	65,9±0,37**	517,693*
	RM7	1.2	82,9±0,60***	73,1±0,39***	75,7±0,37***	76,4±0,89***	35,446***
	RM8	1.1	67,4±0,79**	66,1±1,19**	63,8± 0,4**	69,1±0,16**	76,531***
	FM1	5.6	61,1±0,14**	61,7±0,60**	61,4± 0,37**	60,8±0,76**	506,183*
	FM2	0.9	70,5±0,54***	70,3±0,31***	71,1±0,54***	71,5±0,16***	487,275**
	SM1	1.9	74,1±0,16***	73,8±0,36***	76,3±0,48***	70,9±0,16***	65,166***
	SM2	1.5	70,6±0,18***	70,2±0,31***	71,6±0,60***	70,9±0,54***	70,089***
	SM3	3.6	68,4±0,44**	67,7±0,56**	75,4± 0,16**	751,1±0,48**	356,962**
	SM4	1.1	73,9±0,60***	72,8±0,37***	76,2±0,65***	76,1±0,89***	41,507***
Positive control			Tetracyclin 100***	Tetracyclin 100***	Tetracyclin 100***	Tetracyclin 100***	Ascorbic acid 10,083****

Note: endophytic fungi from root bark (RM1-RM8), endophytic fungi from fruit (FM1-FM2), endophytic fungi from seed (SM1-SM4). The percentage of antibacterial activity is: ***>70% (strong), **50-70% (moderate), and *<50% (weak); for The antioxidant's IC_{50} (mg/mL): ****strong<100 *µ*g/mL; **moderate 100-500 *µ*g/mL; * mild>500 *µ*g/mL; ****very strong<20 *µ*g/mL (Elfita et al. 2023)

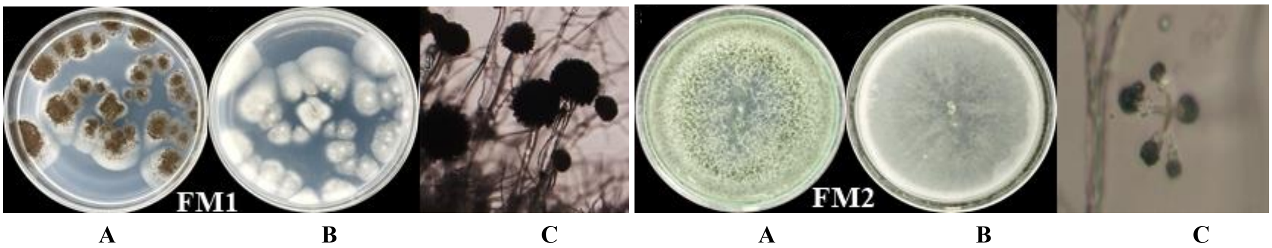

Figure 2. Characteristics of endophytic fungal colonies from cashew fruit. A. Front view, B. Reverse view, C. Microscopic

Chemical profile of endophytic fungus *Aspergillus niger* in root bark, fruit, and seed

The analysis and identification of the chemical content of endophytic fungal extract can be used as a guide for its use as a source of medicinal ingredients and industrial goods, including food, drink, and cosmetics. Moreover, investigating the chemical composition of endophytic fungi might facilitate the development of novel techniques for extracting and purifying compounds and enhance scientific comprehension of these fungi's molecular biology and chemistry.

Gas Chromatography-Mass Spectrometry, or GC-MS, is an analytical technique that detects and identifies chemical compounds in samples. This technique effectively separates compounds based on their volatility and molecular mass, providing a detailed chemical profile. GC-MS is capable of detecting major and minor compounds in complex samples. Three extracts of *A. niger* isolated from various parts of the cashew plant using GC-MS identified several bioactive compounds with potential antibacterial and antioxidant properties and their chemical composition. Morphological analysis showed that *A. niger* was present in three plant parts of cashew, namely root barks (RM1), fruits (FM1), and seeds (SM4).

Figure 4 shows variations in chemical composition. The major compounds of *A. niger* extract from root bark (RM1) were ethoxymethoxyacetic acid, ethyl ester (13.35%), 5-acetoxymethyl-2-furaldehyde (11.92%), and 2,3-butanediol, diacetate (11.62%), as well as trace amounts of methylene diamine, 1-(hydroxymethyl)-1,2-ethanediyl ester (2.54%), and hexadecanoic acid, N, N'-diacetyl- (2.71%). Fruit extract (FM1) was dominated by 5-hydroxymethylfurfural (12.18%) and 5-acetoxymethyl-2-furaldehyde (10.43%), with minor compounds such as ethyl palmitate (2.29%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (2.25%), Ethyl 2-butoxyacetate (2.23%), and 4-Nitrophenyl laurate (2.21%). Seed extract (SM4) contained major compounds such as phthalic acid, di(2-propylpentyl) ester (14.60%), β-sitosterol (11.60%), and n-hexadecanoic acid (8.61%). Minor compounds detected included campesterol (2.27%), tris(1-chloropropan-2-yl) phosphate (2.16%), and Octadecanoic acid (stearic acid) (2.07%). The analysis revealed that the extract of endophytic fungi, i.e., *A. niger,* contained a variety of bioactive compounds with different antioxidant and antibacterial properties.

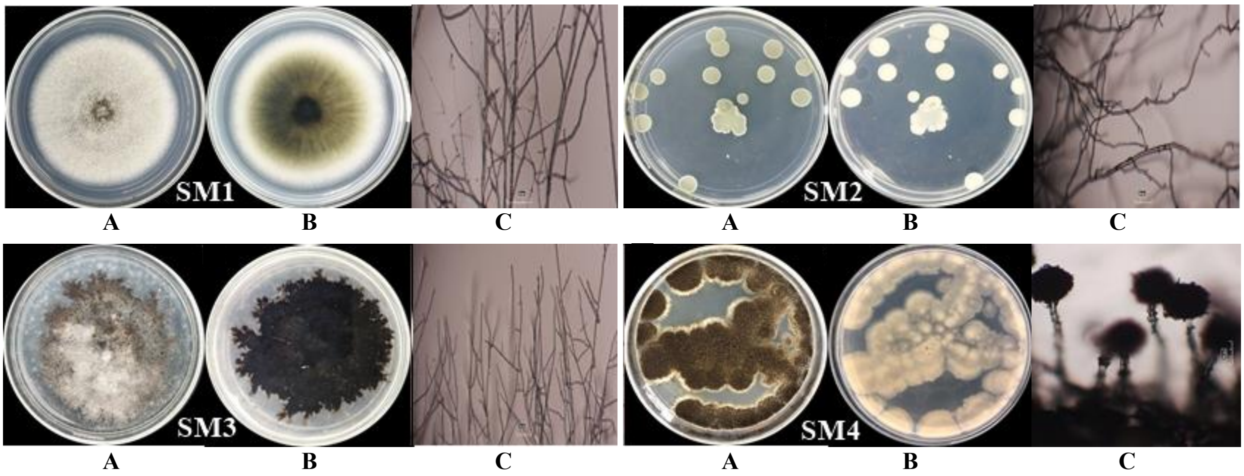

Figure 3. Characteristics of endophytic fungi colonies from cashew seeds. A. Front view, B. Reverse view, C. Microscopic

Discussion

The study's findings showed the biodiversity of endophytic fungi associated with different plant parts of cashew (*Anacardium occidentale*). *Aspergillus* is the most dominant genus in all parts (root barks, fruits, and seeds), while other genera only appear in specific tissues. Diversity Indices showed that the root barks had the highest fungal diversity (H'), and the fruits had the lowest. These values suggest that root barks offer a more supportive environment for diverse fungal communities, possibly due to their closer interaction with the soil and its nutrient-rich nature, compared to the relatively exposed and nutrient-poor root bark and fruit. The stems and fruits of the plant contain lower levels of nutrients relative to the roots, reflecting their reduced ability to assimilate nutrients compared to root tissues (Hashem et al. 2023; Vimal et al. 2024). Stems support and connect plant parts, while fruits focus on reproduction. Both have high air content and little organic matter that can be utilized by microorganisms. Roots have direct access to nutrient sources from the soil, supporting endophytic fungi growth. Endophytic fungal communities are well known to be strongly influenced by the type of plant tissue, with root barks often supporting higher diversity due to their direct exposure to various soil microbes (Hashem et al. 2023; Vimal et al. 2024). The high diversity of endophytic fungi from the root barks also aligns with the findings from the root bark of mangroves, in which root barks function as hotspots for microbial interactions (Sui et al. 2023). Nonetheless, the widespread distribution of *Aspergillus* throughout the plant may suggest that it is a fundamental component of the *A. occidentale* microbiome that can adapt to various plant tissues, which aligns with earlier findings. The low diversity in the fruit may be due to the seasonal nature of the fruit and the limited time for endophytic fungal colonization (Wang et al. 2022; Liu et al. 2024; Öncel and Özkılınç 2025).

The findings of this study demonstrated the potential of endophytic fungal extracts from cashew seeds, fruits, and root barks as a natural source of potent antioxidant and antibacterial chemicals. *Aspergillus niger* (Isolates RM7, FM1, and SM4), an endophytic fungus, is colonized all parts of the plant. The fungus *Aspergillus niger* is an opportunistic pathogen that grows in a variety of environments. Its spores are easily dispersed through the air (aerosol) and might enter the human respiratory system by inhalation, leading to the development of allergies (Mousavi et al. 2016; Latgé and Chamilos 2020; Yu et al. 2021). However, despite being a disease, research shows that *A. niger* may spread quickly and associate with plants; its host specificity is unknown (de Souza Marques Mundim et al. 2022; Garrigues et al. 2022; Lahlali et al. 2022; Silva et al. 2022). It is well known that fungi isolated from medicinal plants exhibit strong bioactivity (Brazkova et al. 2022; Chugh et al. 2022; Rehman et al. 2022; Vaou et al. 2022). It suggests that the fungus *A. niger*, which is present in root barks, fruits, and seeds of *A. occidentale*, can produce secondary metabolites that are also present in its host plant. *A. niger* isolated from medicinal plants has been shown in numerous investigations and possessing antibacterial and antioxidant properties due to the compounds' structural resemblance to those of their host plants (Stan et al. 2021; Rahimi et al. 2022; Wei et al. 2022).

The methanol extract of *A. occidentale* (Table 3) demonstrated substantial antioxidant activity comparable to ascorbic acid and moderate antibacterial activity against the four test pathogens. The presence of this bioactivity is closely related to its chemical content. The root barks, fruits, and seeds of *A. occidentale* predominantly contain secondary metabolites from the polyphenol, tannin, and flavonoid classes. Several studies have also revealed that *A. occidentale* extract contains anacardic acid, cardanol, cardol, and 2-methyl cardol compounds (de Sousa Leite et al. 2016; Salehi et al. 2019; Gutiérrez-Paz et al. 2024). The content of polyphenols, flavonoids, and anacardic acid has been known to have antibacterial and antioxidant activity (Bouarab-Chibane et al. 2019; Makarewicz et al. 2021; Brazkova et al. 2022; Roy et al. 2024).

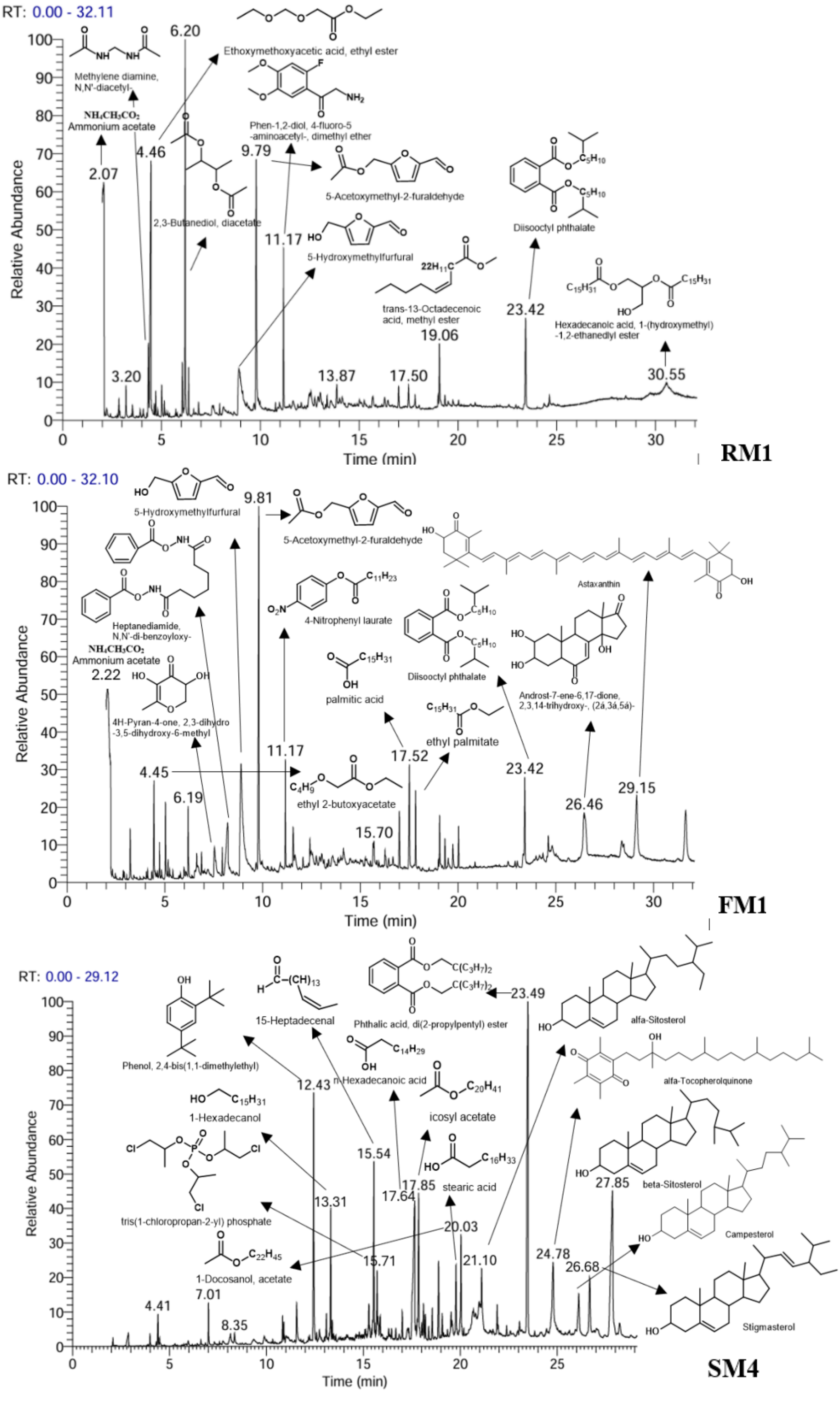

Figure 4. GC-MS chromatogram and identified chemical compounds of endophytic fungal extracts of *Aspergillus niger* from root bark (RM1), fruit (FM1), and seeds (SM4)

The antibacterial activity of endophytic fungal extract ranged in the moderate to strong categories, but its antioxidant activity was categorized into four categories, i.e., weak, moderate, strong, and very strong (Table 7). Isolates RM7, FM1, and SM4 had different antibacterial and antioxidant activities, although the three isolates were identified as *A. niger*. Isolate RM7 has better antibacterial activity (>70%) against the four tested bacteria and antioxidant activity (an IC_{50} value of 35.446 μg/mL) than FM1 and SM4 isolates.

GC-MS analysis revealed that there are similarities and differences in compound content. Several metabolites were present in more than one extract, such as 5-Hydroxymethylfurfural and 5-Acetoxymethyl-2-furaldehyde in root bark (RM1) and fruit (FM1) extracts. These compounds are carbohydrate degradation products often found in plant tissues (Jilani and Oslon 2023). In addition, Diisooctyl phthalate and Ammonium acetate were also found in both organs, which may have come from environmental sources or plant metabolic processes (Prasad et al. 2022). Meanwhile, palmitic acid (n-Hexadecanoic acid) was present in seed extract (SM4) and fruit extract (FM1). Palmitic acid is a common fatty acid in plants and plays a role in cell membrane formation and lipid metabolism (Carta et al. 2017). The differences in compound composition were present in each extract. Seed extract (SM4) contains phytosterols such as β-Sitosterol, α-Sitosterol, stigmasterol, and Campesterol, which are commonly found in plant storage tissues. Fruit extract (FM1) contains Astaxanthin and several furan derivative compounds, often appearing during fruit ripening. Root bark extract (RM1) contains Ethoxymethoxyacetic acid, ethyl ester, 2,3-butanediol, and diacetate, which play a role in interactions with the environment or microorganisms in the soil. Differences in chemical content occur due to the physiological function of plant parts. Seeds function to store food reserves and embryos for germination and plant development. Root barks absorb water and nutrients from the soil and store food reserves and growth hormones. Physiologically, fruit develops from the ovary after pollination and fertilization occur. Fruit is energy storage through sugars, organic acids, and other bioactive compounds essential for seed growth. Differences in the type of endophytic fungi from each part of the plant give each part a unique compound (Lopez and Barclay 2017).

The findings showed that endophytic fungal species in various plant parts may not always exhibit the same level of bioactivity. GC-MS analysis revealed that the extract of *A. niger* isolated from different plant parts have differences and similarities in compound content. Differences in chemical compounds in the *A. niger* extract resulted in differences in antibacterial and antioxidant activities.

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