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Phytochemical Screening and Antioxidant Activity of Endophytic Fungi Isolated from the Nuts of *Anacardium occidentale*

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Abstract

Background: *Anacardium occidentale* (cashew) is an economically important tropical plant valued for its edible nuts and numerous medicinal properties. Beyond the plant itself, its endophytic fungi have attracted increasing attention as potential sources of bioactive secondary metabolites with antioxidant and therapeutic applications. Despite the well-documented phytochemical and antioxidant properties of *Anacardium occidentale*, limited information is available on the phytochemical composition and antioxidant potential of its nut-associated endophytic fungi.
Aims: This study aimed to evaluate the phytochemical composition and antioxidant activity of endophytic fungi isolated from *Anacardium* occidentale (cashew) nuts.
Methods: Four endophytic fungi isolates, named EF1 to EF4, were grown in culture, and their raw extracts were tested for both qualitative and quantitative phytochemical analysis using standard methods. The antioxidant activity was checked using three methods: the DPPH radical scavenging test, finding the IC_{50} value, and measuring the total antioxidant capacity.
Result: All fungal isolates contained important bioactive phytochemicals and exhibited antioxidant activity. EF3 showed the highest levels of phenolics, tannins, and terpenoids, as well as the strongest antioxidant activity, with the lowest IC_{50} value (47.55 µg/mL) and the highest total antioxidant capacity (112.83 mg AAE/g extract) among the fungal isolates.
Conclusion: This research has identified novel fungal-derived natural antioxidants from *Anacardium occidentale* nuts with potential applications in the pharmaceutical, nutraceutical, and food industries.

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INTRODUCTION

Natural chemical substances play essential roles in the discovery and development of therapeutic agents because of their remarkable chemical diversity and broad spectrum of biological activities (Ezeokonkwo & Kabir, 2025; Forman & Zhang, 2021; Lin et al., 2025) In recent years, increasing concern over oxidative stress-related diseases, the rapid emergence of antibiotics opposing to antimicrobials, and the potential dire effects associated with extended use of synthetic antioxidants have intensified the search for fail-safe sustainable natural products (Chang et al., 2020; Kabir & Lawan, 2025; Machado et al., 2023). Consequently, microorganisms capable of producing pharmacologically valuable secondary metabolites have become important targets in natural product research. Despite the recognized medicinal importance of *Anacardium occidentale*, there is limited information on the phytochemical composition and antioxidant activity of endophytic fungi isolated from its nuts, highlighting the need for further investigation into their potential as natural sources of bioactive antioxidant compounds (Bastos et al., 2025; Novianti et al., 2025; Sruthi et al., 2023).

Because they form asymptomatic relationships with healthy plant tissues while producing a variety of structurally diverse secondary metabolites, such as essential oils, alkaloids, phenolic compounds, flavonoids, terpenoids, peptides, and polyketides with antioxidant, antimicrobial, anti-inflammatory, antiviral, anticancer, and other pharmacological activities, endophytic fungi have drawn a lot of attention among microorganisms (Hashem et al., 2023; Mishra et al., 2022; Singh & Kumar, 2023). The metabolic versatility of endophytic fungi is largely attributed to their intimate interaction with host plants, which enables them to produce unique biochemical substances that were lacking or present in lower amounts in the host itself. This characteristic has positioned endophytic fungi as promising alternatives to conventional plant-based sources of bioactive molecules (Mishra et al., 2022).

Anacardium occidentale L. (cashew) is a medicinal cultivated worldwide for its edible nuts and numerous industrial products. Beyond its commercial value, the plant has long been recognized in traditional medicine, where different anatomical parts are used for the management of infections, inflammatory disorders, diabetes, gastrointestinal diseases, and several other health conditions (Malik & Bhadauria, 2020; Shahrajabian & Sun, 2023). Scientific investigations have demonstrated that these therapeutic properties are closely associated with the abundance of phytochemicals naturally occurring within the plant. Nevertheless, while the phytochemistry and pharmacological activities of *A. occidentale* have been extensively documented, the endophytic fungal communities residing within its nuts remain largely unexplored despite their potential to synthesize structurally novel and biologically active metabolites (Liu et al., 2021; Sillu et al., 2025; Singh et al., 2021).

Oxidative stress represents one of the principal mechanisms underlying the progression of many chronic diseases (Danjuma & Lawan, 2025; Sharifi-Rad et al., 2020). When there is a lot of reactive oxygen species being released and the body's natural antioxidant protection is not enough, it leads to damage in proteins, lipids, and DNA.This damage can cause serious health issues such as cancer, heart diseases, diabetes, brain disorders, and problems that come with aging. (Jomova et al., 2023; Leyane et al., 2022). Although synthetic antioxidants are widely used to prevent oxidative deterioration, concerns about their long-term safety have increased interest in natural alternatives. Synthetic antioxidants such as BHA, BHT, TBHQ, and propyl gallate have been linked to potential toxic effects under certain conditions despite their regulated use (Lourenço et al., 2019; Rathee et al., 2023). Consequently, endophytic fungi are being explored as promising sources of safe and effective natural antioxidants for pharmaceutical, food, and nutraceutical applications. These endophytic fungi produce metabolites capable of neutralizing deadly radicals, chelating transition metals, and suppressing oxidative damage through multiple mechanisms(Kıran et al., 2023; Sharifi-Rad et al., 2020; Zahra et al., 2021).

Phytochemical characterization remains an essential step in evaluating the pharmaceutical potential of fungal extracts. Qualitative screening provides information on the classes of metabolites present, whereas quantitative analysis determines their abundance and facilitates correlations between chemical composition and biological activity (Jamal et al., 2022; Kılınc et al., 2023; Sobuj et al., 2023). Such investigations are particularly important for identifying promising fungal isolates that may serve as sustainable sources of bioactive compounds for pharmaceutical, nutraceutical, and biotechnological applications (Danjuma et al., 2025; Jamal et al., 2022).

Although *Anacardium occidentale* has been widely investigated for its phytochemical composition and antioxidant properties, previous researches predominantly focused on plant-derived extracts rather than the endophytic fungi associated with the plant (Novianti et al., 2025; Ojong et al., 2026). In particular, endophytic fungi inhabiting cashew nuts remain largely unexplored, with limited information available on their phytochemical constituents, antioxidant potential, and the relationship between their bioactive metabolites and antioxidant activity (Ponce-Mora et al., 2025). This study seeks to fill this gap by extracting endophytic fungi from A.occidentale nuts, checking their chemical makeup and antioxidant abilities, and offering new ideas about how these fungi could be used as eco-friendly sources of natural antioxidants for medicine, health products, and food.

Figure 1. *Anacardium occidentale* nuts

METHOD

Materials/Reagents

All the chemicals, solvents, microbiological media, and reagents used in this study were of high quality, analytical grade, and purchased from reliable, certified suppliers. Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and Malt Extract Agar (MEA) were used to isolate, grow, and keep the endophytic fungal isolates alive. Organic solvents like methanol were used during the extraction process. Qualitative phytochemical tests were performed using Dragendorff's, Mayer's, Wagner's, and ferric chloride reagents. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin–Ciocalteu reagent, and ascorbic acid as the reference antioxidant (Mishra et al., 2021).

Collection and Preparation of Plant Samples

Healthy, adult nuts from the Anacardium occidentale plant were gathered during the time when the fruits are ripe, from the area around the Federal Polytechnic Idah campus in Kogi State, Nigeria. The collected nuts were carefully moved into clean bags, properly marked, and then taken to the lab. The nuts were cleaned to remove any surface germs in a clean, germ-free environment.First, they were placed in 70% ethanol for one minute.Then, they were put into 2% sodium hypochlorite for three minutes. Finally, they were dipped again in 70% ethanol for 30 seconds. The nuts that had been sterilized were washed three times using sterile distilled water to get rid of any leftover sterilizing substance. To check how well the surface sterilization worked, 100 microliters of rinse water was added to Potato Dextrose Agar (PDA) plates and the plates were kept at a temperature of 28 ± 2°C for 5 to 7 days. There was no growth of microorganisms on the plates, which showed that the surface sterilization was effective.This is because the fungal samples came from inside the nuts, not from their outer surfaces. The nuts were cleaned and then cut into small pieces that were about 5 millimeters by 5 millimeters in size (Sivanesan et al., 2021).

Endophytes Isolation, Purification, and Identification

Endophytes (fungi) were isolated using the tissue-segment inoculation technique. After surface sterilization, small tissue fragments obtained from the cashew nuts were aseptically placed on Potato Dextrose Agar supplemented with an appropriate antibacterial agent to quench the blossoming of bacterial. The seeded plates were hatched at 25–28°C for 5–7 days and monitored daily for fungal emergence. Individual colonies that developed from the tissue segments were repeatedly subcultured onto fresh PDA plates until pure cultures produced (Taheri et al., 2022; Zheng et al., 2021).

Preliminary fungal identification was done using conventional morphological characteristics, including colony appearance, pigmentation, hyphal morphology, conidiophore architecture, and spore characteristics. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified and sequenced for molecular characterization of representative isolates in order to verify species identity. For taxonomy identification, the produced sequences were matched with reference sequences found in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (Novianti et al., 2025).

Extraction of Fungal Secondary Metabolites

Pure fungal isolates grown in the Potato Dextrose Broth under static culture conditions at 25–28°C for 14–21 days for production of secondary metabolites. After incubation the cultures were filtered to remove the mycelial biomass from the fermentation broth. The culture filtrate was extracted three times in succession with the 500 mL ethyl acetate with the aid of a separatory funnel while the mycelia were extracted with methanol as appropriate (Nowak et al., 2024; Saleem et al., 2026).

In order to prevent the thermal degradation of thermolabile metabolites, the organic extracts were extracted collectively and concentrated under decreased pressure using a rotating evaporator that maintained a temperature below 40°C. After that, the crude extracts were weighed, moved to sterile amber vials, and stored at 4°C for additional phytochemical screening and antioxidant activity assessment (Nowak et al., 2024; Saleem et al., 2026).

Qualitative Phytochemical Screening

The endophytic fungal isolates were roughly analysed for the presence of major phytochemicals by the conventional standard methods. Acidification of the extracts with 1% hydrochloric acid, filtration and separate treatment of filtrates with Dragendorff's reagent, Mayer's reagent and Wagner's reagent, revealed the presence of alkaloids. Orange-red, cream and reddish-brown precipitates formed suggested the presence of alkaloids. The Salkowski test was used to evaluate the presence of terpenoids which was characterized by the appearance of reddish-brown interface after the addition of concentrated sulphuric acid to the crude extract (Abdel-Rahman et al., 2019). The Shinoda test was used to identify flavonoids by adding magnesium turnings and concentrated hydrochloric acid to the extract, which produced pink, orange or red colors. Saponins were determined using frothing method which is defined by the persistence of froth after shaking the extract in distilled water vigorously, foam or froth was considered positive. Ferric chloride solution was used for determining both tannins and phenolic compounds; presence of tannins was detected by the formation of a blue-black color while that of phenolic compounds was seen as greenish-black, deep blue, green, or violet coloration was formed (Klangmanee & Athipornchai, 2019; Shaikh & Patil, 2020). For the detection of steroids, the extract was treated with chloroform, acetic anhydrous and concentrated sulphuric acid, and the appearance of bluish green/emerald green colour confirmed the presence of steroids (Shaikh & Patil, 2020). The presence of cardiac glycosides was confirmed by Keller–Killiani test, which involves the reaction of the extract with glacial acetic acid containing ferric chloride and then with concentrated sulphuric acid. Brown ring on the interface and bluish green layer at the upper part of the test tube indicate the presence of cardiac glycosides (Okuna et al., 2024).

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC)

The Folin–Ciocalteu colorimetric technique was used to determine the total phenolic content of each crude fungal extract. A diluted Folin-Ciocalteu reagent was blended with a little amount of each extract before being added to a sodium carbonate solution. After allowing the reaction mixtures to develop color under the proper circumstances, the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid equivalents (GAE) were calculated and displayed as milligrams of gallic acid equivalent per gram of extract (mg GAE per gram of extract) using gallic acid as the calibration standard (Bristy et al., 2022; Sobuj et al., 2023).

Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used to quantify flavonoids. The crude extracts were successively treated with potassium acetate and aluminum chloride while being incubated at room temperature in order to promote the formation of complexes. The flavonoid concentration was then ascertained by measuring the absorbance at 415 nm using a quercetin calibration curve. Milligrams of quercetin equivalents per gram of extract (mg QE/g extract) were used to express all of the results (Baliyan et al., 2022; Nicolescu et al., 2025).

Total Tannin Content (TTC)

The Folin-Denis spectrophotometric technique was used to calculate the total tannin concentration. Before being incubated for color development, the required amounts of the fungal extract were mixed with sodium carbonate solution and folin-Denis reagent. The sample's absorbance was measured at 760 nm, and tannic acid was used to determine the sample's tannin content. Milligrams of tannic acid equivalents per gram of extract (mg TAE/g extract) were used to report the results (Indriaty et al., 2023; Lahare et al., 2021).

Total Alkaloid Content (TAC)

The alkaloid concentration was estimated using the gravimetric alkaline precipitation method. Before filtration, all of the crude extracts were diluted in 10% acetic acid (in ethanol) and kept under extraction conditions. After concentrating the filtrate, all of the alkaloids were precipitated by adding concentrated ammonium hydroxide solution dropwise. After filtering, washing, and allowing the precipitated material to dry to a consistent weight, the percentage alkaloid content (%w/w) of the crude extract was determined (Lahare et al., 2021; Truong et al., 2019).

Total Terpenoid Content (TTeC)

The gravimetric procedure was used to determine the total terpenoid content. The crude fungal extract was extracted with ethanol which was then partitioned in separating funnel with petroleum ether. Petroleum ether fraction, which contains terpenoid constituents was evaporated to dryness and the residue was weighed. The concentration of terpenoids was calculated as % w/w (Farouk et al., 2022; Rangani et al., 2023).

DPPH Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to assess the antioxidant activity of the crude secondary metabolite extracts derived from the endophytic fungal isolates. The procedure outlined by Baliyan et al. (2022) was slightly modified. To avoid photodegradation, a new 0.1 mM DPPH solution was made in methanol and kept out of the light during the experiment. Each fungal extract was serially diluted in methanol at 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL. A total reaction volume of 2.0 mL was obtained for each experiment by combining 1.0 mL of the extract solution with 1.0 mL of the newly made DPPH solution. To enable full interaction between the antioxidant compounds and DPPH radicals, the reaction solutions were thoroughly vortexed and incubated in the dark at room temperature (25 ± 2°C) for 30 minutes. A UV-visible spectrophotometer was used to quantify the absorbance drop at 517 nm following incubation, using methanol as the blank. Ascorbic acid was utilized as the positive control, and a control with 1.0 mL of methanol and 1.0 mL of DPPH solution was made under the same circumstances (Baliyan et al., 2022). The percentage radical scavenging activity was calculated according to the following relationship:

$$\text{Percent Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad \ldots\ldots(1)$$

where $A_{control}$ represents the absorbance of the control reaction and A_{sample} represents the absorbance recorded for the fungal extract (Baliyan et al., 2022; Gulcin & Alwasel, 2023).

Total Antioxidant Capacity (TAC)

Concentration-response curves were constructed by plotting percentage inhibition against extract concentration and the concentration required to obtain 50% inhibition of DPPH radicals (IC_{50}) was estimated. The IC_{50} values were calculated from the inhibition curves by regression analysis. The lower IC_{50} values obtained were interpreted as being more active as antioxidants due to the fact that the extract was more effective at reducing half the free radicals available in the solution (Baliyan et al., 2022; Gulcin & Alwasel, 2023).

Total Antioxidant Capacity (TAC)

The total antioxidant activity of the fungal extracts was measured using the phosphomolybdenum reduction assay method adapted from Hamed et al. (2020) with a few modifications. In brief, 0.3 mL of each extract was thoroughly mixed with 3.0 mL of phosphomolybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate).

After 90 minutes at 95°C in a water bath, reaction mixtures were allowed to cool to room temperature. Using a suitable reagent blank, molybdenum (VI) was reduced to molybdenum (V), and the absorbance was measured spectrophotometrically at 695 nm. Antioxidant capacity was measured in milligrams of ascorbic acid equivalents/g extract (mg AAE/g extract) using a calibration curve for ascorbic acid (Asomadu et al., 2024; Hamed et al., 2020)

RESULTS AND DISCUSSION

Results

Identification of Endophytic Fungi Isolated from *Anacardium occidentale* Nuts

Analysis of the generated internal transcribed spacer (ITS) rDNA sequences of the four fungal isolates was performed using the NCBI GenBank BLAST search engine to identify these isolates at the molecular level. Results obtained showed that the sequences were highly similar (99-100%) to authentic reference sequences and were thus identified as Aspergillus niger (EF1), *Penicillium chrysogenum* (EF2), *Fusarium oxysporum* (EF3), and *Cladosporium cladosporioides* (EF4). Highly identical sequences, complete coverage, and significant E-values were observed. The recovery of the above taxonomically diverse endophytic fungi from the *Anacardium occidentale* nuts emphasizes the presence of a diverse range of fungi associated with the medicinal plant. It is noteworthy that the molecular identification provided an excellent basis for the correlation between the taxonomies and bioactivity of these endophytic fungi; where *Fusarium oxysporum* (EF3) had the highest phytochemical contents and antioxidant activities of the four fungal isolates.

Qualitative Phytochemicals Screening

The qualitative phytochemical profile of the four endophytic fungal isolates is presented in Table 1. Screening showed that alkaloids, terpenoids, flavonoids, tannins, and phenolic compounds were consistently detected in all isolates (EF1–EF4), indicating that these metabolite classes are common constituents of the investigated fungi. The widespread occurrence of these compounds suggests that the isolates possess considerable biosynthetic potential and may serve as important sources of biologically active natural products.

Table 1. Qualitative Phytochemical Screening of Endophytic Fungal Isolates from *Anacardium occidentale* Nuts

Phytochemicals	EF1	EF2	EF3	EF4
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	-	+	-	+
Tannins	+	+	+	+
Phenolic Compounds	+	+	+	+
Glycosides	-	-	+	-
Steroids	-	+	+	-

Key: + = Present; - = absent

Variations were observed for the remaining metabolite classes. Saponins were detected only in EF2 and EF4, whereas EF1 and EF3 tested negative. Glycosides occurred exclusively in EF3, highlighting the distinctive metabolic profile of this isolate. Likewise, steroids were present in EF2

and EF3 but absent from EF1 and EF4. These differences emphasize the metabolic diversity that exists among endophytic fungi colonizing the same host tissue, reflecting species-specific biosynthetic pathways and secondary metabolite production.

Quantitative Phytochemicals Screening

Table 2. Quantitative Phytochemical Composition of Crude Extracts from Endophytic Fungal Isolates of Anacardium occidentale Nuts

Parameter	Unit	EF1	EF2	EF3	EF4
Total Phenolic Content (TPC)	mg GAE/g extract	53.22 ± 1.43	41.67 ± 0.04	60.82 ± 1.14	49.15 ± 1.07
Total Flavonoid Content (TFC)	mg QE/g extract	31.97 ± 0.44	32.21 ± 0.82	31.48 ± 0.69	24.93 ± 0.81
Total Tannin Content (TTC)	mg TAE/g extract	20.01 ± 0.12	15.32 ± 0.42	22.51 ± 0.61	17.45 ± 0.49
Total Alkaloid Content (TAC)	% (w/w)	6.74± 0.36	3.95 ± 0.12	5.61 ± 0.18	4.26 ± 0.13
Total Terpenoid Content (TTeC)	% (w/w)	5.74 ± 0.75	6.38 ± 0.19	8.27 ± 0.24	6.85 ± 0.20

The quantitative phytochemical analysis of the endophytic fungal isolates (EF1–EF4) from *Anacardium occidentale* nuts revealed variations in the concentrations of the investigated bioactive compounds Table 2. The total phenolic content (TPC) ranged from 41.67 ± 0.04 to 60.82 ± 1.14 mg GAE/g extract, with EF3 recording the highest value, followed by EF1, EF4, and EF2. The total flavonoid content (TFC) ranged from 24.93 ± 0.81 to 32.21 ± 0.82 mg QE/g extract, with EF2 exhibiting the highest concentration, while EF4 had the lowest. Similarly, the total tannin content (TTC) varied between 15.32 ± 0.42 and 22.51 ± 0.61 mg TAE/g extract, with EF3 showing the highest value. The total alkaloid content (TAC) ranged from 3.95 ± 0.12 to 6.74 ± 0.36% (w/w), with EF1 containing the highest concentration, whereas EF2 recorded the lowest. Likewise, the total terpenoid content (TTeC) ranged from 5.74 ± 0.75 to 8.27 ± 0.24% (w/w), with EF3 exhibiting the highest level. Overall, EF3 possessed the richest phytochemical profile due to its high phenolic, tannin, and terpenoid contents, while EF1 was distinguished by its high alkaloid content and EF2 by its relatively high flavonoid content. These findings indicate that the endophytic fungal isolates are valuable sources of bioactive secondary metabolites and may possess significant antioxidant and other pharmacological properties (Gupta et al., 2023).

DPPH Radical Scavenging Assay

Table 3. DPPH Radical Scavenging Activity (% Inhibition) of Crude Extracts from Endophytic Fungal Isolates of Anacardium occidentale Nuts

Sample	Conc. (µg/mL)							
	7.8	15.6	31.2	62.5	125	250	500	1000
EF1	22.22	30.81	34.66	44.00	51.29	60.03	64.17	67.50
EF2	27.47	33.00	46.17	53.01	74.55	83.90	88.14	81.62
EF3	24.18	33.46	43.89	58.16	71.42	82.53	89.74	94.31
EF4	28.22	35.98	40.71	55.80	63.47	70.33	75.00	79.08
Ascorbic acid	24.00	60.36	77.99	82.15	92.36	94.88	95.00	96.52

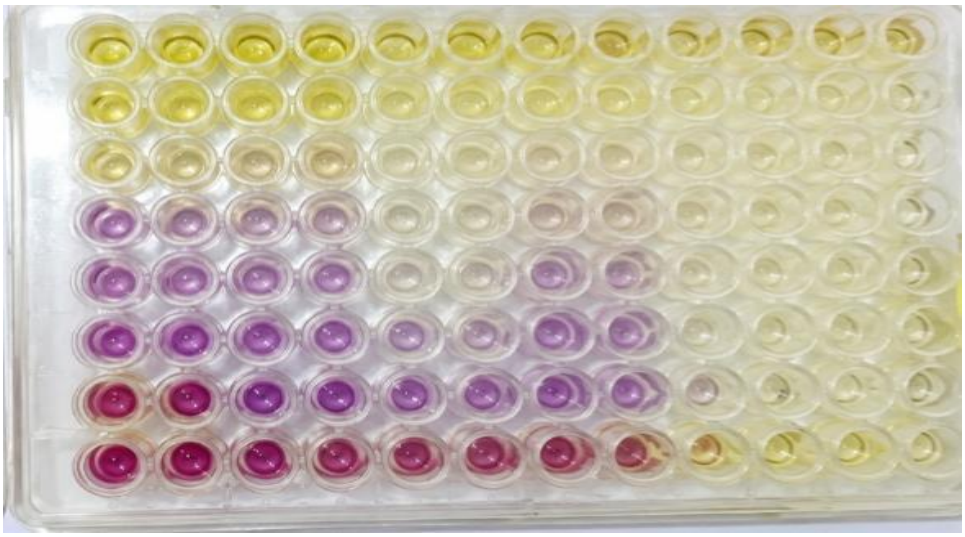

Figure 2. Plate showing DPPH reaction with sample

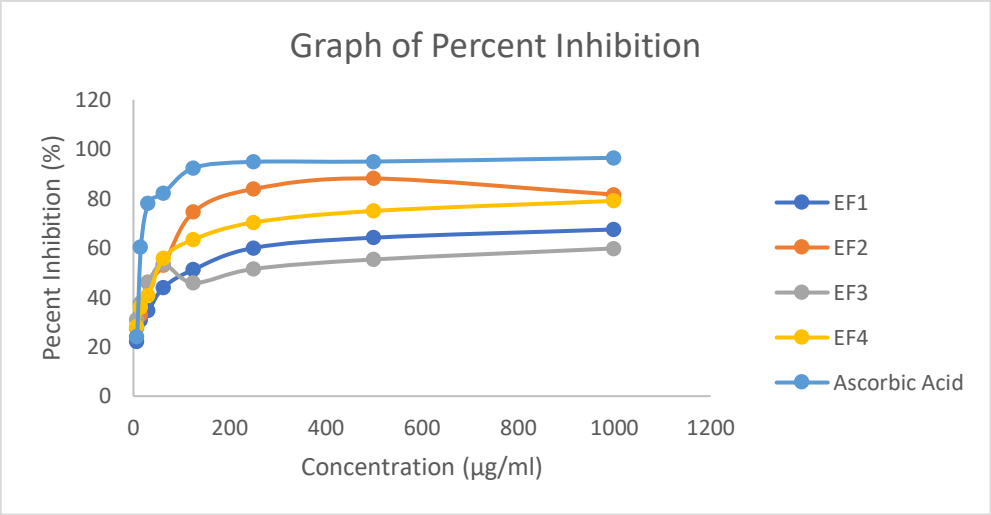

Figure 3: Graph of percent inhibition of the samples and the standard

The DPPH radical scavenging abilities of the crude extracts from the four endophytic fungal isolates are shown in Table 3 and Figure 3. All the extracts showed some antioxidant activity across all tested concentrations, showing they can give away electrons or hydrogen atoms to neutralize DPPH free radicals. In general, the ability to remove free radicals went up as the concentration of the extract increased, showing that the antioxidant effect depends on how much of the extract is present.

Table 4. IC_{50} Values of Crude Extracts and Ascorbic Acid Determined by the DPPH Assay

Sample	IC50 (µg/mL)
EF1	113.94
EF2	48.73
EF3	47.55
EF4	50.47
Ascorbic Acid	13.38

The antioxidant strength of the fungal extracts found inside plants was checked more closely by looking at their IC_{50} values.These values show how much of the extract is needed to remove 50% of the DPPH free radicals. Lower IC_{50} values mean the antioxidant is more effective because less of the extract is needed to produce the same level of inhibition. As expected, ascorbic acid exhibited the strongest antioxidant activity, with the lowest IC_{50} value (13.38 µg/mL), confirming its effectiveness as the reference antioxidant Table 4.

Among the fungal isolates, EF3 demonstrated the greatest antioxidant potency, recording the lowest IC_{50} value (47.55 µg/mL), closely followed by EF2 (48.73 µg/mL) and EF4 (50.47 µg/mL). In contrast, EF1 exhibited the highest IC_{50} value (113.94 µg/mL), indicating comparatively weaker radical scavenging activity. These findings suggest that EF3, EF2, and EF4 possess substantially higher antioxidant efficiency than EF1.

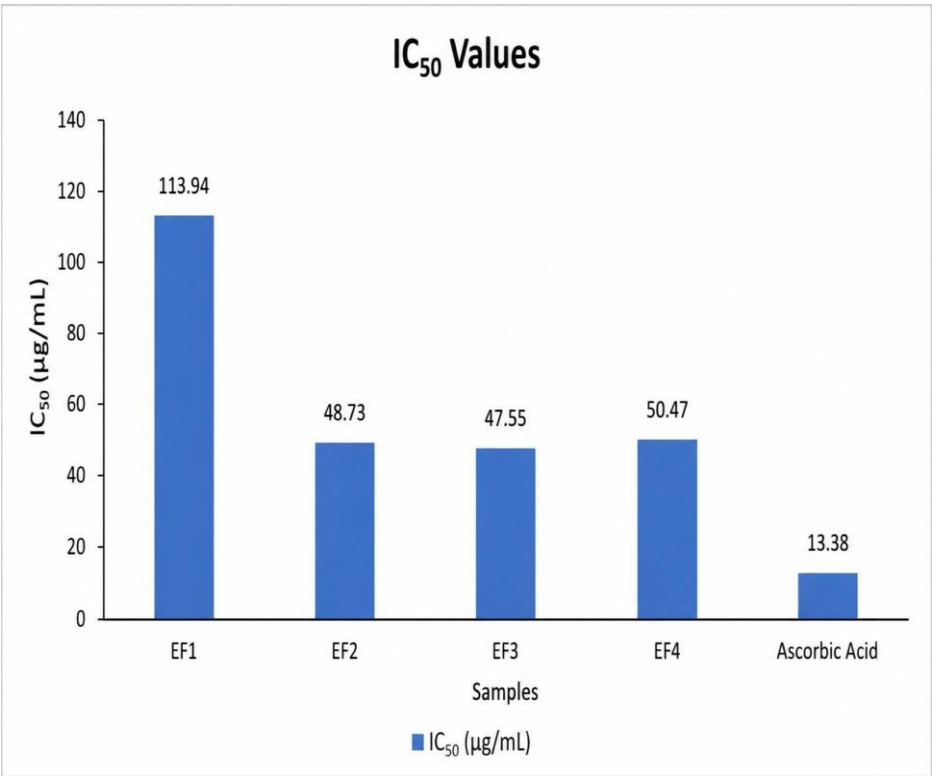

Figure 4. IC50 values of the samples and the standard

Total Antioxidant Capacity

Table 5. Total Antioxidant Capacity of Crude Extracts from Endophytic Fungal Isolates of Anacardium occidentale Nuts

Sample	Total Antioxidant Capacity (mg AAE/g extract)
EF1	96.42
EF2	84.57
EF3	112.83
EF4	91.26
Ascorbic Acid	145.68

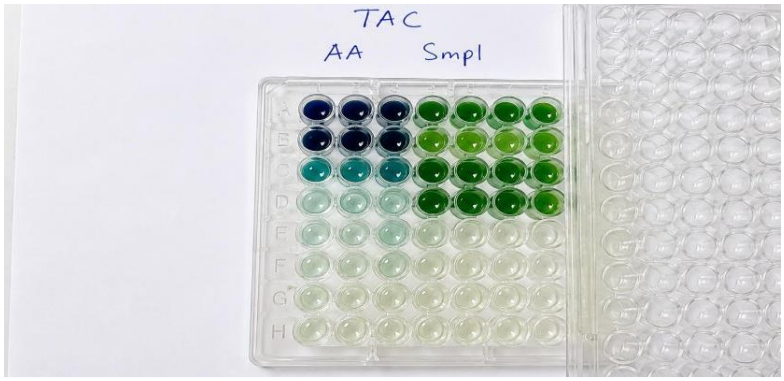

Figure 5. Total Antioxidant Capacity sample plate

Table 5 and Figure 5 show the overall antioxidant activity of the endophytic fungal extracts as determined by the phosphomolybdenum technique. The isolates differed significantly from one another, indicating variances in antioxidant activity and overall reducing power. The ability of antioxidant chemicals to change molybdenum (VI) into molybdenum (V), which indicates the extracts' total antioxidant activity, is the activity being tested. As would be predicted given that ascorbic acid is the reference antioxidant, its total antioxidant capacity was the highest (145.68 mg AAE/g extract). The antioxidant activity among the fungal were highest in EF3 (112.83 mg AAE/g extract), followed by EF1 (96.42 mg AAE/g extract), EF4 (91.26 mg AAE/g extract) and EF2 (84.57 mg AAE/g extract). The results show that all four isolates have considerable antioxidant activity, with EF3 having the highest antioxidant activity overall.

Discussion

The presence of a lot of phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids is especially important because these chemicals are widely known for their antioxidant, antimicrobial, anti-inflammatory, and other health-related benefits. So, the results from this study show that the fungal isolates have a good mix of plant-based chemicals, which suggests they could be useful for medicine and might be worth studying more in areas like drug development and biotechnology (Sruthi et al., 2023).

The present findings agree with those of (Sruthi et al., 2023), who demonstrated that *Anacardium occidentale* nut testa contains abundant phenolic compounds, flavonoids, condensed tannins, catechin, epicatechin, epicatechin gallate, and procyanidins. Their study further established that the richness of these phytochemicals was responsible for the remarkable antioxidant activity of cashew-derived materials, emphasizing their importance as potential sources of natural bioactive compounds for pharmaceutical and nutraceutical applications.

Endophytic fungal isolates are a rich source of structurally varied secondary metabolites with significant pharmacological and antioxidant properties, according to earlier research. For example, Ancheeva et al, (2020); Mishra et al. (2025); Zheng et al. (2021) reported that endophytic fungi produce bioactive compounds such as phenolics, flavonoids, alkaloids, terpenoids, polyketides, xanthones, and coumarins with strong antioxidant, antimicrobial, anti-inflammatory, antiviral, anticancer, antimalarial, and immunosuppressive properties. These studies also highlighted the fact that endophytic fungi are an important and sustainable source of novel natural products with considerable potential for pharmaceutical, nutraceutical, and biotechnological applications.

Among the fungal isolates, EF2 consistently displayed the greatest percentage inhibition across most of the tested concentrations, reaching 88.14% at 500µg/mL before declining slightly to 81.62% at 1000µg/mL. The reduction observed at the highest concentration may reflect saturation of the reaction system or interactions among extract constituents that reduced scavenging efficiency under highly concentrated conditions. EF4 exhibited the second-highest antioxidant activity, whereas EF1 produced moderate radical scavenging effects. By comparison, EF3 showed the lowest percentage inhibition across most concentrations evaluated (Solomakou et al., 2025).

As expected, ascorbic acid demonstrated superior antioxidant performance throughout the assay, with percentage inhibition increasing from 24.00% at 7.8µg/mL to 96.52% at 1000µg/mL. The consistently higher activity of the standard antioxidant confirms the validity and reliability of the experimental procedure. The observed antioxidant activities are likely attributable to the diverse phytochemical constituents detected in the fungal extracts, particularly phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids, all of which have been reported to possess effective free radical scavenging properties. The concentration-dependent response further suggests that these metabolites collectively contribute to the antioxidant potential of the isolates. For instance, Amirzakariya and Shakeri (2022) reported that antioxidant activity of the endophytic fungal extracts has been attributed to various phytochemical constituents, especially phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids in previous studies. Based on a study by Mishra et al. (2020), these classes of secondary metabolites are the major contributors to the free radical scavenging capacity of endophytic fungi by mechanisms such as hydrogen atom donation, electron transfer, and metal ion chelation. Similarly, Amirzakariya and Shakeri (2022) also noted that terpenoids along with phenolics and alkaloids produced by endophytic fungi had high antioxidant

and other biological activities. Recent reviews by Gamkrelidze et al. (2026) have further emphasized that the synergistic interactions among these phytochemicals enhance the overall antioxidant potential of fungal extracts, with higher metabolite concentrations generally producing stronger free radical scavenging effects in a concentration-dependent manner. These findings support the view that the collective action of multiple secondary metabolites, rather than a single compound, is responsible for the potent antioxidant properties observed in endophytic fungal isolates.

The present findings are consistent with those reported by Ganbold et al. (2025), who demonstrated that ethanolic extracts of *Anacardium occidentale* kernels possess considerable antioxidant activity in the DPPH assay and attributed this biological effect primarily to their phenolic constituents. The agreement between both studies reinforces the importance of cashew-associated natural products as valuable sources of antioxidant compounds with potential pharmaceutical and nutraceutical applications.

The phytochemical makeup of EF3 is consistent with its strong antioxidant function. According to quantitative phytochemical analysis, EF3 has the highest quantities of terpenoids, tannins, and total phenolics all of which are known to contribute to antioxidant activity. While tannins and terpenoids further improve antioxidant defense by complementing radical scavenging and metal-chelating processes, phenolic substances rapidly donate hydrogen atoms or electrons to eliminate reactive oxygen species. The combined action of these metabolites likely accounts for the relatively low IC_{50} value observed for EF3.

Although EF2 exhibited the highest percentage DPPH inhibition at higher extract concentrations, its IC_{50} value was only slightly higher than that of EF3. This indicates that while EF2 achieved greater maximum radical scavenging activity at elevated concentrations, EF3 reached the 50% inhibition threshold with marginally greater efficiency. Therefore, IC_{50} provides a more reliable indicator of antioxidant potency than percentage inhibition at a single concentration because it considers the overall dose–response relationship rather than the response at one concentration alone.

The present findings support previous reports demonstrating that antioxidant activity in endophytic fungi is strongly associated with the accumulation of phenolic and other bioactive secondary metabolites (Ganbold et al., 2025; Zahra et al., 2021). The potential of endophytic fungi isolated from Anacardium occidentale nuts as promising natural sources of antioxidant chemicals with potential pharmacological, nutraceutical, and biotechnological applications is highlighted by the comparatively low IC_{50} values obtained for EF2, EF3, and EF4.

The high total antioxidant activity shown by EF3 is in accordance with the quantitative phytochemical profile which exhibited the highest level of total phenolics, tannins, and terpenoids content for EF3 among all the investigated isolates. The wide-spread knowledge of the antioxidant activity of phenolic compounds is based on their electron donation properties, the ability to stop the chain propagation of free radicals, and the ability to stabilize ROS. Similarly, tannins and terpenoids contribute to the antioxidant defence system in complementary ways, such as in their ability to scavenge radicals and chelate metal ions. The synergistic effect of these metabolites may account for the greater antioxidant activity of EF3.

Interestingly, EF2 exhibited the highest percentage inhibition in the DPPH assay at several concentrations while EF3 exhibited a lower antioxidant capacity than EF2. The observation implies that antioxidant activity is not only based on the level of free radical scavenging obtained from one of the assays but also on the overall reducing power of all antioxidant components contained in the extract. Various antioxidants assays have different modes of action, so the use of multiple assays is more representative of the antioxidant capacity (Munteanu & Apetrei, 2021; Sadeer et al., 2020). Previous studies such as those of Munteanu & Apetrei, (2021), They have said that you can't properly measure antioxidant activity with just one test because different antioxidant tests look at different ways antioxidants work, like electron transfer, hydrogen atom transfer, and reducing metal ions. They said that using several different tests like DPPH, FRAP, ABTS, ORAC, and CUPRAC gives a better and more accurate picture of how well something can act as an antioxidant.These tests check both the ability to remove free radicals and the power to reduce substances.

The present results corroborate previous studies showing that antioxidant activity of endophytic fungi is closely related with the synthesis of phenolic and other secondary metabolites (Ganbold et al., 2025; Zahra et al., 2021) The moderate-to-high antioxidant potentials noted for the EF3 may

suggest that the endophytes of A. occidentale are valuable sources of natural antioxidants for use in pharmaceutical, nutraceutical, food, and cosmetic industries.

Implications

The results of this study contribute to existing knowledge of endophytic fungi of *Anacardium occidentale* nuts and highlight their potential as a source of natural antioxidant compounds. The observed substantial phytochemical diversity and antioxidant activities of the fungal isolates indicate the potential of these microorganisms in decreasing the need for synthetic antioxidants in pharmaceutical, nutraceutical, food and cosmetic industries. Moreover, the isolation of fungal bioprospecting strains with strong antioxidant activity, offers scientific support for future bioprospecting research that could lead to the isolation of new bioactive metabolites with pharmaceutical and industrial applications.

Research contribution

The present study is one of the many in the literature that describe the phytochemical composition and antioxidant activities of endophytic fungi from *Anacardium occidentale* nuts. It gives comparative qualitative and quantitative phytochemical profile of the fungal isolates and quantifies the antioxidant activities by DPPH radical scavenging and total antioxidant capacity assays. The study revealed that EF3 was the most potential isolate producing antioxidant activities, therefore, it was recommended to be further investigated in a scientific manner. The results also contribute to the very little information available on endophytic fungi from cashew nut and its potential as source of valuable bioactive compounds for pharmaceutical and biotechnological applications using sustainable sources.

Suggestions

Future research is required to precisely determine the endophytic fungus isolates' taxonomic identity through molecular identification utilizing DNA sequencing technology. Advanced spectroscopic techniques such as LC-MS/MS, GC-MS, and NMR should be used in conjunction with bioassay-guided fractionation to isolate and characterize the individual antioxidant molecules responsible for the observed activity. Additionally, it is advised that in vitro and in vivo tests be used to evaluate the pharmacological activity, toxicity, and safety profile of the fungal metabolites. The medicinal and industrial uses of endophytic fungi would increase with more research into how to optimize fermentation conditions to enhance metabolite production and other biological activities like antibacterial, anti-inflammatory, anticancer, and enzyme inhibitory properties.

CONCLUSION

This study shows that the fungi living inside cashew nuts (Anacardium occidentale) are good sources of natural chemicals that have strong antioxidant properties. The study highlights how these fungi living inside plants can make different chemicals that help protect against harmful free radicals. The isolate EF3 was determined to be the most effective for future research because it showed strong antioxidant activity and had a high amount of phytochemicals. This study helps us learn more about how these endophytes from Anacardium occidentale can be used in areas like medicine, health products, food, and beauty care. Future studies on this topic will focus on isolating and identifying active plant chemicals, understanding how they work, and checking their safety and effectiveness as natural antioxidants.

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AUTHOR CONTRIBUTION STATEMENT

JP conducted the research and generated the experimental data while KD wrote the manuscript. Both authors read and approved the final version of the manuscript.

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