RESEARCH PAPER

Preliminary Screening and Antioxidant activity of Endophytic fungi isolated from Ampelocissus latifolia (Roxb.) Planch.

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ABSTRACT
Ampelocissus latifolia (Roxb.) Planch., a medicinal climber of the family Vitaceae, is known for its traditional therapeutic uses and rich phytochemical profile, including phenolics and flavonoids. In this study, five morphologically distinct endophytic fungi (AL01-01: *Penicillium* sp., AL01-04: *Aspergillus niger*, AL02-01: *Fusarium moniliforme*, AL02-02: *Alternaria* spp., AL02-03: *Acremonium strictum*) were isolated from its leaves. Microscopic identification, phytochemical screening, and quantification assays were performed to evaluate the presence of secondary metabolites and antioxidant potential. Phytochemical analysis revealed the presence of saponins, phenols, tannins, flavonoids, terpenoids, and alkaloids, with AL02-01 and AL02-03 showing the richest profiles. Total phenolic content (TPC) and total flavonoid content (TFC) increased with extract concentration, with AL01 isolates exhibiting higher TPC and TFC than AL02 isolates. Total antioxidant capacity (TAC), DPPH radical scavenging activity, and FRAP reducing power assays demonstrated that AL02 isolates, particularly AL02-01, AL02-02, and AL02-03, possessed strong antioxidant potential, whereas AL01 isolates showed moderate activity. Overall, the study highlights that endophytic fungi from *A. latifolia* are promising sources of bioactive metabolites with significant antioxidant properties, supporting their potential for pharmaceutical and nutraceutical applications..

Keywords: *Vitaceae, Therapeutic uses, Endophytic fungi, Antioxidant potential, Profiles, Phytochemicals, Traditional, Quantification, Leaves, Secondary metabolites*.
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INTRODUCTION

Ampelocissus latifolia (*Roxb*.) Planch. (family *Vitaceae*) is a large, herbaceous, twining climber commonly referred to as wild grape, native to the Indian subcontinent and parts of Southeast Asia. It bears hollow stems, lobed leaves, and small grape-like berries. Initially described as *Vitis latifolia*, it was later transferred to the genus *Ampelocissus* (**Kew Science, 2023**). Traditionally, *A. latifolia* has been used in indigenous medicine for a wide range of ailments. Ethnobotanical reports document its application in the treatment of wounds, skin diseases, dysentery, diarrhoea, bone fractures, dental problems, rheumatism, and for maternal health during childbirth, with different plant parts (roots, leaves, bark) employed in local remedies (**Bhargava and Singh, 2018; Sharma *et al.,* 2021**).

Phytochemical analyses reveal the presence of phenolics, flavonoids, tannins, and other secondary metabolites. Experimental studies indicate antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties, supporting some of the plant's traditional claims (**Chaudhuri and Ray, 2020; Sharma *et al.,* 2021**). Recent work has also highlighted its antibacterial activity against clinically relevant strains (**Khushbu *et al.,* 2025**).

Mostly medicinal plants, especially those in the family *Vitaceae*, are promising hosts for fungal endophytes with potential bioactive properties. However, to date there is no published work reporting the isolation, characterization, or bioactivity of endophytic fungi specifically from *Ampelocissus latifolia* (*Roxb*.) *Planch.* Despite its ethnomedicinal relevance and demonstrated antimicrobial activity in extracts of its tuberous roots and roots (**Rajak *et al.,* 2023; Khushbu *et al.,* 2025**), studies have not yet addressed fungal endophytes inhabiting its leaves, stems, bark, or root tissues.

Primary and secondary metabolites of endophytic fungi

Endophytic fungi are known to synthesize a wide range of primary metabolites such as enzymes, amino acids and organic acids, along with secondary metabolites including alkaloids, phenolics, terpenoids and polyketides that contribute to host defense and medicinal properties (**Alam, 2021; Wen *et al.,* 2022; Liu *et al.,* 2021**). Considering that *A. latifolia* itself is rich in phenolics and flavonoids, its endophytic fungi represent an underexplored reservoir for bioactive metabolites of pharmacological significance.

Antioxidant activity of endophytic fungi isolated from *A. latifolia*

Endophytic fungi associated with medicinal plants such as *Ampelocissus latifolia* are known to synthesize diverse bioactive metabolites, including antioxidants that play a significant role in mitigating oxidative stress. Oxidative stress, caused by the overproduction of reactive oxygen species (ROS), is linked with cellular damage and various chronic diseases, making antioxidants of natural origin

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highly valuable (**Apak *et al*., 2016**). Endophytes from *A. latifolia* have demonstrated strong antioxidant properties through assays such as DPPH, ABTS, and ferric reducing antioxidant power (FRAP), indicating their ability to scavenge free radicals and reduce oxidative damage (**Deshmukh *et al.,* 2018**). These antioxidant metabolites include phenolic compounds, flavonoids, and other secondary metabolites, which are comparable to those produced by their host plants (**Huang *et al.,* 2019**). The discovery of antioxidant-producing endophytes from *A. latifolia* highlights their potential as sustainable sources of natural antioxidants for pharmaceutical and nutraceutical applications (**Rana *et al.,* 2021**).

2. Materials and methods

Sample collection

Fresh and healthy leaves of *Ampelocissus latifolia L*. were collected directly from Jaipur region (Rajasthan). Immediately after collection, the leaves were placed in sterile, airtight polythene bags to avoid external microbial contamination and to preserve their physiological condition during transport.

Isolation of Endophytic fungi

Collected fresh healthy leaves were processed for endophytic fungi isolation following **Araújo *et al.* (2002)**. Leaves were washed, surface-sterilized (75% ethanol, 5% sodium hypochlorite, and sterile water), and ~1 cm segments were placed on PDA with gentamycin to inhibit bacteria. Plates were incubated at 25–28°C for 15–20 days, and fungal growth was subcultured for purification. PDA slants stored at 4°C were used to preserve the pure cultures.

2.3 Microscopic identification of isolated Endophytic Fungi

Fungal specimens were prepared for microscopic observation using the lactophenol cotton blue (LPCB) staining method as described by **Barnett and Hunter (1998)** with minor modifications. A drop of LPCB was placed on a clean glass slide, and a small portion of mycelium from the actively growing colony margin was transferred aseptically. The fungal material was gently teased apart with a sterile needle to separate hyphae and spores, after which a cover slip was placed at an angle to avoid air bubbles. The slide was observed under a compound microscope, first at 10× to locate structures and then at higher magnifications (40× and 100×) to study morphological details such as hyphae, conidiophores, spores, and reproductive structures **(Ellis *et al.,* 2007; Samson *et al*., 2014).** This method enabled clear visualization of diagnostic features necessary for fungal identification.

Phytochemical screening of selected isolates

(a) Fungal extracts Preparation

Fungal extracts were prepared by cultivating isolates in liquid media such as Potato Dextrose Broth (PDB) or Sabouraud Dextrose Broth, which promote both growth and metabolite production. Cultures were incubated under optimal conditions for 1–2 weeks to allow sufficient biomass and metabolite accumulation **(Patel *et al.,* 2017).** After incubation, the culture broth was filtered to separate biomass from the metabolite-rich filtrate. Intracellular metabolites were extracted from the biomass using polar solvents (e.g., methanol, ethanol), followed by homogenization, filtration, and concentration **(Gupta *et al.*, 2018).** Extracts were stored at −20 °C to −80 °C to preserve bioactivity.

(b) Preliminary tests

Test for Flavonoids:

Flavonoids in the fungal extract were detected using the method of **Harborne (1998).** One milliliter of extract was treated with a few drops of 10% NaOH, producing a yellow coloration due to flavonoid–alkali complex formation. The addition of dilute HCl decolorized the solution, confirming the presence of flavonoids.

Test for Phenols:

Phenolic compounds were detected qualitatively by adding a few drops of 5% ferric chloride ($FeCl_3$) solution to 1 mL of the fungal extract dissolved in distilled water **Harborne, 1998**. Phenolic hydroxyl groups form colored complexes with Fe^{3+} ions, producing characteristic green, blue, or purple hues. The development of a dark green coloration was considered a positive result, with color intensity reflecting the type and concentration of phenolic compounds present.

Test for Alkaloids:

Alkaloids were detected using **Hager's test**. Approximately 2 mL of slightly warm hydrochloric acid (HCl) was added to the extract to convert any alkaloids into their water-soluble hydrochloride salts. Subsequently, 2 mL of Hager's reagent, a saturated solution of picric acid, was added. The formation of a yellow precipitate indicated the presence of alkaloid compounds.

Test for Saponin:

Saponin were identified by mixing 5 mL of fungal extract with an equal volume of distilled water and shaking vigorously for 30–60 seconds. The formation of persistent froth lasting 7–10 minutes was considered a positive indication of saponin presence.

Test for Tannins:

To detect tannins, 1 mL of fungal extract was diluted with 2 mL of distilled water, stirred, and filtered. Two milliliters of the filtrate were mixed with a few drops of 1% ferric chloride solution. A blue-black, green, or blue-green coloration indicated the presence of tannins, with hydrolyzable tannins producing blue-black and condensed tannins producing greenish hues.

Test for Terpenoids:

Terpenoids were tested by mixing 2 mL of extract with an equal volume of chloroform, followed by careful addition of 3 mL concentrated sulfuric acid along the sides of the test tube. The formation of a reddish-brown layer at the interface indicated the presence of terpenoids.

(c) Quantification Test

Total phenolic content (Stock solution of 10mg/ml):

The total phenolic content (TPC) of fungal extracts was determined using the Folin–Ciocalteu (F–C) assay **(Singleton *et al*., 1999)** to assess antioxidant potential, as phenolics correlate with radical-scavenging activity **(Prabakaran *et al.,* 2019).** Aliquots of 100–300 µL extract (10 mg/mL) were diluted to 1 mL with distilled water, followed by addition of 2.5 mL diluted F–C reagent (1:10) and 2 mL of 7.5% Na_2CO_3. After vortexing, mixtures were incubated at 50 °C for 15 min, and absorbance was recorded at 765 nm. TPC was expressed as gallic acid equivalents (GAE) using a standard calibration curve.

Total Flavonoid Content (TFC):

The total flavonoid content of the extracts was determined using the aluminum chloride (AlCl3) colorimetric method, as described by **Ordonez *et al.*, 2006** with slight modifications. Flavonoids form stable complexes with AlCl3, producing a yellow coloration proportional to flavonoid concentration. Aliquots of 100, 200, and 300 µL of the fungal extract (10 mg/mL stock) were adjusted to 1 mL with distilled water, and 0.5 mL of 2% AlCl3 solution in ethanol was added to each tube. The reaction mixtures were thoroughly mixed and incubated at room temperature for 60 minutes to allow complete complex formation. Absorbance was measured at 420 nm against a reagent blank. TFC was expressed as quercetin equivalents (QE) using a calibration curve and the content was calculated using the equation:

$$Y = 0.01x + 0.05$$

Where (x) represents absorbance and (Y) represents the quercetin equivalent (mg/g).

Antioxidant activity of isolated endophytic Fungus isolates

(a) DPPH radical scavenging activity (stock solution of 1mg/ml):

The free radical scavenging activity of the fungal extract was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, following **Prieto *et al.*, 1999** with minor modifications. This assay is based on the reduction of the purple-colored DPPH radical by antioxidants, which results in a decrease in absorbance at 517 nm. Various concentrations of the fungal extract were prepared in a DMSO–distilled water mixture to ensure solubility. To each sample, 1 mL of 0.2 mM DPPH methanolic solution was added, and the mixtures were incubated in the dark at room temperature for 30 minutes. The reduction in absorbance relative to the control (DPPH solution without extract) indicated radical scavenging activity, expressed as percentage inhibition using the formula:

$$I\% = \frac{\{A_b - A_s\}}{A_b} \times 100$$

Where (A_b) is the absorbance of the control and (A_s) is the absorbance of the sample. Ascorbic acid was used as a positive control.

(b) Reducing Power Assay:

The ferric reducing potential of the fungal extract was determined using the method of **Oyaizu, 1986** with slight modifications. Aliquots of 100, 200, and 300 µL of the extract (stock 10 mg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide [$K_3Fe(CN)_6$]. The mixtures were incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of 10% trichloroacetic acid to terminate the reaction. After centrifugation, 2.5 mL of the supernatant was mixed with 0.5 mL of 0.1% ferric chloride. The formation of a Prussian blue complex was measured at 700 nm, with higher absorbance indicating greater reducing power and electron-donating ability of the extract.

(c) Total Antioxidant Capacity (Phospho-molybdenum Assay):

The total antioxidant capacity of the fungal extract was assessed using the phosphomolybdenum method, as described by **Guha *et al.*, 2010** with minor modifications. The assay is based on the reduction of molybdenum (VI) to molybdenum (V) by antioxidants, forming a green phosphate–molybdenum (V) complex. A stock solution of the extract (1 mg/mL) was diluted to various concentrations (100, 200, 300 µL) and mixed with 3.3 mL of a reagent containing ammonium molybdate, sodium phosphate, and concentrated sulfuric acid. The mixtures were incubated in a water bath at 95°C for 60 minutes, cooled to room temperature, and absorbance was measured at 695 nm. A reagent blank without extract was used to correct for background absorbance. The intensity of the green complex reflected the total antioxidant capacity of the fungal extract.

3. Results

3.1 Isolation and Morphological Identification of Endophytic Fungi from *Ampelocissus latifolia* Leaves

In the present study, a total of five morphologically distinct endophytic fungal isolates (AL01-01, AL01-04, AL02-01, AL02-02, and AL02-03) were obtained from 150 leaf segments of *Ampelocissus latifolia*. Based on microscopic examination and morphological characteristics, the isolates were identified as *Penicillium* sp. (AL01-01), *Aspergillus niger* (AL01-04), *Fusarium moniliforme* (AL02-01), *Alternaria* spp. (AL02-02), and *Acremonium strictum* (AL02-03).

3.2 Preliminary screening of selected isolates fungal isolates

The phytochemical screening of fungal extracts revealed the presence of several bioactive compounds such as saponins, phenols, tannins, flavonoids, terpenoids, and alkaloids in different isolates. Isolate **AL01-01** showed the presence of flavonoids, terpenoids, and alkaloids, while saponins, phenols, and tannins were absent. Isolate **AL01-04** exhibited positive results for phenols, tannins, flavonoids, terpenoids, and alkaloids, but no saponins were detected. Isolate **AL02-01** contained all the tested phytochemicals, suggesting it is a rich source of diverse bioactive compounds. In **AL02-02**, saponins, flavonoids, terpenoids, and alkaloids were present, while phenols and tannins were absent. Isolate **AL02-03** was the most promising, showing strong positive (++) results for phenols, tannins, and flavonoids along with the presence of saponins, terpenoids, and alkaloids.

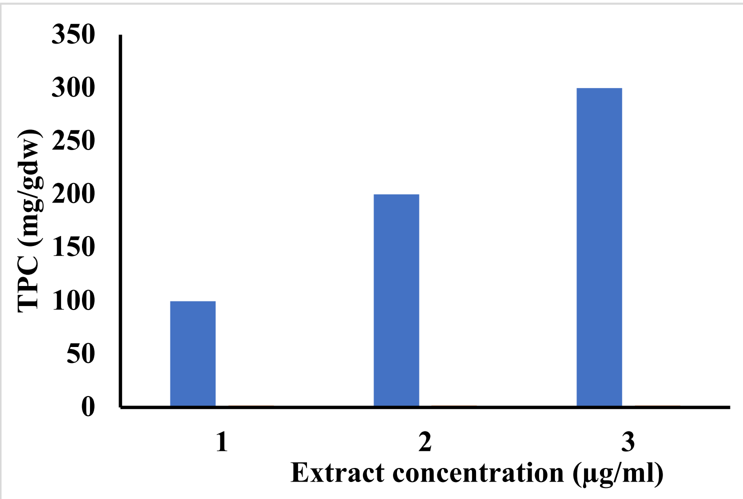
Table 1: Preliminary screening of selected isolates fungal isolates

Endophytic fungi isolates	Saponin test	Phenol test	Tannin test	Flavonoid test	Terpenoid test	Alkaloid test
AL01 - 01	_	_	_	+	+	+
AL01 - 04	_	+	+	+	+	+
AL02 - 01	+	+	+	+	+	+
AL02 – 02	+	_	_	+	+	+
AL02 – 03	+	++	++	++	+	+

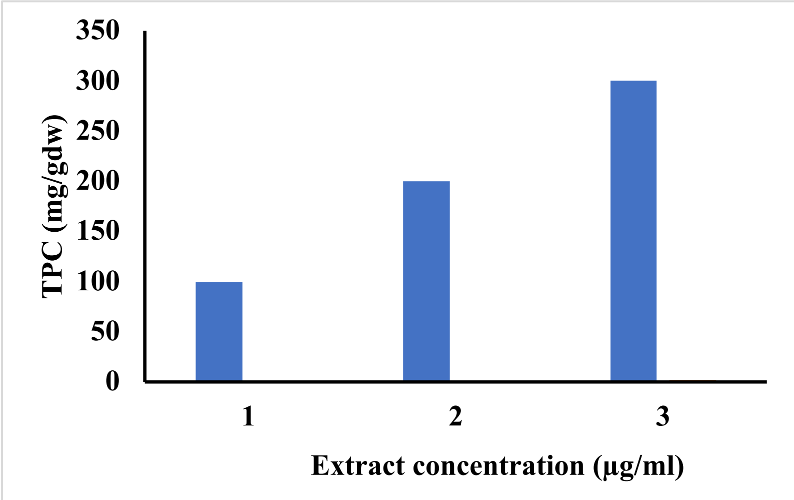
Quantification Phytochemical test of endophytic fungal isolates
(a) Total Phenolic content:
The total phenolic content (TPC) of fungal extracts, expressed as mg/g dry weight (dw), varied among the isolates and increased with rising extract concentration (100–300 µg/mL). At **100 µg/mL**, the highest phenolic activity was recorded in **AL01-01 (1.741 mg/g dw),** followed by **AL01-04 (1.637 mg/g dw),** whereas **AL02-02 (0.548 mg/g dw)** showed the lowest value. At **200 µg/mL**, a similar trend was observed, with **AL01-01 (1.756 mg/g dw)** and **AL01-04 (1.672 mg/g dw)** maintaining higher activity compared to other isolates. At the maximum tested concentration **(300 µg/mL), AL01-01 (1.976 mg/g dw)** and **AL01-04 (1.918 mg/g dw)** again exhibited the highest phenolic content, while **AL02-02 (0.804 mg/g dw)** remained the lowest (Table 2).

Table 2: Total Phenolic Activity of isolated endophytic fungal strains

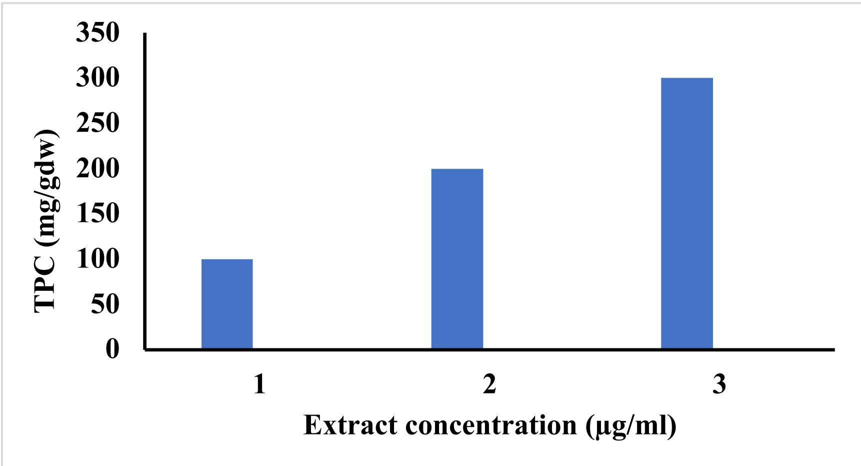
Extract conc. (µg/ml)	TPC (mg/g dw)				
	AL01-01	AL01-04	AL02-01	AL02-02	AL02-03
100	1.741	1.637	0.585	0.548	0.612
200	1.756	1.672	0.788	0.560	0.825
300	1.976	1.918	0.996	0.804	1.014



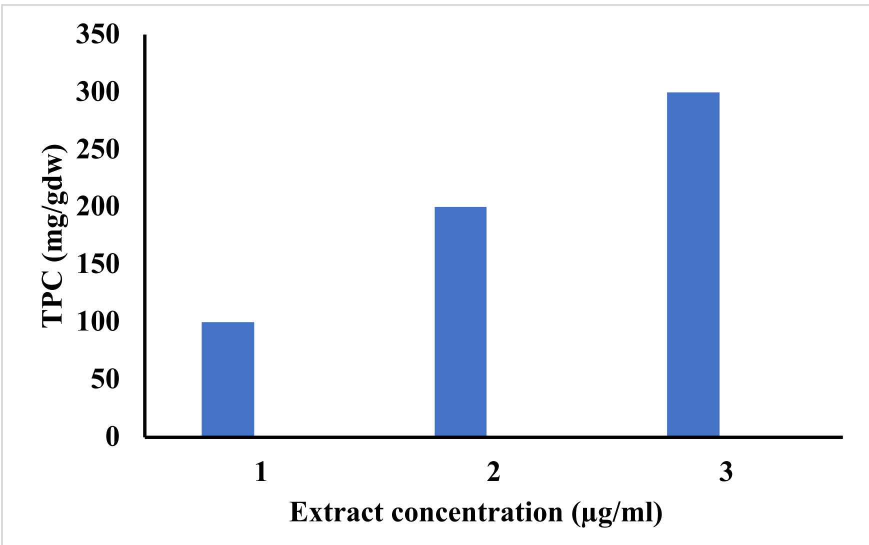
Graph 1: Total Phenolic Activity of isolated endophytic fungal strain- AL01-01



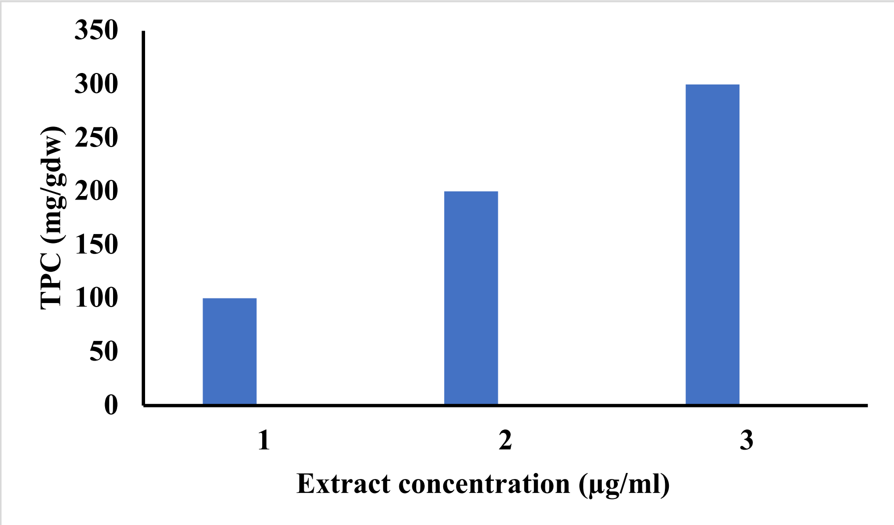
Graph 2: Total Phenolic Activity of isolated endophytic fungal strain- AL01-04



Graph 3: Total Phenolic Activity of isolated endophytic fungal strain- AL02-01



Graph 4: Total Phenolic Activity of isolated endophytic fungal strain- AL02-02



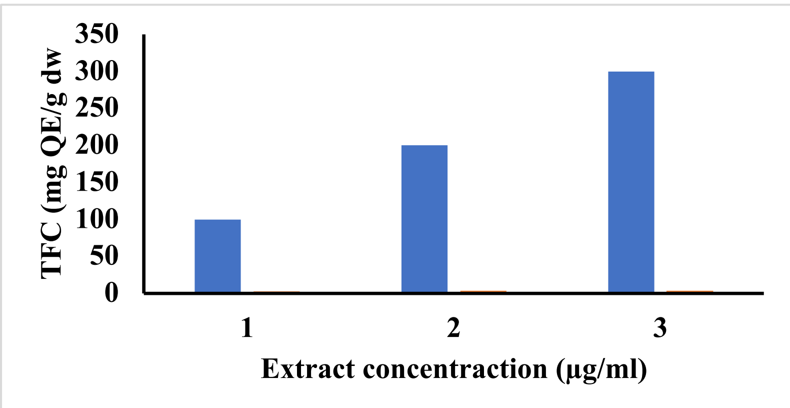
Graph 5: Total Phenolic Activity of isolated endophytic fungal strain- AL02-03

(b) Total Flavonoid content:

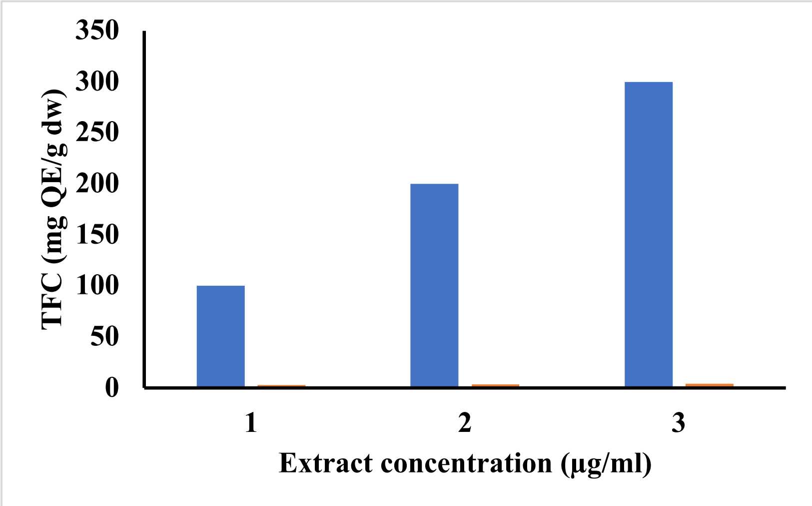
The total flavonoid content (TFC) of fungal extracts, expressed as mg quercetin equivalent per g dry weight (mg QE/g dw), increased with extract concentration (100–300 µg/mL) in all isolates. At **100 µg/mL**, the highest flavonoid content was observed in **AL01-04 (3.096 mg QE/g dw),** followed closely by **AL01-01 (3.000 mg QE/g dw),** while the lowest was detected in **AL02-02 (2.506 mg QE/g dw).** At **200 µg/mL**, flavonoid levels increased in all isolates, with **AL01-01 (3.318 mg QE/g dw)** and **AL01-04 (3.300 mg QE/g dw)** remaining superior compared to the others. At the maximum concentration **(300 µg/mL), AL01-04 (3.880 mg QE/g dw)** showed the highest flavonoid accumulation, followed by **AL01-01 (3.512 mg QE/g dw),** whereas **AL02-01 (3.068 mg QE/g dw)** exhibited the lowest content (Table 3).

Table 3: Total Flavonoids Activity of isolated endophytic fungal strains

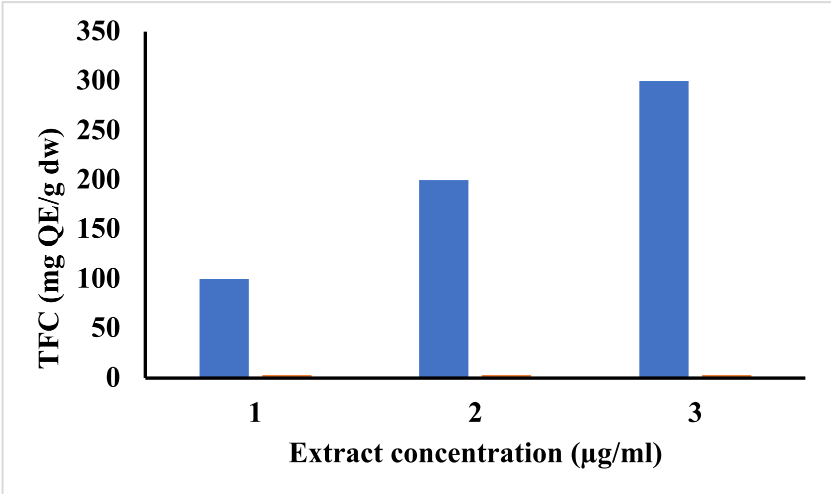
Extract conc. (µg/ml)	**TFC (mg QE / g dw)**				
	AL01-01	**AL01-04**	**AL02-01**	**AL02-02**	**AL02-03**
100	3.0	3.096	2.944	2.942	2.506
200	3.318	3.300	3.030	3.148	3.204
300	3.512	3.880	3.068	3.484	3.536



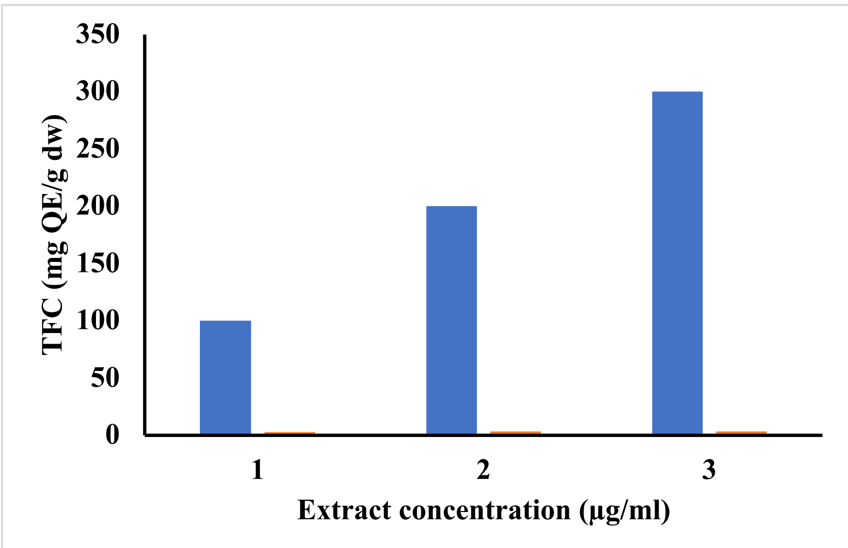
Graph 6: Total Flavonoids Activity of isolated endophytic fungal strain- AL01-01



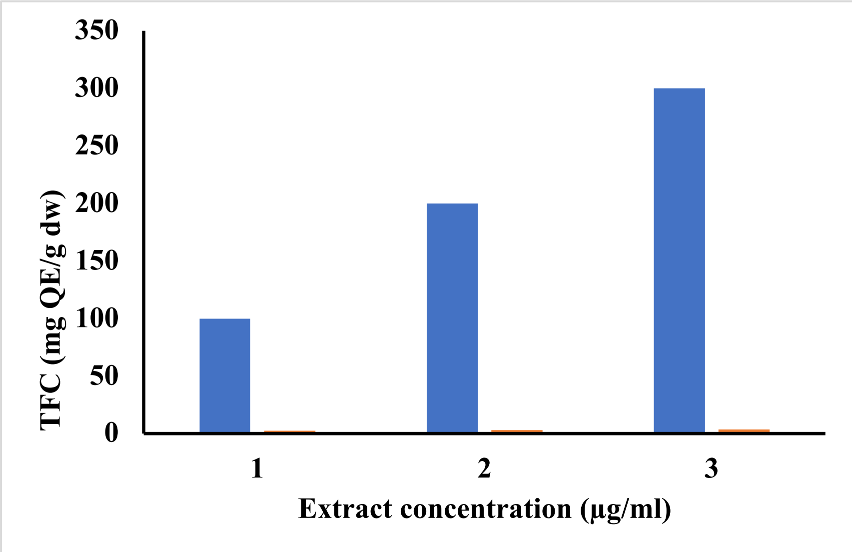
Graph 7: Total Flavonoids Activity of isolated endophytic fungal strain- AL01-04



Graph 8: Total Flavonoids Activity of isolated endophytic fungal strain- AL02-01



Graph 9: Total Flavonoids Activity of isolated endophytic fungal strain- AL02-02



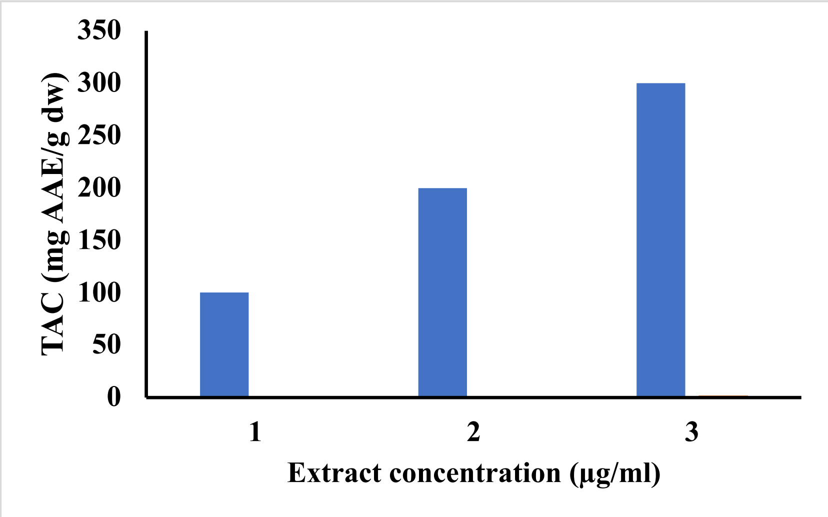
Graph 10: Total Flavonoids Activity of isolated endophytic fungal strain- AL02-03

Antioxidant activity of endophytic fungal isolates

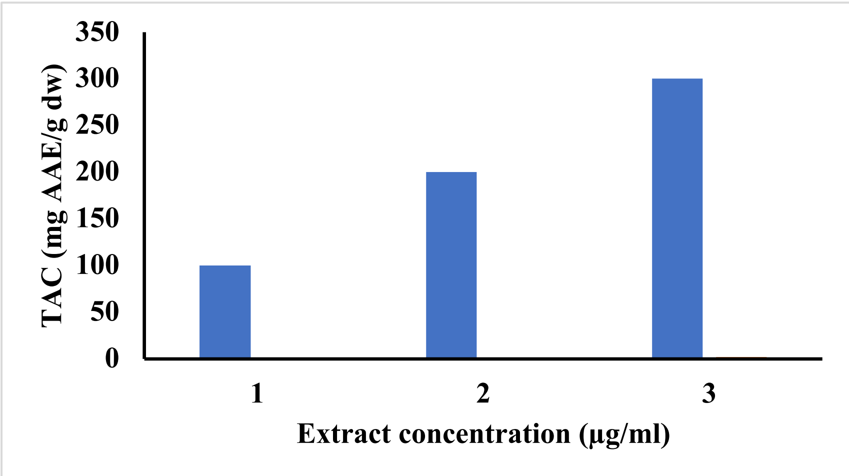
(a) Total antioxidant assay:
The total antioxidant capacity (TAC), expressed as mg ascorbic acid equivalent per g dry weight (mg AAE/g dw), varied among the fungal isolates and generally increased with extract concentration. At **100 µg/mL**, the highest TAC was recorded in **AL02-02 (1.56 mg AAE/g dw)** and **AL02-03 (1.53 mg AAE/g dw),** followed closely by **AL02-01 (1.47 mg AAE/g dw),** while **AL01-04 (1.14 mg AAE/g dw)** showed the lowest value. At **200 µg/mL**, TAC values increased in all isolates, with **AL01-01 (2.94 mg AAE/g dw)** showing a sharp rise compared to others, whereas the AL02 group showed only moderate increments (1.61–1.67 mg AAE/g dw). At the maximum concentration **(300 µg/mL), AL01-01 (2.96 mg AAE/g dw)** remained the most active isolate, followed by **AL02-02 (2.11 mg AAE/g dw)** and **AL02-03 (2.06 mg AAE/g dw).** Again, **AL01-04 (1.95 mg AAE/g dw)** showed the lowest antioxidant capacity among all strains (Table 4).

Table 4: Total antioxidant activity of isolated endophytic fungal strains

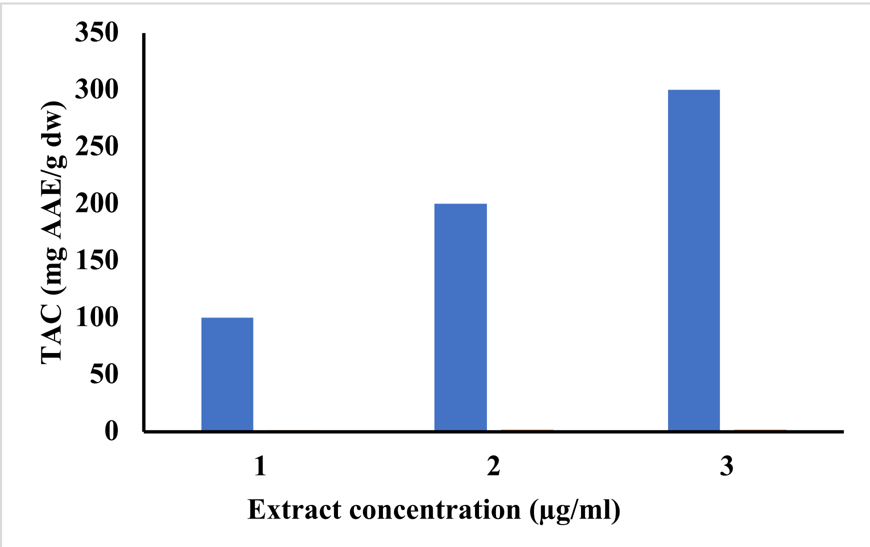
Extract Conc. (µg/ml)	**TAC (mg AAE/g dw)**				
	AL01-01	**AL01-04**	**AL02-01**	**AL02-02**	**AL02-03**
100	1.40	1.14	1.47	1.56	1.53
200	2.94	1.71	1.67	1.61	1.65
300	2.96	1.95	1.72	2.11	2.06



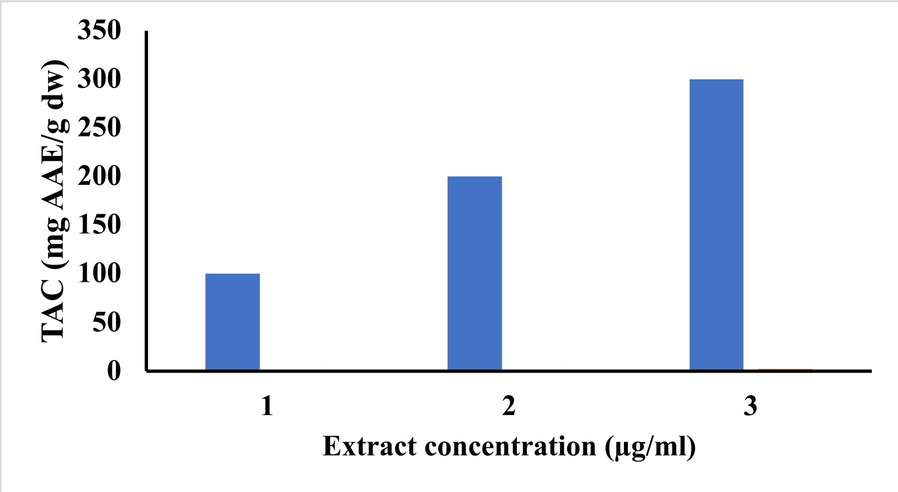
Graph 11: Total antioxidant activity of isolated endophytic fungal strain- AL01-01



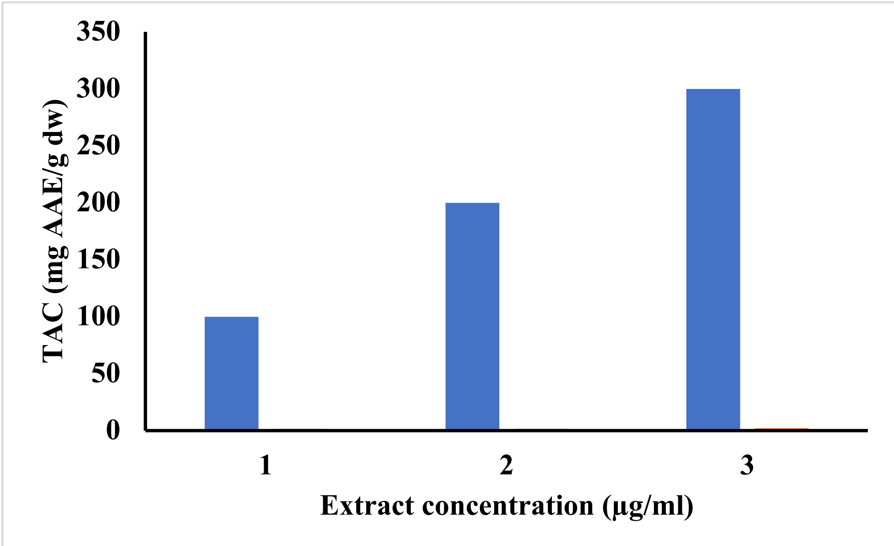
Graph 12: Total antioxidant activity of isolated endophytic fungal strain- AL01-04



Graph 13 Total antioxidant activity of isolated endophytic fungal strain- AL02-01



Graph 14: Total antioxidant activity of isolated endophytic fungal strain- AL02-02



Graph 15: Total antioxidant activity of isolated endophytic fungal strain- AL02-03

(b) DPPH antioxidant activity:

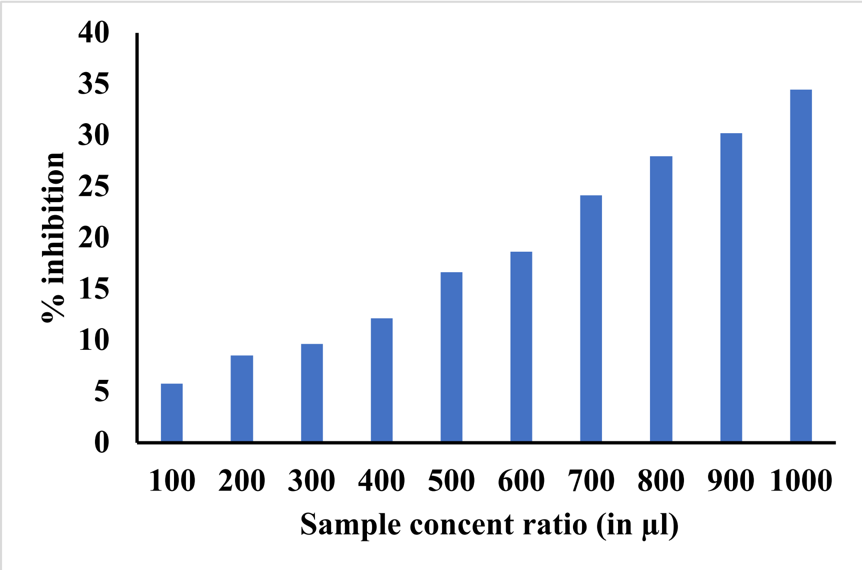
The DPPH radical scavenging activity, expressed as percentage inhibition, increased in a **concentration-dependent manner** for all fungal isolates (100–1000 µL). At the lowest concentration **(100 µL), AL02-01 (22.03%)** exhibited the highest inhibition, followed by **AL02-02 (17.83%)** and **AL02-03 (17.08%),** while **AL01-01 (5.8%)** and **AL01-04 (9.12%)** showed comparatively lower activity. As the concentration increased, all isolates showed enhanced scavenging activity. At **500 µL**, inhibition ranged from **AL01-01 (16.67%)** to **AL02-01 (29.86%),** and at the maximum concentration **(1000 µL**), the highest activity was observed in **AL02-02 (43.28%),** followed closely by **AL02-03 (42.53%)** and **AL02-01 (40.02%). AL01-01 (34.47%)** and **AL01-04 (35.56%)** displayed moderate inhibition (Table 5).

Overall, isolates **AL02-01, AL02-02, and AL02-03** demonstrated stronger DPPH radical scavenging activity compared to AL01 isolates, indicating superior antioxidant potential.

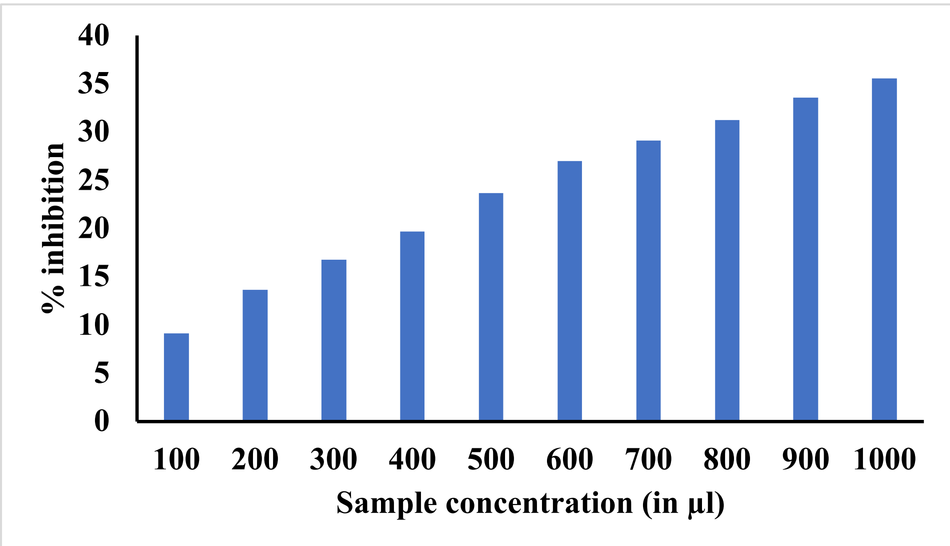
Table 5: DPPH Antioxidant activity of isolated endophytic fungal strains

Sample concentration (in µl)	% inhibition				
	AL01-01	AL01-04	AL02-01	AL02-02	AL02-03
100	5.8	9.12	22.03	17.83	17.08
200	8.5	13.68	24.26	21.09	19.89

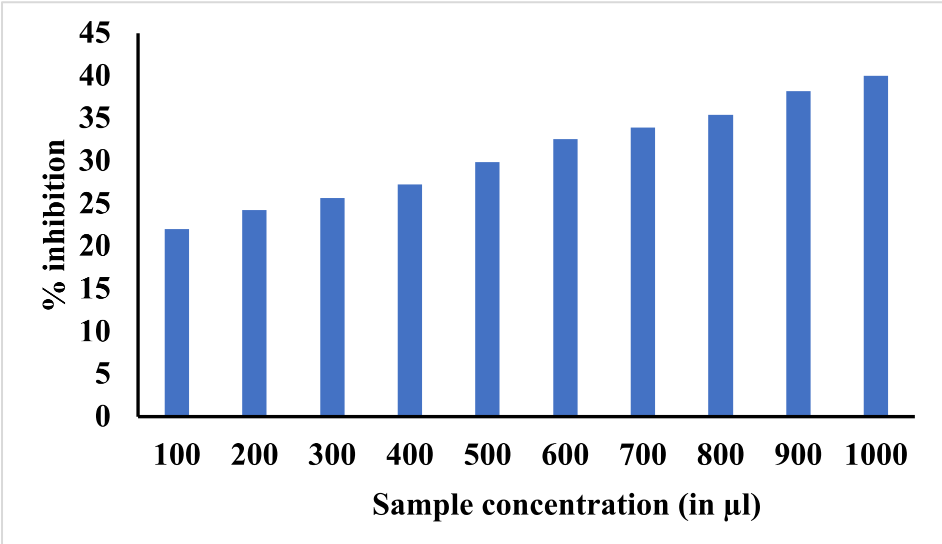
300	9.64	16.79	25.66	23.22	22.60
400	12.13	19.69	27.26	26.59	25.90
500	16.67	23.69	29.86	28.92	28.22
600	18.66	27.00	32.55	31.31	30.70
700	24.15	29.13	33.90	34.37	33.73
800	27.99	31.25	35.45	37.63	36.78
900	30.22	33.59	38.20	40.53	39.66
1000	34.47	35.56	40.02	43.28	42.53



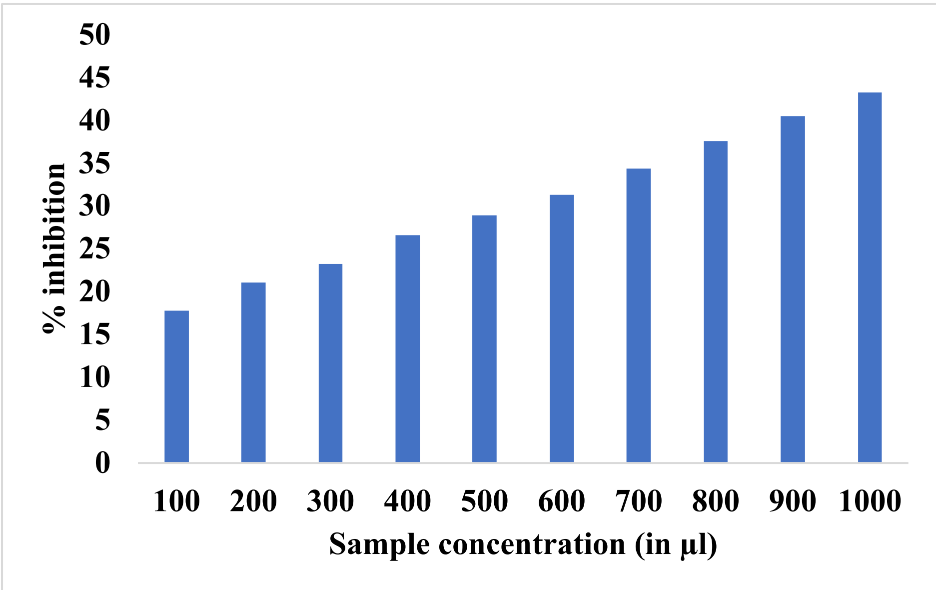
Graph16: DPPH Antioxidant activity of isolated endophytic fungal strain- AL01-01



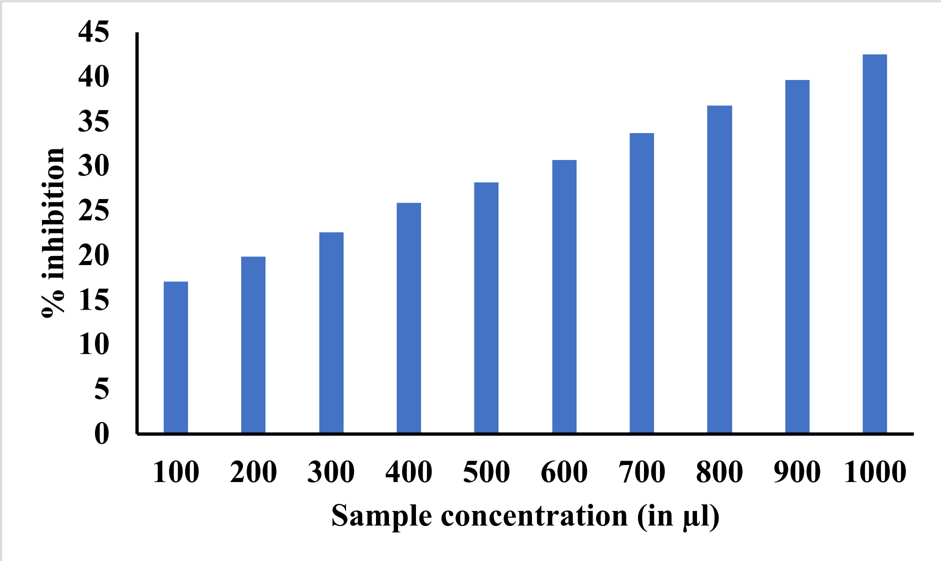
Graph17: DPPH Antioxidant activity of isolated endophytic fungal strain- AL01-04



Graph18: DPPH Antioxidant activity of isolated endophytic fungal strain- AL02-01



Graph19: DPPH Antioxidant activity of isolated endophytic fungal strain- AL02-02



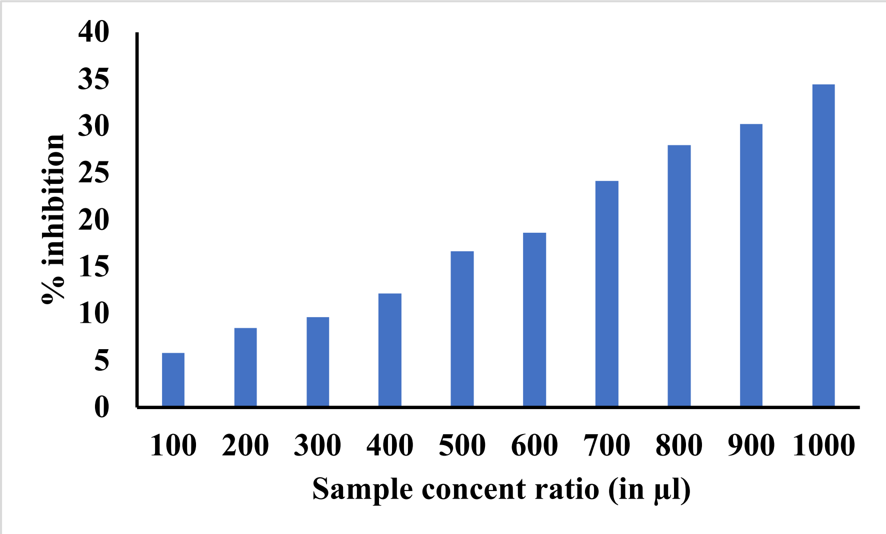
Graph20: DPPH Antioxidant activity of isolated endophytic fungal strain- AL02-03

(c) FRAP antioxidant activity:

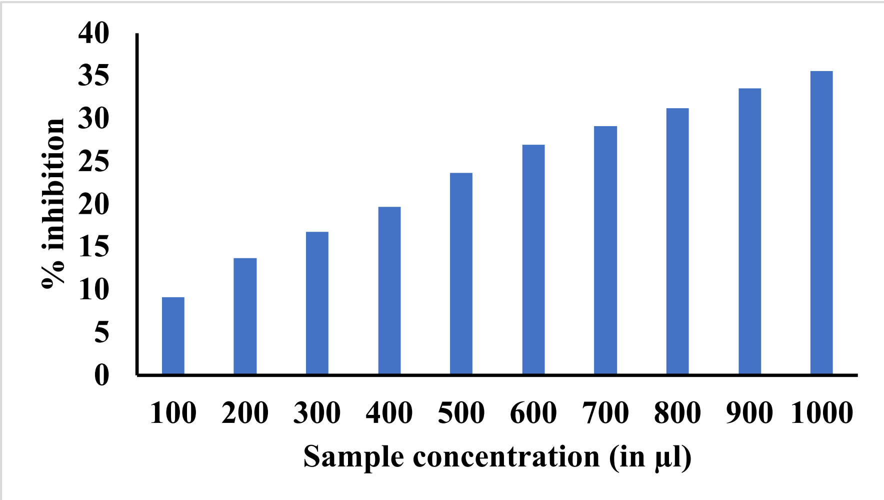
The FRAP assay, expressed in µM Fe^{2+} equivalents, revealed differences in the reducing power of fungal extracts across isolates and concentrations (100–300 µL). At the lowest concentration **(100 µL), AL02-01 (314.45 µM)** showed the highest reducing power, followed by **AL01-01 (289.90 µM),** while **AL02-03 (267.27 µM)** and **AL02-02 (275.36 µM)** exhibited the lowest activity. At **200 µL,** the FRAP values increased for all isolates, with **AL02-01 (349 µM)** and **AL01-01 (327.18 µM)** maintaining the highest reducing power. Similarly, at **300 µL, AL02-01 (382.63 µM)** exhibited the strongest antioxidant activity, followed by **AL01-01 (328.99 µM),** whereas **AL02-02 (287.18 µM)** and **AL02-03 (290 µM)** showed comparatively lower activity (Table 6).

Table 6: FRAP Antioxidant activity of isolated endophytic fungal strains

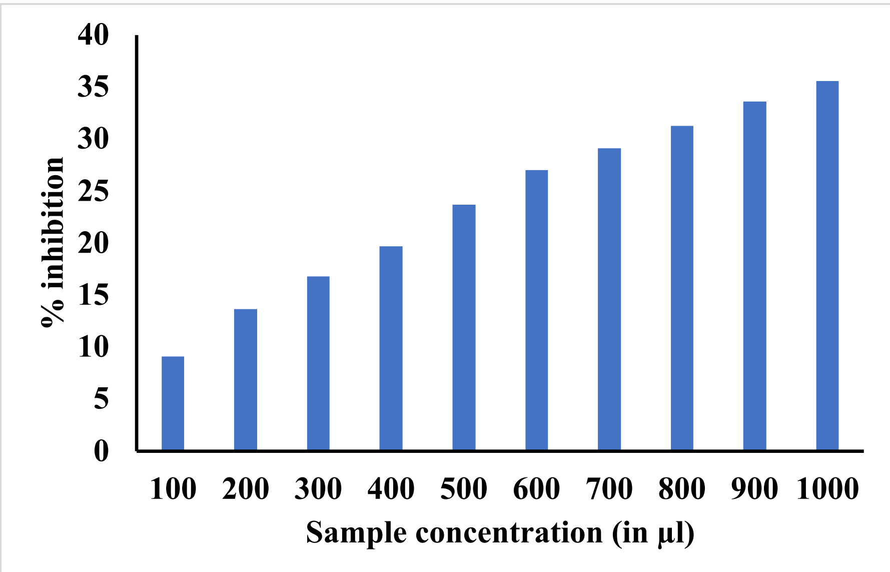
Sample concentration (in µl)	**FRAP value (µM) Y= 0.0011X + 0.1041**				
	AL01-01	**AL01-04**	**AL02-01**	**AL02-02**	**AL02-03**
100	289.90	280.82	314.45	275.36	267.27
200	327.18	308.09	349	278.09	279.09
300	328.99	309	382.63	287.18	290



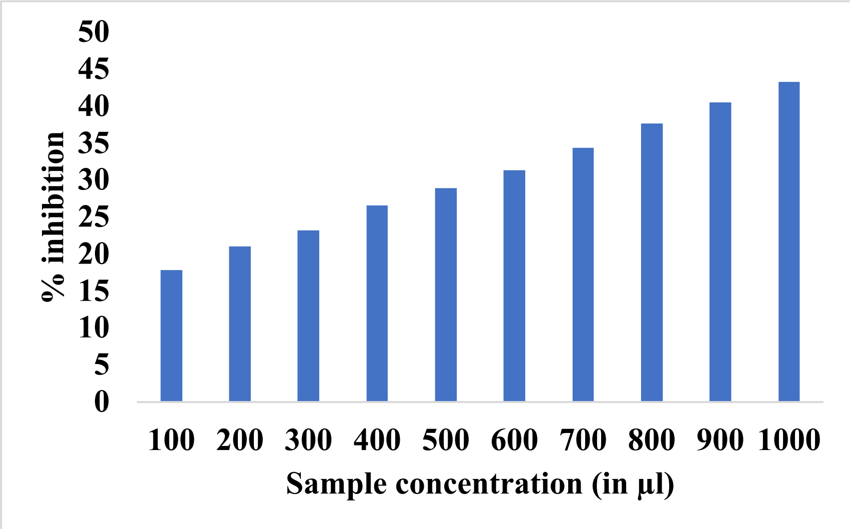
Graph 21: FRAP Antioxidant activity of isolated endophytic fungal strain- AL01-01



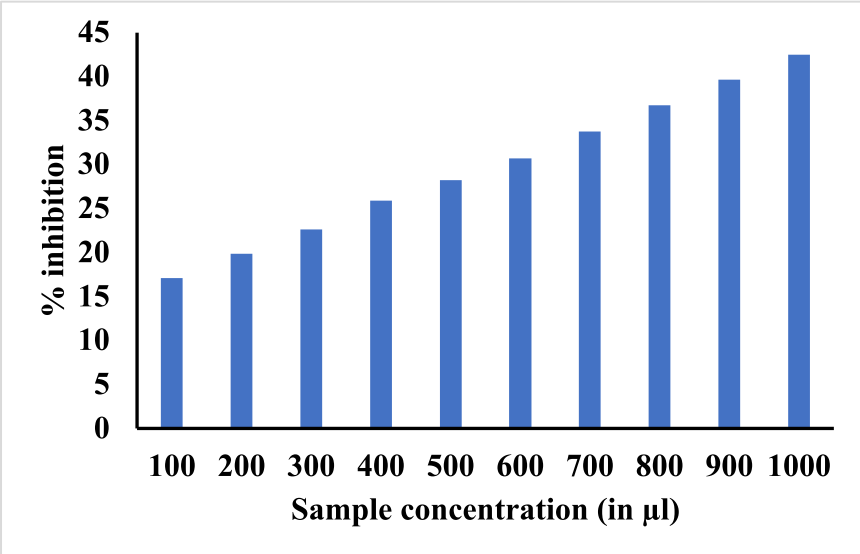
Graph 22: FRAP Antioxidant activity of isolated endophytic fungal strain- AL01-04



Graph 23: FRAP Antioxidant activity of isolated endophytic fungal strain- AL02-01



Graph 24: FRAP Antioxidant activity of isolated endophytic fungal strain- AL02-02



Graph 25: FRAP Antioxidant activity of isolated endophytic fungal strain- AL02-03

4. Discussion

The present investigation revealed that *Ampelocissus latifolia* leaves harbor diverse endophytic fungi belonging to the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, and *Acremonium*. These genera are frequently reported as dominant endophytes in medicinal plants and are known producers of bioactive secondary metabolites (Alam, 2021; Gupta et al., 2018). The isolation of five morphologically distinct strains from 150 leaf segments indicates a considerable colonization frequency and supports the concept that medicinal plants act as reservoirs of metabolically versatile endophytic fungi.

Preliminary phytochemical screening demonstrated the presence of phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids in the fungal extracts, with AL02-01 and AL02-03 showing the richest metabolite profiles. These findings are consistent with the report of Deshmukh et al. (2018), who documented that endophytic fungi isolated from medicinal plants produce diverse phenolic and flavonoid compounds contributing to antioxidant activity. Similarly, Prabakaran et al. (2019) observed a positive correlation between total phenolic content (TPC) and antioxidant potential in fungal endophytes, supporting our observation that isolates with higher phenolic and flavonoid content exhibited stronger antioxidant responses. Quantitative analysis in the present study showed that AL01 isolates (particularly AL01-01 and AL01-04) possessed higher TPC and TFC values compared to AL02 isolates. However, antioxidant assays (DPPH and FRAP) revealed that AL02-01, AL02-02, and AL02-03 exhibited superior radical scavenging and reducing power activity. This variation suggests that antioxidant potential is not solely dependent on total phenolic or flavonoid concentration but may also involve the qualitative nature of metabolites and synergistic interactions among secondary compounds. A similar observation was reported by Guha et al. (2010), where total antioxidant capacity measured by the phosphor molybdenum assay did not always directly correspond to phenolic concentration, indicating the contribution of multiple antioxidant mechanisms.

The DPPH assay results demonstrated a clear concentration-dependent increase in radical scavenging activity, with AL02-02 (43.28%) and AL02-03 (42.53%) showing the highest inhibition at 1000 µL. Comparable findings were reported by Deshmukh et al. (2018), who highlighted that endophytic fungi from medicinal hosts exhibit strong DPPH scavenging activity due to phenolic and flavonoid metabolites. Likewise, FRAP assay results confirmed that AL02-01 displayed the highest reducing power (382.63 µM at 300 µL), aligning with the findings of Prabakaran et al. (2019), who emphasized that fungal endophytes with elevated reducing power are promising sources of natural antioxidants.

Overall, the present study is in agreement with previous reports that endophytic fungi from medicinal plants are valuable sources of antioxidant compounds (Alam, 2021; Wen et al., 2022). However, this is among the first reports describing the isolation and antioxidant profiling of endophytic fungi specifically from *Ampelocissus latifolia*. The strain-specific variability observed in our results further supports the hypothesis that host–endophyte interactions influence metabolite biosynthesis. Therefore, the potent antioxidant activity exhibited by AL02 isolates highlights their potential for further purification, characterization, and application in pharmaceutical and nutraceutical formulations.

5. Conclusion

The present study demonstrates that the leaves of *Ampelocissus latifolia* host diverse endophytic fungi capable of producing a variety of bioactive secondary metabolites, including phenolics, flavonoids, saponins, alkaloids, terpenoids, and tannins. Quantitative analyses revealed that AL01 isolates were rich in phenolic and flavonoid compounds, whereas AL02 isolates exhibited stronger antioxidant activities, as evidenced by DPPH and FRAP assays. The findings underscore the strain-specific variability in metabolite production and antioxidant potential, highlighting endophytic fungi from *A. latifolia* as valuable natural reservoirs for novel antioxidants. These isolates could serve as sustainable sources for developing natural antioxidant formulations with potential therapeutic applications..

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