Culturable endophytic fungal assemblages from *Styrax sumatrana* and *Stryax benzoin* and their potential as antifungal, antioxidant, and alpha-glucosidase inhibitory resources

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Benzoin resin, produced by the native Indonesian trees *Styrax sumatrana* and *Stryax benzoin*, has been incorporated into medical practices to treat wounds, erythema, and many other conditions for centuries. Endophytic fungi that reside within medicinal plants have antimicrobial, antioxidant, and α-glucosidase inhibitory capacities, contributing to plant health and derivative products. In this study, we determined the antifungal, antioxidant, and α-glucosidase inhibitory capacities of endophytic fungal isolates from three different tissues (leaves, bark, and stems) of *S. sumatrana* and *S. benzoin* trees. The genera of fungal isolates were determined by phylogenetic analysis of internal transcribed spacer sequences. A total of 58 fungal isolates were classified into 15 different fungal genera from eight taxonomic orders—Hypocreales, Botryosphaeriales, Glomerellales, Diaphorales, Pleosporales, Eurotiales, Xylariales, and Mucorales—with a pattern of host species specificity. Among these isolates, *Neopestalotiopsis* sp. 6431 was notably potent in 2,2-diphenyl-1-picrylhydrazyl inhibition (25.04 ± 0.27 mgTE/g), and α-glucosidase inhibitory activity (52.15 ± 10.08%).
antioxidant power-based antioxidant activity (197.49 ± 8.65 mgTE/g), and α-glucosidase inhibitory activity (52.88 ± 4.93%). This study revealed that Trichoderma sp. 6407, Phyllosticta sp. 6454, and Neopestalotiopsis sp. 6431 exhibited antifungal, antioxidant, and α-glucosidase inhibitory activities.

KEYWORDS
medicinal plant, benzoin resin, antifungal, antioxidant, Trichoderma, Phyllosticta, Neopestalotiopsis

Introduction

Humankind has long used fragrances for health, beauty, and ceremonial purposes. The burning of fragrant incense has been observed in numerous cultures and religious ceremonies (Nir, 2004; Smit, 2004; Baum, 2013; Ergin, 2014; le Maguer, 2015; Milburn, 2016; Gershon, 2019). Ancient Greek, early Christian, Jewish, and Islamic societies used or use various fragrances, including balsamic resin, in their ritual practices (Haran, 1960; Nir, 2004; Smit, 2004; Ergin, 2014; le Maguer, 2015; Gershon, 2019). Balsamic resin has been traditionally used in Chinese culture for ceremonial and medicinal purposes, and the tradition has spread to other Asian countries (Milburn, 2016). Such practices have made the substance a valuable trading commodity (Kashio and Johnson, 2001; Ergin, 2014; le Maguer, 2015).

Styrax trees produce a balsamic resin known as benzoin resin (Kashio and Johnson, 2001). Styrax sumatranus and Styrax benzoin are native Indonesian trees that are widely cultivated in North Sumatra, Indonesia, where their resin has been traditionally used for herbal remedies and cultural ceremonies. For such applications, benzoin resin is valuable as a nontimber forest product (Nurwahyuni et al., 2021). Benzoin resin and its derivatives are incorporated into incense, cosmetics, and pharmaceutical products for their anti-inflammatory, antioxidant, and antimicrobial properties (Sharif et al., 2016; Hidayat et al., 2018, 2019b). The radical scavenging activity of resin from S. sumatranus has been reported to have high potency as an antioxidant; hence, it is a good candidate as a natural antioxidant resource (Nurwahyuni et al., 2021). Despite supporting the livelihood of 70% of local people in North Sumatra, Indonesia (Iswanto et al., 2016), and their value as a natural antioxidant resource, Styrax plantations for benzoin resin have gradually decreased because of land conversion (Saputra and Lee, 2021). Such limitations in plantation areas necessitate innovations in the sustainable production of benzoin resins and their derivatives.

In most plants, endophytic fungi that colonize the inner tissue play significant ecological roles, such as strengthening plant defenses against pathogens and abiotic stressors (Arnold et al., 2003; Schardl et al., 2004; Mejia et al., 2008). Particularly, in medicinal plants, these fungi have been reported to have distinctive relationships with their hosts; they influence the plant’s secondary metabolite production and antioxidant enzyme functioning and even incorporate their own metabolites into the host plant’s tissues, which consequently enhances the plant’s ability to withstand stress (Zhao et al., 2010; Ogbe et al., 2020). However, whether benzoin resin is synthesized and accumulated by endophytic fungi that reside within these plants remains an intriguing question.

Naturally sourced antioxidants have desirable properties that may reduce the use of chemically synthesized additives in food products (Brewer, 2011). The carcinogenic potential and other health risks of synthetic additives have limited their use and instigated the search for natural antioxidants (Capitani et al., 2013). A similar trend has been observed for cosmetics and pharmaceutical products. If the capacity of such functional substances from endophytic fungi is better than that of the host plant, endophytic fungi could be a more manageable and sustainable source option. Endophytic fungi isolated from medicinal plants, such as Pinus roxburghii, Ginkgo biloba, Rauwolfia tetraphylla, and agarwood-producing trees Aquilaria and Gymnop is have been reported to have antimicrobial and antioxidant capacities (Xiao et al., 2013; Bhardwaj et al., 2015; Alurappa and Chowdappa, 2018). However, information on endophytic fungi from S. sumatranus and S. benzoin remains scarce (Elfiati et al., 2021; Slamet et al., 2021). Considering the well-known role of endophytic fungi in enhancing plant defense against pathogens and the antioxidant function of benzoin resin (Hidayat et al., 2018), this study aimed to determine the antifungal, antioxidant, and alpha-glucosidase inhibitory activities of endophytic fungi isolated from the leaves, stems, and bark of S. sumatranus and S. benzoin trees.

Materials and methods

Sample collection and endophytic fungal isolation

Asymptomatic (healthy) 25- to 30-year-old S. benzoin and S. sumatranus trees with heights between 15 and 20 m and
breast-high diameters between 15 and 25 cm were selected for this study (Slamet et al., 2021). Fragments were collected from plantations in North Sumatra Province, Indonesia, at elevations of 800–1,000 m asl and temperatures between 15 and 24°C. Tree fragments or plant organs (leaves, stems, or bark) were surface sterilized, and their inner parts were cut and planted aseptically on four chloramphenicol-supplemented isolation media: potato dextrose agar, Pachlewski, yeast dextrose agar, and yeast malt extract (Atlas, 2004; Hidayat et al., 2019a, 2021a); thereafter, successful endophytic fungal isolation was validated (Hidayat et al., 2019a, 2021a). The obtained fungal isolates were deposited in the Indonesian Tropical Forest Culture Collection.

Antifungal assay

A dual culture assay was used to screen fungal isolates for their ability to suppress mycelial growth of the plant pathogenic fungi *Fusarium* sp. (INTROF CC 0509) and *Trichoderma viride*, and *Aspergillus niger*. *T. viride*, and *A. niger* cultures were obtained from IPB University Culture Collection. Five-millimeter-diameter agar discs of endophytic and pathogenic fungal cultures were co-inoculated 3 cm apart on potato dextrose agar plates (90 mm diameter) and incubated at 25°C for 7 days. The percentage of inhibition (%I) was calculated using the following formula:

\[
%I = \left( \frac{J_c - J_t}{J_c} \right) \times 100\%
\]

where \(J_c\) is the pathogen’s radial outward growth (control) and \(J_t\) is its radial growth in the direction of the endophytic fungi (Hajieghrari et al., 2010). Each treatment was repeated five times.

Antioxidant and antidiabetic assays

Prior to phytochemical assays for bioactive compounds, fungal extracts were prepared according to the method described by Hidayat et al. (2019a), with three replicates for each assay. Antioxidant activities were determined using three approaches: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power (FRAP).

Determination of antioxidant activity via DPPH depletion followed the protocol described by Hidayat et al. (2021b). The effective concentration at which 50% of the radicals were scavenged (IC\(_{50}\) value) was obtained by interpolation from the linear regression analysis (Dewi et al., 2013). Concentrations of DPPH in ppm (based on IC\(_{50}\) estimation, \(E\)) were ranked as follows: \(E < 50\), very strong; \(50 \leq E < 100\), strong; \(100 \leq E < 150\), moderate; \(150 \leq E < 200\), weak; and \(E \geq 200\), undetected.

Measurement of antioxidant activity using the ABTS approach was based on Trolox equivalent antioxidant capacity. The activity was expressed as Trolox equivalent in fungal extracts (mg TE/g), where the capacity of the sample to neutralize ABTS radicals is equivalent to that of Trolox (Wang et al., 2013). ABTS radical solution was prepared as follows: ABTS solution (7 mM) was mixed with potassium persulfate (140 mM) at a 62.5:1 ratio for the stock solution. The mixture was then kept in a dark room at 25°C for 16 h. For measurement, an ABTS radical solution with a 0.7 absorbance value at 734 nm was prepared. The assay was performed by mixing sample solution (5 μL, 1 mg/mL) with 200 μL of ABTS radical solution. The mixture was kept in a dark room for 6 min before absorbance reading at 734 nm. A Trolox solution with a range of concentrations was used to construct the standard curve.

Antioxidant capacity was determined using the FRAP approach according to the methods described by Dudonné et al. (2009) with slight modifications. FRAP reagent was prepared with 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine, and 20 mM FeCl\(_3\) in a 10:1:1 ratio. Fungal
Fungi isolated from *Styrax sumatrana* and *Styrax benzoin*, their molecular phylogeny, and origin-based clustering

Fifty-eight fungal isolates were obtained from the bark, stems, and leaves of *S. sumatrana* and *S. benzoin* trees. Thirty-eight isolates were obtained from 14 bark, eight stem, and nine leaf samples of *S. sumatrana* grown in four types of isolating media. Twenty isolates from four bark, nine stem, and seven leaf samples of *S. benzoin* were obtained using the same set of agar media.

The genera of all 58 isolates were determined based on ITS sequences. The closest matching genera based on sequence similarity are shown in Figure 1. Almost all isolates belonged to the phylum Ascomycota, except *Lichtheimia* sp. 6410, which belonged to the phylum Mucoromycota. The 58 fungi identified in this study were classified into 15 genera belonging to 12 families that belong to eight orders: Hypocreales, Botryosphaeriales, Glomerellales, Diaphortales, Pleosporales, Eurotiales, Xylariales, and Mucorales (Figure 1). *Fusarium*, *Pestalotiopsis*, and *Neopestalotiopsis* were the most common genera, with 20, 9, and 8 isolates, respectively.

Six possible combinations of two variables (host species and host organ) from which endophytic fungal genera originated are presented in Figure 2. A shorter Euclidean distance indicates a closer relationship; that is, the two variables share more similarities. Conversely, a longer Euclidean distance indicates greater heterogeneity and fewer similarities. Clustering analysis of endophytic fungal species based on the combination of plant organs and host plant species revealed two large groups. Host plant species seemed to be more important than plant organs in determining the grouping. Fungal communities residing in the leaves, bark, and stem of *S. sumatrana* tended to have more similarities and were thus grouped into one distinct cluster. The other cluster consisted of fungal communities residing in the leaves, bark, and stem of *S. benzoin*. A slightly different pattern was observed in each host plant species: in *S. benzoin*, fungal diversities in bark and stem organs showed more similarities to those residing in leaves, whereas in *S. sumatrana*, fungal diversities in bark...
FIGURE 1
Neighbor-joining phylogenetic tree of 58 endophytic fungi isolated from Styrax sumatrana and Styrax benzoin and their respective reference based on fungal internal transcribed spacer sequence. Fungi from this study are presented as their isolate number followed by their National Center for Biotechnology Information accession number, whereas for reference sequences, species name, and accession number are given (Supplementary Table 1).
and leaves showed more similarities to each other than to those in the stem.

Antagonistic activity of endophytic fungal isolates against plant pathogenic fungi

All 58 endophytic fungal isolates exhibited in vitro growth inhibition of three plant-pathogenic fungi. The percentage inhibition ranged from 10.27 to 78.59%, 18.33 to 82.67%, and 6.31 to 73.57% for *Fusarium* sp., *T. viride*, and *A. niger*, respectively (Supplementary Table 2). Three most inhibiting isolates against *Fusarium* sp. were *Trichoderma* sp. 6395, *Trichoderma* sp. 6411, and *Trichoderma* sp. 6407, with averages of 78.59 ± 1.03%, 74.41 ± 3.61%, and 73.57 ± 7.56% inhibition, respectively. *Trichoderma* sp. 6407, *Fusarium* sp. 6413, and *Fusarium* sp. 6445 were the most inhibiting against *T. viride*, with 82.67 ± 4.94%, 76.00 ± 2.79%, and 69.00 ± 5.35% inhibition, respectively. *A. niger, Trichoderma* sp. 6407, *Neofusicoccum* sp. 6399, and *Trichoderma* sp. 6396 were the most inhibiting (73.57 ± 7.56%, 62.66 ± 3.25%, and 59.77 ± 10.50%, respectively) (Table 1).

Corroborating Wheeler and Hocking (1993), *Trichoderma* sp. 6407, which inhibited all three pathogenic fungi, displayed interaction type B against *T. viride*, where both fungi inhibited each other's growth and created less than 2 mm space between their colonies (Figure 3A, left). Both *Fusarium* spp. 6413 and 6445 exhibited interaction type C against *T. viride*, where the growth of each endophytic fungus was lower than that of the pathogen, followed by slower fungal growth when a barrier between colonies was apparent (Figure 3A, center and right). *Neofusicoccum* sp. 6399 and *A. niger* pathogen appeared to have type D interaction, where both cultures inhibited each other and created a space of 2 mm or more between colonies (Figure 3B, center). Interaction type E was observed in *Trichoderma* sp. 6395 and pathogenic *Fusarium* sp. dual cultures, where the pathogen's growth was smaller and covered by endophytic fungi; however, the growth of both fungi decreased, and a barrier between colonies was apparent (Figure 3C, center). Interaction type F was observed in the remaining dual cultures, where pathogen growth was smaller than that in the endophytic fungal cultures, which later covered the pathogen's colony (Figure 3C, center and right, and Figure 3B, left and right).

Antioxidant activity of endophytic fungal extracts

Antioxidant activities of the endophytic fungal extracts were measured using DPPH, ABTS, and FRAP assays.
TABLE 1. The high antagonistic activity of endophytic fungal isolates against plant pathogenic fungi, *Fusarium* sp, *Trichoderma viride*, and *Aspergillus niger* using dual culture assay*.

| Host plant      | Plant organ | Isolate number | Closest genus       | *Fusarium* sp. | *Trichoderma viride* | *Aspergillus niger* |
|-----------------|-------------|----------------|---------------------|----------------|---------------------|---------------------|
| *Styrax sumatran* | Bark        | 6395           | *Trichoderma*       | 78.59 ± 1.03   | 38.67 ± 3.80        | 48.00 ± 13.83       |
|                 |             | 6396           | *Trichoderma*       | 35.27 ± 19.14  | 58.67 ± 4.47        | 59.77 ± 10.50       |
|                 |             | 6411           | *Trichoderma*       | 74.41 ± 3.61   | 63.33 ± 1.18        | 53.63 ± 14.39       |
|                 | Stem        | 6407           | *Trichoderma*       | 73.57 ± 7.56   | 82.67 ± 4.94        | 73.57 ± 7.56        |
|                 |             | 6413           | *Fusarium*          | 65.00 ± 1.60   | 76.00 ± 2.79        | 55.10 ± 4.98        |
|                 |             | 6445           | *Fusarium*          | 63.43 ± 2.10   | 69.00 ± 5.35        | 44.49 ± 14.38       |
| *Styrax benzoin* | Stem        | 6399           | *Neofusicoccum*     | 56.99 ± 18.96  | 59.00 ± 4.01        | 62.66 ± 3.25        |

* A complete list of 58 endophytic fungal isolates against plant pathogenic fungi is presented in Supplementary Table 2; **values are presented as mean ± standard deviation, which were performed in triplicate. The three isolates that showed the highest values of pathogenic fungal growth inhibition are presented in bold font.

![FIGURE 3](image-url)  
**FIGURE 3** Inhibition of pathogenic fungi (placed on the left side of the plate); inhibition of (A) *Trichoderma viride*, (B) *Aspergillus niger*, and (C) *Fusarium* sp. by endophytic fungi (placed on the right side of the plate) in dual culture plates. The three highest inhibition percentages for each pathogen were obtained from isolates (left to right): (A) 6407, 6413, and 6445; (B) 6407, 6399, and 6396; (C) 6395, 6411, and 6407.

( Supplementary Table 2). Seven isolates with the highest values were exclusive for each method. The remaining three isolates were able to perform among the highest levels for the two methods. *Neopestalotiopsis* sp. 6431 extract performed the best in DPPH inhibition, with an average of 49.65 ± 0.80% or IC50 estimated concentration of 100.71 ppm, followed by *Trichoderma* sp. 6395, *Phyllosticta* sp. 6454, and *Fusarium* sp. 6430 extracts, tied at 37.59% or IC50 estimated concentration of 133.02 ppm. As previously mentioned, *Trichoderma* sp. 6395 was the most inhibiting in the dual culture assay against pathogenic *Fusarium* sp.

Estimated IC50 concentrations were all categorized as medium. For ABTS-based antioxidant activity, isolate 6412 extract led to an average of 28.41 ± 0.04 mg TE/g, closely followed by *Fusarium* sp. 6430 and *Phyllosticta* sp. 6454 extracts, with averages of 27.74 ± 0.34 and 25.04 ± 0.27 mg TE/g, respectively. The last two isolates were among the highest achievers of DPPH inhibition. *Neopestalotiopsis* sp. 6431, *Neopestalotiopsis* sp. 6450, and *Neofusicoccum* sp. 6399 were the three isolates with the highest antioxidant activities for the FRAP method: 197.49 ± 8.65, 167.39 ± 8.57, and 152.05 ± 32.40 mg TE/g, respectively. Moreover, the *Neopestalotiopsis* sp. 6431 extract exhibited the highest activity for both the DPPH and FRAP methods (Table 2).

An entirely different set of isolates, *Colletotrichum* sp. 6439, *Fusarium* sp. 6456, and *Fusarium* sp. 6444, produced extracts that contained the three highest concentrations of flavonoid compounds (155.79 ± 7.47, 108.86 ± 94.86, and 88.86 ± 14.49 mg QE/g, respectively) (Table 3). As for the total phenolic compounds, the three highest producers were fungal extracts from *Phyllosticta* sp. 6454, *Neopestalotiopsis* sp. 6431, and *Fusarium* sp. 6430 (110.87 ± 18.52, 43.02 ± 1.25, and 41.33 ± 1.50 mg GAE/g, respectively). These isolates were also the most inhibiting in the DPPH-based antioxidant capacity assay (Table 2).

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of the 58 endophytic fungal isolates ranged from 0.00 to 65.00% (Supplementary Table 2). Fungal extracts of *Pestalotiopsis* sp. 6416, *Neopestalotiopsis* sp. 6431, and *Phyllosticta* sp. 6454 were the most inhibiting at 65.00 ± 0.28%, 52.88 ± 4.93%, and 52.15 ± 10.08%, respectively (Table 4). *Phyllosticta* sp. 6454 repeatedly resurfaced among the top three isolates for DPPH inhibition, ABTS-based antioxidant capacity, phenolic content, and α-glucosidase inhibitory activity. Similarly, *Neopestalotiopsis* sp. 6431 was the top performer in four assays: DPPH inhibition, FRAP-based antioxidant
were performed in triplicate for each fungal extract at a final concentration of 100 µg/mL. QE: quercetin equivalent; GAE: gallic acid equivalent. The three isolates that achieved the highest values in the phytochemical assay are presented in bold font.

**TABLE 2** Fungal isolates with high antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assay.

| Host plant      | Plant organ | Isolate number | Closest genus   | DPPH Inhibition (%)** | IC50 (ppm) | ABTS (mg TE/g) | FRAP (mg TE/g) |
|-----------------|-------------|----------------|-----------------|-----------------------|------------|---------------|---------------|
| Styrax sumatrana| Bark        | 6395           | Trichoderma     | 37.59 ± 0.24          | 133.02     | 2.72 ± 0.06   | 15.27 ± 0.16  |
|                 |             | 6412           | Fusarium        | 26.24 ± 11.89         | 190.54     | 28.41 ± 0.04  | 93.99 ± 3.86  |
|                 | Stem        | 6454           | Phyllosticta    | 37.59 ± 0.05          | 133.02     | 25.04 ± 0.27  | 36.37 ± 2.22  |
|                 |             | 6430           | Fusarium        | 37.59 ± 0.56          | 133.02     | 27.74 ± 0.34  | 40.20 ± 7.69  |
| Styrax benzoin  | Stem        | 6399           | Neofusicoccum   | ND                    | ND         | 22.21 ± 0.89  | 152.05 ± 32.40 |
|                 |             | 6431           | Neopestalotiopsis | 49.65 ± 0.80         | 100.71     | 3.35 ± 0.14   | 197.49 ± 8.65  |
|                 |             | 6450           | Neopestalotiopsis | 7.27 ± 6.98         | 687.41     | 18.71 ± 1.36  | 167.39 ± 8.57  |

* A complete list of 58 endophytic fungal isolates against plant pathogenic fungi is presented in Supplementary Table 2; ** values are presented as mean ± standard deviation, which were performed in triplicate. Three isolates that achieved the highest values of antioxidant activity are presented in bold font; ***the final concentration of each fungal extract was applied at 100 µg/mL; ND: not detected; TE: Trolox equivalent.

**TABLE 3** Fungal isolates with high content of flavonoid by using aluminum chloride assay, and phenol by using Folin–Ciocalteau reagent assay.

| Host plant      | Plant organ | Isolate number | Closest genus   | Flavonoid (mg QE/g) | Phenol (mg GAE/g) |
|-----------------|-------------|----------------|-----------------|---------------------|-------------------|
| Styrax sumatrana| Bark        | 6444           | Fusarium        | 88.86 ± 14.49       | 27.10 ± 1.33      |
|                 |             | 6454           | Phyllosticta    | 47.88 ± 8.52        | 110.87 ± 18.52    |
|                 | Stem        | 6430           | Fusarium        | 30.72 ± 7.00        | 41.33 ± 1.50      |
|                 | Leaf        | 6439           | Colletotrichum  | 155.79 ± 7.47       | 3.88 ± 0.20       |
|                 | Stem        | 6456           | Neopestalotiopsis | 108.86 ± 94.86    | 6.21 ± 0.32       |
| Styrax benzoin  | Stem        | 6431           | Neopestalotiopsis | 33.52 ± 0.28       | 43.02 ± 1.25      |

* A complete list of 58 endophytic fungal isolates against plant pathogenic fungi is presented in Supplementary Table 2; ** values are presented as mean ± standard deviation, which were performed in triplicate. QE: quercetin equivalent; GAE: gallic acid equivalent. The three isolates that achieved the highest values in the phytochemical assay are presented in bold font.

**TABLE 4** Fungal isolates with high α-glucosidase inhibition determined by p-nitrophenyl-α-D-glucopyranoside as substrate.

| Host plant      | Plant organ | Isolate number | Closest genus   | α-glucosidase inhibition (%)** |
|-----------------|-------------|----------------|-----------------|-------------------------------|
| Styrax sumatrana| Bark        | 6416           | Pestalotiopsis  | 65.00 ± 0.28                 |
|                 |             | 6454           | Phyllosticta    | 52.15 ± 10.08                |
| Styrax benzoin  | Stem        | 6431           | Neopestalotiopsis | 52.88 ± 4.93                |

* A complete list of 58 endophytic fungal isolates active against plant pathogenic fungi are presented in Supplementary Table 2; ** values are presented as mean ± standard deviation and were performed in triplicate for each fungal extract at a final concentration of 100 µg/mL.

**Detection of bioactive compounds**

Based on the LC-HRMS results from base line (Figure 4A) and fungal crude extracts (Figures 4B–D), the crude extract of *Trichoderma* sp. 6407, which showed prominent antifungal activities, had eight peaks in total, with four main peaks indicating the active compounds (Figure 4B). The first four major peaks occurred at a retention time (tR) of 7.87 min, with a molecular ion (m/z) value of 279.2321 [M+H]+, and molecular formula C18H20O2. Bioactive compounds with such properties were predicted to be methyl hydroxysterpurate ethylidene acetel or xylarinorditerpene Q (Figures 4B, 5A1). The second four major peaks occurred at tR = 8.67 min, with a m/z value of 319.2251 [M+H]+, and molecular formula C18H32O2, which could be (1S,2S)-3-oxo-2-pentylcyclopentane-1-octanoic acid or 3,7-dimethyl-9-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)nona-1,6-dien-3-ol (Figures 4B, 5A2). The third four major peaks occurred at tR = 9.98 min, with an m/z value of 395.3310 [M+H]+, and molecular formula C28H42O, which were predicted to be ergosta-4,6,8,22E-tetraen-11β-OL (Figures 4B, 5A3). The last detected major peak...
occurred at $t_R = 10.91$ min, with a predicted $m/z$ value of 281.2459 [M+H$^+$], and molecular formula $C_{18}H_{32}O_2$, which were approximated to be 4-Me-6E,8E-16:2 methyl ester or 4-methyl-7,11-heptadecadienoic acid (Figures 4B, 5A4).

Several ionic predictions and molecular formulas for *Phyllosticta* sp. 6454 extracts, which showed antioxidant and α-glucosidase activity, were identified (Figures 4C, 5B). The LC–HRMS results showed at least 10 peaks for active compounds. The four largest peaks had a $t_R$ of 7.43, 8.25, 10.13, and 10.19 min (Figure 4C), with the following respective $m/z$ values and molecular formulas: $m/z = 463.1601$ [M+Na$^+$] and molecular formula $C_{27}H_{32}N_2O_4$ (predicted to be...
FIGURE 5
Mass chromatogram of metabolite compounds from extracted isolates Trichoderma sp. 6407 (A1–4), Phyllosticta sp. 6454 (B1–4), and Neopestalotiopsis sp. (C1–3).
SF2809-IV (4-hydroxy-3-[[3-(2-hydroxyethyl)-1H-indol-2-yl]-[(4-hydroxyphenyl)methyl]-1-methylquinolin-2-one] compound; m/z = 475.3167 [M+Na]+ and molecular formula C_{30}H_{44}O_{3} (predicted to be igniarine); m/z = 337.1663 [M+H]+ and molecular formula C_{12}H_{24}O_{6} (predicted to be corynechromone or cytosprone); and m/z = 808.3502 [M+Na]+ and molecular formula C_{37}H_{21}N_{7}O_{12} (Figures SB1–4).

For the active compounds in Neopestalotiopsis sp. 6431 extracts, which may be responsible for the exhibited antioxidant activities, at least nine peaks were predicted (Figure 4D). The three largest peaks were detected at a tR of 3.36, 10.92, and 11.05 min (Figure 4D), with the following respective m/z values and molecular formulas: m/z = 211.1445 [M+H]+ and molecular formula C_{11}H_{16}N_{2}O_{3}; m/z = 808.3502 [M+Na]+ and molecular formula C_{37}H_{21}N_{7}O_{12}; and m/z = 626.3495 [M+Na]+ and molecular formula C_{26}H_{46}N_{7}O_{4} (Figures SC1–3).

Discussion

All the closest matches of the isolates belong to the phylum Ascomycota, except for Lichtheimia, which belongs to Mucoromycota. The dominance of Ascomycota in endophytic fungal assemblages has also been observed elsewhere (Hamzah et al., 2018; Zhou et al., 2018; Nguyen et al., 2021). This study complements previous reports on endophytic fungi isolated from S. sumatrana and S. benzoin and adds the newly reported genera Lichtheimia, Penicillium, Pseudopestalotiopsis, and Colletotrichum from S. sumatrana and Botryosphaeria from S. benzoin (Hidayat et al., 2021a; Slamet et al., 2021). All of the closest-matched genera have been previously reported as endophytes (Shetty et al., 2016; Huang et al., 2020). Various factors are involved in the dynamics of endophytic fungal communities, including macroenvironmental factors such as season, geographic location (Mishra et al., 2012; Slamet et al., 2021), and water availability (Costa et al., 2018). Each microenvironmental biotic factor, including host species, host tissue/organ (Moricca et al., 2012; Slamet et al., 2021), and other coexisting endophytes or pathogens (Sicard et al., 2007), adds to the community dynamics. These complex mechanisms may act as selection pressures for endophytic fungi, leading to host species and/or host organ specificities (Slamet et al., 2021).

Clustering the endophytic fungal genera based on the plant host species and plant organs demonstrated a specific pattern only for the plant host species. Endophytic fungal communities in the bark and stems of S. benzoin clustered within the shortest Euclidean distance, indicating higher genus similarity and homogeneity between them. The fungal community in the leaves of the same host species was clustered at a longer distance but was still within the same cluster. Although these organs have different structures (Romero, 2014), the closer proximity of the bark and stem may accommodate fungal mycelia extending between these two organs and consequently having more shared species. In the other cluster, the fungal community residing in the bark and leaf organs of S. sumatrana shared more similarities than those residing in the stems of the same host plant species. This pattern of similarities indicates the tendency of fungal genus specificity to be based on host plant species. These observations are based on the genus taxonomic rank, which is limited to a less restrictive interpretation. Multi-locus taxonomy identification and/or an inclusive metagenomic approach is recommended for further studies to scrutinize these mechanisms.

Trichoderma sp. 6407, isolated from the stems of S. sumatrana, consistently showed the highest antifungal activity against all tested pathogenic fungi. Plant-protective members of the Trichoderma genus, including T. pubescens, produce a group of polypeptide antibiotics that may contribute to their antagonistic potential against fungal diseases in grapevine trunks (Degenkolb et al., 2006). Other species of Trichoderma have been shown to reduce disease severity by inhibiting the growth of the pathogenic fungus Rhizoctonia solani (Molan et al., 2008). The predicted methyl hydroxyesterpurate ethyldiene acetal or xylarinorditerpene Q compounds from Trichoderma sp. 6407 extracts were previously reported to be isolated from endophytic fungi and also have antifungal properties (Xie and Li, 1992; Wu et al., 2014; Chen et al., 2020). The predicted (1S,2S)-3-oxo-2-pentylcyclopentane-1-octanoic acid or 3,7-dimethyl-9-(2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)nona-1,6-dien-3-ol compounds have also been reported to be produced by endophytic fungi (Miersch et al., 1999; Lin et al., 2016). The prediction ergosta-4,6,8,22E-tetraen-11β-OL compound has previously been reported to be isolated from the fruiting body of Coprinus setulosus (Ma et al., 2018), whereas, the postulated 4-Me-6E,8E-16:2 methyl ester or 4-methyl-7,11-heptadecadienoic acid compounds were reported to be isolated from liquid cultures of Clonostachys rosea and Sperothrrix sp. and have the potential to inhibit the growth of MFC-7 cancer cells, F. oxysporum f. sp. lycopersici, T. viride, and Bacillus subtilis (Choudhury et al., 1994; Dias et al., 2015). In this study, the metabolites produced by Trichoderma sp. 6407 displayed four major peaks; however, other minor peaks still have the potential to represent other active compounds. Considering the vital role of endophytic fungi in strengthening plant defense against pathogens and promoting overall health (Mejia et al., 2008), purification and elucidation of the active compounds produced by Trichoderma sp. 6407 and further investigation of antifungal mechanisms are required to optimize their biocontrol potential.

Phyllosticta sp. 6454, which was isolated from the bark of S. sumatrana, is characterized by its high antioxidant and α-glucosidase activity. This genus is a widely distributed fungal endophyte and is found in 70 plant families
was also previously discovered in the bark of *S. benzoin* and was among the most commonly isolated genera in this study. This genus has been reported to produce antimicrobial and antioxidant agents, such as eugenol, myristaldehyde, lauric acid, and caprylic acid (Tanapichatsakul et al., 2019). However, based on the LC–HRMS results of the present study, secondary metabolites from *Phyllosticta* with antimicrobial activities have been previously reported (Taher et al., 2022) and may be related to the phenol and flavonoid contents of *Phyllosticta* sp. 6454 extracts.

**Neopestalotiopsis** sp. 6431, isolated from the stems of *S. benzoin*, also exhibited high antioxidant activity. *Neopestalotiopsis* is a common endophytic fungal genus (Hamzah et al., 2018; Azuddin et al., 2021) and was among the most commonly isolated genera in this study. This genus was also previously discovered in the bark of *S. benzoin* (Ilyas et al., 2019). A member of the genus *Neopestalotiopsis* has been reported to produce antimicrobial and antioxidant agents, such as eugenol, myristaldehyde, lauric acid, and caprylic acid (Tanapichatsakul et al., 2019). However, based on the LC–HRMS results of the present study, *Neopestalotiopsis* sp. 6431 may have produced different secondary metabolites.

**Fusarium** sp. 6430 isolated from the stems of *S. sumatrana* was the most frequently isolated genus in this study and displayed high antioxidant activity. This genus has been reported to exude diverse bioactive compounds and exert biocatalytic functions to enhance plant health. Secondary metabolites, aza-anthraquinones, isolated from an endophytic *F. solani* strain, the crude extract of which shows antimicrobial and antioxidant activities, have been reported to be potent bioactive compounds for anticancer and antimicrobial agents (Khan et al., 2018). *Fusarium* sp. evinced the production of a new antifungal and antimalarial cyclodepsipeptide, known as fusaripeptide (Ibrahim et al., 2018). The observed antioxidant activity and phenol content of *Phyllosticta* sp. 6454, *Neopestalotiopsis* sp. 6431, and *Fusarium* sp. 6430 suggests that these isolates are strong candidates for natural antioxidant sources. Future studies, including methods of purifying bioactive compounds, are required to further optimize their potential as bioresources.

Antioxidants are compounds that inhibit the initiation or propagation of chain oxidation reactions. The chemical structure of antioxidants, source of free radicals, and physicochemical properties of different sample preparations can provide different test results for antioxidant activity (Craft et al., 2012). Therefore, it is necessary to analyze the antioxidant activity of a specific sample type. In this study, antioxidant testing using DPPH, ABTS, and FRAP assays was conducted. The DPPH assay measures the ability of compounds to donate hydrogen to a stable DPPH• molecule, resulting in the formation of a purple color (520 nm). In contrast, the ABTS cation radical (ABTS••+), which absorbs light at 743 nm, changes to stable ABTS by accepting hydrogen from antioxidant compounds, resulting in solution decolorization (Chu et al., 2000). This method is based on a reduction reaction in an acidic atmosphere to a yellow Fe³⁺ (potassium hexacyanoferrate) complex compound to a bluish-green Fe²⁺ complex compound owing to electrons donated by antioxidant compounds (Craft et al., 2012).

Furthermore, we investigated the α-glucosidase inhibitory activity of the fungal extracts in this study. Phenolic compounds are antioxidants and can also inhibit natural α-glucosidase enzymes because they inhibit carbide enzymes owing to their ability to bind proteins (Zhang et al., 2015). This assay is based on the formation of p-nitrophenol, which results from the cleavage of p-nitrophenyl-α-D-glucopranose at 410 nm.

These findings lay the groundwork for further studies that will identify the compounds responsible for the observed antioxidant and α-glucosidase inhibitory activities and their underlying mechanisms, which will potentially guide the optimization of their production.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

**Author contributions**

DE, SF, LR, AA, RD, HR, MT, MR, AS, and AH contributed to the conceptualization, methodology, experiment, validation, analysis, resources, writing, review, and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Alurappa, R., and Chowdappa, S. (2018). Antimicrobial activity and phytochemical analysis of endophytic fungal extracts isolated from ethno-pharmaceutical plant Rauwolfia tetraphylla L. J. Pure Appl. Microbiol. 12, 317–332. doi: 10.22207/PAM.12.1.38

Arndt, A. E., Mejia, L. C., Kyflo, D., Rojas, E. I., Maynard, Z., Robbins, N., et al. (2003). Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl Acad. Sci. U.S.A. 100, 15649–15654. doi: 10.1073/PNAS.2533483100

Atlas, R. M. (2004). Handbook of microbiological media. Great Britain: Taylor & Francis. doi: 10.1201/9781420039726

Azuddin, N. F., Mohd, M. H., Rosely, N. F. N., Mansor, A., and Zakaria, L. (2021). Molecular phylogeny of endophytic fungi from rattan (Calamus castaneus griff.) spines and their antagonistic activities against plant pathogenic fungi. J. Fungi 7:301. doi: 10.3390/JOF7040301

Baum, J. M. (2013). From incense to idolatry: The reformation of olfaction in late medieval german ritual. Sixteenth Century J. 44, 323–344.

Bhardwaj, A., Sharma, D., Jadon, N., and Agrawal, P. K. (2015). Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of Pinus roxburghii. Arch. Clin. Microbiol. 6, 1–9.

Brewer, M. S. (2011). Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. Comput. Rev. Food Sci. Food Saf. 10, 221–247. doi: 10.1111/J.1541-4337.2011.00156.X

Capitani, C. D., Hatano, M. K., Marques, M. F., and Castro, J. A. (2013). Effects of optimized mixtures containing phenolic compounds on the oxidative stability of sausages. Food Sci. Technol. Int. 19, 69–77. doi: 10.1177/1702173912442184

Chen, H. P., Zhao, Z. Z., Cheng, G. G., Zhao, K., Han, K. Y., Zhou, L., et al. (2020). Immunosuppressive nor-isopimarane diterpenes from cultures of Fuscaria terreus. J. Nat. Prod. 83, 401–412. doi: 10.1021/acs.jnatprod.9b00889

Choudhury, S. R., Traquair, J. A., and Jarvis, W. R. (1994). 4-methyl-7,11-heptadecadienal and 4-methyl-7,11-heptadecadienonic acid: New antibiotics from Sperotheix flocculosa and Sperotheix rugulosa. J. Nat. Prod. 57, 700–704. doi: 10.1021/np50018a003

Chu, Y. H., Chang, C. L., and Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. 80, 561–566. doi: 10.1002/(SICI)1097-0010(200004)80:5<561::AID-JFA574-3.0.CO;2-4

Costa, D., Tavares, R. M., Baptista, P., and Lino-Neto, T. (2018). Diversity of fungal endophytic community in Quercus suber L. under different climate scenarios. Revista de Ciências Agrárias 41, 22–30. doi: 10.19048/RCA.17063

Craft, B. D., Kerrhard, A. L., Amarowicz, R., and Pegg, R. B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. Comput. Rev. Food Sci. Food Saf. 11, 148–173. doi: 10.1111/J.1541-4337.2011.00173.X

Degenkolb, T., Grafenthal, T., Berg, A., Nirenberg, H. I., Gams, W., and Brücker, H. (2006). Peptidomics: Screening for polypeptide antibiotics (peptibiotics) from plant-protective Trichoderma species. Chem. Biodivers. 3, 593–610. doi: 10.1002/CBDV.200690063

Dewi, R. T., Tachibana, S., and Darmawan, A. (2013). Effect on α-galactosidase inhibition and antioxidant activities of butyrolactone derivatives from Aspergillus terreus MC7:51. Med. Chem. Res. 23, 454–460. doi: 10.1007/S00044-013-0659-4

Dewi, R. T., Primahana, G., Septama, A. W., Angelina, M., Melliawati, L., Fairiah, S., et al. (2022). Quality control standardization of indonesian noni fruit (Morinda citrifolia) extract and evaluation of their angiotensin-converting enzyme inhibitory activity. Pharmacua 69, 709–717. doi: 10.3987/Pharmacua.69.e86854

Dias, A. C. D. S., Ruiz, N., Coutinet-Mossion, A., Bertrand, S., Dubois, M., Pouchas, Y. F., et al. (2015). The marine-derived fungus Clonostachys rosea, source of a rare conjugated 4-me-6L,8E-hexadecadienonic acid reducing viability of MCF-7 breast cancer cells and gene expression of lipogenic enzymes. Mar. Drug. 13, 4934–4948. doi: 10.3390/MD1308563

Dodunni, S., Vitrac, X., Coutriés, P., Woillez, M., and Méribon, J. M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 57, 1768–1774. doi: 10.1021/JF80311IR

Effati, D., Susilowati, D., and Hidayat, A. (2021). Isolation of endophytic fungi from styrax sumatrana tree from hambang basundutana, north sumatra. Earth Environ. Sci. 782:2045. doi: 10.1088/1755-1315/782/4/042045

Ergin, N. (2014). The fragrance of the divine: Ottoman incense burners and their context. Art Bull. 96, 70–97. doi: 10.1080/00043079.2014.877506

Gershon, I. (2019). Smells like divine spirit. Available online at: https://daily-jstor.org/smells-like-divine-spirit/ (accessed March 27, 2022).

Hajighatr, R., Torabi-Giglou, M., Mohammadi, M. R., and Devari, M. (2010). Biological potential of some iranian trichoderma isolates in the control of soil borne plant pathogenic fungi. Atr. J. Biotechnol. 7, 967–972. doi: 10.4314/sb.v7i7.58586

Hamah, T. N. T., Lee, S. Y., Hidayat, A., Terhem, R., Faridah-Hanum, I., and Mohamed, R. (2018). Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, rhizophora mucronata, and identification of potential antagonists against the soil-borne fungus, Fusarium solani. Front. Microbiol. 9:1707. doi: 10.3389/FMICB.2018.01707

Haran, M. (1960). The uses of incense in the ancient israelite ritual. Vetus Testamentum 10, 129. doi: 10.2307/1516131

Hidayat, A., Iswanto, A. H., Susilowati, A., and Rachmat, H. H. (2018). Radical scavenging activity of kemeyan resin produced by an Indonesian native plant, Styrax sumatrana. J. Korean Wood Sci. Technol. 46, 346–354. doi: 10.5658/WOOD.2018.46.4.346

Hidayat, A., Susilowati, A., Faulina, S. A., Effati, D., Imanuddin, R., and Turjaman, M. (2021a). Diversity of endophytic fungi isolated from benzoin-producing tree styrax sumatrana. Earth Environ. Sci. 762, 12002. doi: 10.1080/1755-1315/762/12002

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.974526/full#supplementary-material
Hidayat, A., Turjaman, M., Qamary, R., Imamuddin, R., Tohir, D., Rahmanto, R. G. H., et al. (2021b). Bioactive composition, antifungal, antioxidant, and anticancer potential of agarwood essential oil from deodorized (Gyrpaceae spp.) of papua island (indonesia). J. Appl. Pharm. Sci. 11, 070–078. doi: 10.7324/IAPS.2021.1101010

Hidayat, A., Turjaman, M., Faulina, S. A., Ridwan, F., Aryanto, Najmul, et al. (2021a). Antifungal and antifungal activity of endophytic fungi associated with agarwood trees. J. Korean Wood Sci. Technol. 47, 459–471. doi: 10.5658/WOOD.2019.47.4.459

Hidayat, N., Yati, K., Krisiani, E. A., and Gozun, M. (2019). Extraction and antioxidant activity test of black sumatra incense. AIP Conf. Proceed. 2193, 30017. doi: 10.1063/5.033954

Huang, L. Q., Niu, Y. C., Su, L., Deng, H., and Lyu, H. (2020). The potential of endophytic fungi isolated from cucurbit plants for biocontrol of soilborne fungal diseases of cucumber. Microbiol. Res. 231, 126369. doi: 10.1016/j.micres.2019.126369

Ibrahim, S. R. M., Abdallah, H. M., Elkahtay, E. S., Musayri, N. M., Asfour, H. Z., Zayed, M. F., et al. (2018). Fusariparptide a: New antifungal and anti-malarial cyclodepsipeptide from the endophytic fungus Fusarium sp. J. Asian Nat. Prod. Res. 20, 75–85. doi: 10.1080/14786419.2017.1320989

Ilays, M., Praptiw, Wulanaisi, D., Fathomni, A., and Agusta, A. (2019). An assembly of fungal endophytes isolated from medicinal plants collected from toba and samosir, north sumatra. Earth Environ. Sci. 308, 012070. doi: 10.1088/1755-1315/308/1/012070

Iswanto, A. H., Sulsiswati, A., Azhar, I., Riswan, S., Tarigan, J. E., et al. (2016). Physical and morphological properties of local styx woods from north tapanuli in indonesia. J. Korean Wood Sci. Technol. 44, 539–550. doi: 10.5658/WOOD.2016.44.5.59

Karamac, M., Kostiska, A., and Pegg, R. B. (2005). Comparison of radical-scavenging activities for selected phenolic acids. Polish J. Food Nutr. Sci. 55, 165–170.

Kashio, M., and Johnson, D. V. (2001). Monograph on benzoin (balsamic resin from styxus species. food and agriculture organization of the united nations. regional office for asia and the pacific. Bangkok: RAP. Publication, 21.

Khan, N., Afroz, F., Begum, M. N., Roy Rony, S., Sharmin, S., Moni, F., et al. (2018). Antioxidant and antimicrobial activity of endophytic fungus Phyllosticta sp. isolated from Aloe vera with antimicrobial activity on diabetic fallopiae. L67 isolated from aloe vera with antimicrobial activity. L. M., et al. (2021). Diversity of endophytic fungal species from styrax benzoin sp. isolated from timber forest product. J. Physics Conf. Ser. 914, 12041. doi: 10.1088/1755-1315/914/1/012041

Smit, P. B. (2004). Incense revisited: Reviewing the evidence for incense as a clue to the christian provenance of the greek life of adam and eve. Novum Testamentum 16, 335–340. doi: 10.1163/1472-6882-S.1201

Shetty, K. G., Ravigeoneira, D., Jayachandran, K., and Walker, D. (2016). Isolation and molecular characterization of the fungal endophytic microbiome from conventionally and organically grown avocado trees in south florid. Mycol. Progress 15, 977–980. doi: 10.1007/s11571-016-1219-3

Sicard, D., Peñnings, P. S., Grasséclent, C., Acosta, J., Kalz, O., and Shykoff, J. A. (2007). Specialization and local adaptation of a fungal parasite on two host plant species as revealed by two fitness traits. Evolution 61, 27–41. doi: 10.1111/j.1558-5566.2007.00103.X

Smlet, W. Y., Faulina, S. A., Hidayat, A., Suliswati, A., Elfaati, D., Bahayu, L. M., et al. (2021). Diversity of endophytic fungal species from styxus benzoin found in benzoin-producing locations in north sumatra. Earth Environ. Sci. 914, 12041. doi: 10.1088/1755-1315/914/12/12041

Srimvisan, K., Jagadish, L. K., Shenhagaramar, R., and Muthumary, J. (2010). Antioxidant activity of endophytic fungi Phyllosticta sp. isolated from Guazumam tomentosa. J. Pharm. Sci. 37, 34–71.

Taher, M. A., Tan, W. N., Chen, Y. J., Leong, C. C., Rashid, A. S., and Tong, W. Y. (2022). Metabolites characterisation of endophytic Phyllosticta falkiae L67 isolated from aloe vera with antimicrobial activity on diabetic wound microorganisms. Nat. Prod. Res. 2022, 1–6. doi: 10.1080/17556764.2022.2103172

Tambe, Y. D., and Bhambar, S. R. (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus tilicous Linn. wood extracts. RRPPP 2, 41–47.

Tamura, K., Nii, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. U.S.A. 101, 11030–11035. doi: 10.1073/PNAS.0420610101

Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. doi: 10.1093/molbev/msab120

Tanapichatskul, K., Khruengsai, S., Mongoot, S., and Pripdeevee, P. (2019). Production of eugenol from fungal endophytes Neosartorya fischeri and Diapetera sp. isolated from Cinnamomum laurentii leaves. PeerJ 7:e6427. doi: 10.7717/PEERJ/7833

Tani, M., Harimaya, K., Gyoob, Y., Sasaki, T., Takenouchi, O., Kawamura, T., et al. (2004). SF2809 compounds, novel chymase inhibitors from Cephalosporium sp. isolated from Cinnumomum laurae leaf. Acta Pharmacol. Sin. 6, 443–452. doi: 10.1111/j.1745-3610.2003.t01277

Wang, S. S., Wang, D. M., Pu, W. J., and Li, D. W. (2013). Phytochemical profiles, antioxidant and antimicrobial activities of three Potentilla species. BMC Complement. Altern. Med. 13.1–11. doi: 10.1186/1472-6882-13-321

Wheeler, K. A., and Hocking, A. D. (1993). Interactions among xerophile fungi associated with dried salted fish. J. Appl. Bacteriol. 75, 164–169. doi: 10.1111/1365-2672.993.B01030X

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). “Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics,” in PCR protocols,
Wikee, S., Lombard, L., Crous, P. W., Nakashima, C., Motohashi, K., Chukeatirote, E., et al. (2013). Phyllosticta capitatae, a widespread endophyte of plants. *Fungal Divers.* 60, 91–105. doi: 10.1007/S13225-013-0235-8

Wu, S. H., He, J., Li, X. N., Huang, R., Song, F., Chen, Y. W., et al. (2014). Guaiene sesquiterpenes and isopimarane diterpenes from an endophytic fungus *Sylaria* sp. *Phytochemistry* 105, 197–204. doi: 10.1016/j.phytochem.2014.04.016

Xiao, Y., Li, H. X., Li, C., Wang, J. X., Li, J., Wang, M. H., et al. (2013). Antifungal screening of endophytic fungi from ginkgo biloba for discovery of potent anti-phytopathogenic fungicides. *FEMS Microbiol. Lett.* 339, 130–136. doi: 10.1111/1574-6968.12065

Xie, J. L., and Li, L. P. (1992). New metabolites from the fungus *Stereum purpureum*. *Chin. J. Chem.* 10, 537–543. doi: 10.1002/cjoc.19920100610

Zhang, H., Wang, G., Beta, T., and Dong, J. (2015). Inhibitory properties of aqueous ethanol extracts of propolis on alpha-glucosidase. *Evid. Based Complement. Alternat. Med.* 2015:383. doi: 10.1155/2015/587383

Zhao, J., Zhou, L., Wang, J., Shan, T., Zhao, J., Zhou, L., et al. (2010). Endophytic fungi for producing bioactive compounds originally from their host plants. *Curr. Res. Technol. Educ. Trop. Appl. Microbiol. Microbial. Biotechnol.* 1, 567–576.

Zhou, J., Diao, X., Wang, T., Chen, G., Lin, Q., Yang, X., et al. (2018). Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China sea. *PLoS One.* 13:e0197359. doi: 10.1371/JOURNAL.PONE.0197359