Trop J Nat Prod Res, July 2024; 8(7):7899-7908 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Comparison of Phytocompounds and Antibacterial Activity of *Moringa oleifera* Leaves and its Endophytic Fungi on Different Environment Conditions

Eka Sukmawaty[1,6], Ahyar Ahmad[2, 3 *], Abdul Karim[2], Zaraswati Dwyana[4], Harningsih Karim[5], Masriany[6], Zulkarnain[6], Devi Armita[6], Rahmat F Alir[6], Siti H Larekeng[7,8]

[1]*Doctoral Program, Chemistry Department, Hasanuddin University, Makassar 90245, Indonesia*
[2]*Chemistry Department Hasanuddin University, Makassar 90245, Indonesia*
[3]*Puslitbang Biopolimer dan Bioproduk, Institute for Research and Community Services (LPPM), Hasanuddin University, Makassar 90245, Indonesia*
[4]*Biology Department, Hasanuddin University, Makassar 90245, Indonesia*
[5]*Pharmacy Department, School of Pharmacy Yamasi, Makassar 90222, Indonesia*
[6]*Biology Department, UIN Alauddin Makassar, Makassar 92118, Indonesia*
[7]*Research Collaboration Center for KARST Microbes BRIN-LPPM, Hasanuddin University, Makassar 90245, Indonesia*
[8]*Faculty of Forestry, Hasanuddin University, Makassar 90245, Indonesia*

ARTICLE INFO

Article history:
Received 06 June 2024
Revised : 15 June 2024
Accepted: 12 July 2024
Published online 01 August 2024

ABSTRACT

The bioactivity of plant secondary metabolites is influenced by the plants' environment and the symbiotic endophytic fungi in their tissues. This study aims to compare the effect of differences in agro-climatic conditions and geographical location on phytocompound and the antibacterial activity of *Moringa oleifera* methanol extract and their endophytic fungi. Samples were collected from 2 locations with different environmental conditions. The phytocompound analysis was conducted with FTIR and GCMS. The antibacterial activity was evaluated to the tested bacteria *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis*. Analysis of phytocompound revealed endophytic fungi have greater compounds than the leaves. Antibacterial activity in endophyte fungi and leaves in location 2 is higher than in location 2. Two isolates showed the best antibacterial activity from location 1 and location 2. EN5 and J14 had inhibition zones respectively against *E. coli* (30.5±0.7; 20±0), *S. aureus* (33.5±1.7; 19±4.2), *S. typhi* (30.5±0.7; 20±0) and against *B. subtilis*. (15.3±1.4; 11.5±0.7). Based on this, it was concluded that differences in environmental conditions affect the phytocompounds and bioactivity of Moringa leaves and their colonizing endophytic fungi.

Keywords: Bioactivity, Biocompounds, Microbe symbiont, Phytochemistry

Introduction

Moringa oleifera Lam is one of the main plants in the Moringaceae family. It is also known as 'drumstick' and magic tree. Each part is used for one purpose or another and has medicinal activity. These plants are an extraordinary source of nutrients and bioactive compounds.[1] This plant grows in tropical or arid areas of Asia and Africa with other shrubs.[2] Several bioactive compounds have been identified in Moringa leaves, namely vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, and oxalate and phytate.[3] Some of these compounds showed positive results when tested for various biological activities such as antibacterial, antifungal, anticancer, and other bioactivities.[4,5] The search for moringa bioactive compounds is mainly focused on the leaves.[6]

*Corresponding author. Email: ahyarahmad@unhas.ac.id
Tel: +62-411-586498

Citation: Sukmawaty E, Ahmad A, Karim A, Dwyana Z, Karim H, Zulkarnain M, Armita D, Alir RF, Larekeng SH. The Comparison of Phytocompounds and Antibacterial Activity of *Moringa oleifera* Leaves and its Endophytic Fungi on Different Environment Conditions. Trop J Nat Prod Res. 2024; 8(7):7899-7908 https://doi.org/10.26538/tjnpr/v8i7.37

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Moringa leaf extract exhibits inhibitory activity against Gram-negative and Gram-positive pathogenic bacteria as well as against pathogenic fungi such as *Klebsiella* sp, *Pseudomonas aeruginosa*, *Trichoderma* sp, *Aspergillus flavus*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Candida* sp, and *Escherichia coli*.[7,8,9] Metabolites produced by plants are physiological responses influenced by abiotic factors such as climate and geographical conditions.[10,11] Environment and agro-climatic conditions affect the composition of compounds and therapeutic components of medicinal plant species.[12] The resulting secondary metabolite profile variations can affect their biological activity.[13] Differences in the phytochemical content of *M. oleifera* grown from different places have been investigated previously. The concentrations of flavonoids, alkaloids, saponins, tannins, and glycosides of *M. oleifera* seeds are influenced by geographical location.[14] The tocopherol content of *M. oleifera* young leaves obtained from mountainous areas showed higher concentrations than those from coastal areas.[15] This study shows that geographical and environmental differences affect the antibacterial activity of active plant compounds. Therefore, choosing agroclimatic conditions that provide optimal compound activity is essential.

Currently, the search for sources of bioactive compounds has shifted to endophytic microbes as the search for natural compounds from plants has increased.[16] Endophytes are an alternative for producing bioactive compounds of plant origin derived from slow-growing and endangered plants. They are a solution when secondary metabolites are not commercially available and are difficult to synthesize because they are heavy molecules or molecules with complex structures.[17,18] Endophytic fungi inhabit plants without causing any symptoms. Several studies have reported the beneficial role of endophytic symbiosis, which positively influences the physiological activities of host plants, such as growth hormone production, increasing host

adaptation to abiotic stress, and producing stress-adapter metabolites that protect host plants from invasion by herbivores and pathogenic microbe.[19,20]

Numerous researchers have highlighted that these fungi act as chemical factories within plants, suggesting that the metabolites produced by cultured fungi could serve as alternatives to synthetic compounds and antibiotics. This is particularly important because microorganisms tend to develop resistance to synthetic antibiotics over time, and such compounds can accumulate in the environment without decomposition, posing risks to human and animal health. Endophytic fungi can produce bioactive compounds similar to the pharmacological activity of their host plants, making them attractive sources of secondary metabolites.[21,22] *M. oleifera* endophytes exhibit antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* growth.[23] Moreover, as with plants as hosts, the production of endophytic metabolites is influenced by agro-climatic and geographical conditions such as climatic conditions, geographic location, and soil conditions where endophytes colonize the host.[24] The interactions between host endophytes are influenced and shaped by the corporation of micro-ecological niches formed by the host plant's internal and external environment.[25]

Research on the effect of the environment on the biological activity of *M. oleifera* has been described previously. However, the impact on the endophytes that inhabit it still needs further study and comparison with the host. This study will reveal the comparison of the effect of agroclimatic and geographical conditions on the antibacterial activity of the *M. oleifera* leaves as the host and their endophytic fungus as new insight into endophytic fungi and host interaction.

Materials and Methods

Plant Collection

Sampling of Moringa leaves was conducted in two districts in South Sulawesi Province, Indonesia, in June 2022. The first location is Banca Hamlet, Bontongan Village, Baraka District, Enrekang Regency (3°17'32 "S, 119°46'16"E). The second location is Kassika Hamlet, Palaju Village, Arungkeke District, Jeneponto Regency (5 °40'17 "S, 119°48'19"E). Young and fresh leaves of moringa with bright green colours taken from 3-5 stalks under the shoots were used for this study. The samples were placed in a plastic container in a cool box and kept in the Laboratory for further research. Measurements of soil temperature, soil pH, soil moisture, and light intensity were carried out as indicators of environmental factors that affect the synthesis of secondary metabolites in Moringa plants. Soil temperature and pH were measured using a soil tester (Takemura, Indonesia). Air temperature, humidity, and light intensity measurements were carried out using an envirometer (Dekko, Indonesia), and altitude measurements were carried out using a GPS map (Garmin, Indonesia).

Extraction of the Metabolite Compounds

Extraction of the Moringa oleifera leaves compounds

Moringa leaves separated from the leaf stalks were macerated with 96% methanol (Fulltime, ACS) for three days. Then, the extract was filtered, and the filtrate obtained was evaporated using a rotary evaporator (Buchi, Swiss) to obtain the concentrated extract.

Extraction of the Moringa oleifera Endophytic compounds

Moringa oleifera endophytic fungi were cultured on Potato Dextrose Broth for 7 days at 30 °C. It was filtered, and the mycelium separated. The filtrate-containing metabolites compound was extracted with chloroform by liquid-liquid extraction. The filtrate obtained was evaporated to dryness using a rotary evaporator (Buchi, Swiss) to obtain concentrated extract.

Endophytic Fungi Isolation

The plant sample was surface sterilized following the method previously described[26] by immersing the sample in 70% ethanol (Fulltime, ACS) for 1-2 minutes, followed by immersion in 4% NaOCl for 1 minute, rinsing several times with sterile distilled water, and then drying on sterile filter paper. Samples were cut into ± 1 cm pieces and spread on PDA media (Merck) to which chloramphenicol had been added. The last sterile distilled water rinse was spread on PDA media (Merck) as a negative control. The medium was incubated for seven days at room temperature. Surface sterilization was successful if no colonies were found on the media control. The fungus colonies that grew were then purified based on the morphological characteristics of the colonies. Each pure isolate was characterized macroscopically and microscopically (Nikon, U.S.A). Colonies showing the same macroscopic characteristics were categorized as one isolate. Isolates from location 1 were coded with EN, and isolates from location 2 were coded with J. Microscopic observation was carried out by placing the isolate on a glass slide and dripping it with lactophenol cotton blue dye (Merck). The slide was closed and observed under a microscope with 40x magnification.

Antibacterial Activity Test

The antibacterial activity test was conducted to investigate the capability of endophytic fungi and Moringa Leaves to inhibit pathogen bacteria. The test bacteria used were *Escherichia coli* and *Salmonella* sp from the Gram-negative group and *Bacillus subtilis* and *Staphylococcus aureus* from the Gram-positive group. Chloramphenicol was used as the positive control.

Antibacterial Activity Test of M. oleifera leaves extract.

The antibacterial test was carried out using the Kirby-Bauer disc diffusion method. The test solution was made by dissolving 5 g of Moringa methanol extract in 5 mL of distilled water and shaking it until homogeneous. Different concentrations (25%, 50%, 75%, and 100%) of the test solutions were prepared, and the paper discs were soaked at each concentration. They were placed on MHA media (Merck) containing 0.1 mL of each bacterial culture, then incubated (Thermo Fisher Scientific, U.S) at 37°C for 24 hours. Testing was carried out in triplicate. The criteria inhibition are > 20 mm = Very strong***, 10-20 mm = Strong**, 5-10 mm =Medium*, >5 mm =No response.[28]

Antibacterial Activity Test of endophytic fungi

Antibacterial activity was tested using a modified agar plug diffusion method.[27] Endophytic moulds growing on PDA media (Merck) were collected using a cork borer with a diameter of 6 mm. The agar pieces were placed upside down on the MHA media (Merck), which had been inoculated with the test bacteria and incubated for 24 hours. After 24 hours, clear zones were observed. Testing was carried out in triplicate.

Identification of functional groups using FTIR

FTIR identification was conducted to investigate the functional group of the phytocompound on both extract sample. The Moringa leaves extract (1.5–2.0 g) and endophytic fungi were mixed gently with 200 mg of solid KBr and ground to make pellets. A standard device was used to pellet under vacuum and pressure (75 $kN.cm^{-2}$) for 2-3 minutes. This pellet was then used for functional group spectral analysis with FTIR (Thermo Fisher Scientific, U.S). The spectral resolution was 4 cm^{-1} with a 400-4000 cm^{-1} scanning range.

Identification of compounds by GC-MS

Identification of secondary metabolite compounds was carried out using GC-MS. The extract was injected into the GC-MS (Shimadzu, Japan). The injection temperature was set at 250 °C. The GC-MS column temperature was set at 70 °C and held for 1 minute, then increased to 7 °C at a speed of 7 °C /minute, then increased again at a speed of 7°C minute until the temperature was 250 °C and maintained for 5 minutes. The mass spectrum of GC-MS was interpreted using the National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns. The mass spectrum of the unknown component was compared to that of the known components in the NIST library to confirm the names, molecular weights, and structures of the test materials.

Identification of endophytic fungi

Identification of endophytic mould species showing the best

antibacterial activity was carried out by molecular identification using primers ITS1(5' –TCCGTAGGTGAACCTGCGG– 3') and ITS 4 (5' – TCTCCGCTTATTGATATGC – 3'). Fungal DNA extraction was performed using the Quick-DNA Fungal Miniprep Kit by Zymo Research, D6005. PCR amplification was conducted using 2× MyTaq HS Red Mix (Bioline, BIO25048). Phylogenic tree construction was carried out with MEGA 11 software (https://www.megasoftware.net/)

Data Analysis

The Environmental data were analyzed statistically with an independent Sample Test using IBM SPSS ver. 22. Significance at the 0.05 level.

Results and Discussion

Environmental data measurements were carried out to determine differences in environmental and geographical conditions at the two locations. Location 1 was Enrekang Regency, and location 2 was Jeneponto Regency. The measurement results showed that the temperature, humidity, and altitude significantly differed at both locations. Location 1 was higher than location 2, while the pH showed no difference at both locations (Table 1). The difference between both locations affects the diversity and bioactivity of plants and their endophytic fungi as an abiotic factor.

There were 34 endophytic fungi of *Moringa* leaves successfully isolated from both locations. Nineteen endophytic fungi isolates were from location 1, and 15 isolates from location 2. Each isolate showed different macroscopic and microscopic characters (Table 2).

Table 1. Environmental data at the sampling location (mean ± SD)

Location	Temperature (°C)	pH	Humidity (%)	Altitude (m AMSL)
Location 1	21.7±0.57*	7±0.0	2.8±0.76*	1335*
Location 2	27.7±0.57*	7±0.0	1.2±0.28*	12*

Table 2. Microscopic characters of *M. oleifera* leaf endophytic fungi. EN code from location 1, J code from location 2

Isolates	**Morphology Characteristic**			
	Colony Color	**Reverse color**	**Texture**	**Topography**
EN1	Green	Yellow	Velvety	Umbonate
EN2	Cream Chocolate	Black	Velvety	Flat
EN3	White-Orange	White-Orange	Granular	Flat
EN4	White	White	Velvety	Flat
EN5	White	Chocolate	Velvety	Flat
EN 6	Chocolate	Orange	Woolly	Flat
EN 7	Dark Grey	Black	Woolly	Flat
EN 8	White Chocolate	Dark Chocolate	Velvety	Flat
EN 9	Greenish White	Blackish Green	Velvety	Flat
EN 10	Grey-White	Yellowish White	Velvety	Rugose
EN 11	White	White	Velvety	Flat
EN 12	White	Yellow	Velvety	Flat
EN 13	Greenish Green	Green-White	Velvety	Flat
EN 14	White	Yellow	Woolly	Flat
EN 15	White	Yellowish White	Granular	Flat
EN 16	White	White	Cottony	Flat
EN 17	Blackish White	Brown-Black	Velvety	Flat
EN 18	Dark Grey	Black Ash	Woolly	Flat
EN 19	Black	Yellow-Black	Velvety	Flat
JP1	White-Orange	White-Orange	Granular	Flat
JP2	White-Gray	Grey	Granular	Umbonate
JP3	Grey	Black	Velvety	Flat
JP4	Grey Black	Black	Velvety	Flat
JP5	Greyish White	Greyish White	Velvety	Umbonate
JP6	Black	Black	Velvety	Flat
JP7	Grey	Black	Velvety	Flat
JP8	Dark Grey	Green-Black	Woolly	Flat

JP9	White	White	Velvety	Flat
JP10	Dark Grey	White Black	Woolly	Flat
JP11	Black, Orange White	White-Orange	Velvety	Flat
JP12	Yellowish White	Yellowish White	Velvety	Flat
JP13	Black, Orange, White	White, Grey, Black	Velvety	Flat
JP14	Dark Grey-White	Blackish White	Woolly	Umbonate
JP15	Grey	White	Velvety	Flat

The macroscopic characteristics observed were colour, texture, topography, exudate droplets, radial lines, and concentric lines on the fungus colonies growing on PDA media. The microscopic characters observed were hyphae, conidia, conidia shape, and other characteristics. Several biotic and abiotic factors influence endophytic colonization as a form of symbiosis with host plants, not only influenced by the type of symbiont interaction but also by environmental factors and geographic gradients. Environmental conditions affecting endophytic structures in host plants include temperature, humidity, light, geographical location, and vegetation.[29] In this study, environmental factors were observed to influence the presence of endophytic fungi on Moringa leaves: temperature, pH, humidity, and altitude.

This study showed differences in the diversity of endophytic fungi found at both locations. In addition, based on the morphological characterization of the fungi growing on PDA media, they did not show the presence of identical isolates at both locations. According to Jiang *et al*.[30], species and community structure of endophytic fungi from different areas show a low level of similarity even in the same plant species. Diversity of endophytic fungi characterized by differences in morphological characteristics was found more in location 1 (19 isolates) with high altitude, lower temperature, and higher soil moisture than in location 2 (15 isolates). The soil pH measurements did not show differences between the two locations, so the pH did not affect the differences in the diversity and populations of the endophytic fungi found. Therefore, the environmental factors discussed in this study are altitude, temperature, and humidity.

The previous study revealed that fungi isolated from *Poa* species at high altitude locations more than at low altitude[31], similar to the result obtained in this study. Although different isolates were reported in *Pinus ponderosa,* the trend of leaf endophytic diversity was higher at low elevations.[32] Several mechanisms can explain variations in the diversity and composition of endophytic fungi in plants at different environmental elevation gradients. Variations in the diversity of fungi may reflect the selection of similar host plants by the symbiont fungi at various altitudes.[33] Plants may choose symbiont functions based on the cost-benefit context obtained under certain environmental conditions.*[34]* Another mechanism is that the structure of the plant-fungal symbiont community is determined by selecting the right environment for biotic and abiotic adaptation to various gradients.[35]

Endophytic foliar colonizes plants through stomata. Its distribution can be through wind-borne spores and is influenced by external environmental factors such as precipitation, temperature, and relative humidity.[36] Temperature affects endophytic fungal communities within the same plant species at different locations on a large or local scale.[37] The highest diversity of endophytic fungi was found under cold conditions.[38] Apart from temperature, humidity, and elevation, soil nutrition also influences the production of plant secondary metabolites and indirectly influences the diversity structure and populations of endophytic fungi. However, in this study, no analysis of soil nutrition was carried out, so further analysis was needed to reveal the effect of the interaction of biotic and abiotic factors on the community structure of endophytic fungi. Because the interaction of biotic and abiotic factors that affect the composition of the endophytic fungal community is very complex, it is sometimes difficult to predict the response of the fungal symbiont community to climate differences.[39]

Endophytic-plant interaction is closely related to the production of secondary metabolites. As a symbiont that has inhabited plant tissues for a long time, coevolution occurs between the two and transfers horizontally, causing endophytic fungi to be able to produce secondary metabolites similar to their hosts.[40,41,42] *M. oleifera*, a host of isolated endophytic fungi, is a plant that produces various antibacterial metabolite compounds against pathogenic bacteria.[43,44,45,46] This study compared the antibacterial capabilities of methanol extracts from moringa leaves with their endophytic fungi in inhibiting Gram-positive and Gram-negative pathogenic bacteria. The antibacterial activity assay of *M. oleifera* extract was carried out using the Kirby-Bauer disc diffusion method. In contrast, the antibacterial assay of endophytic fungi was carried out using the agar plug diffusion method. This method is carried out by placing a plug piece of agar culture media on MHA media previously inoculated with the test bacteria. During its growth, the fungus produces compounds that diffuse into the media. When placed on the MHA medium, the compounds diffused from the agar plug, and the secreted compounds' antimicrobial activity was detected by forming a clear zone around the agar plug. The endophytic fungi isolate that showed the best inhibition at both locations were EN5 and J14 (Figure 1).

Antibacterial assay results indicated that environmental conditions significantly influenced the antibacterial activity of compounds produced by the Moringa plant as the host. While both Moringa extracts from Location 1 and Location 2 exhibited inhibitory activity against Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) and Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), Moringa extracts from location 1 demonstrated a comparatively higher antibacterial activity across all tested concentrations against the pathogenic bacteria compared to location 2 (Figure 2).

This finding aligns with previous research suggesting that plants grown at higher altitudes are rich sources of bioactive compounds.[47] Plant extracts originating from cold and wet areas showed more potent antibacterial activity than those from dry and hotter locations.[48] For instance, air humidity, longitudes, and altitudes of collecting locations positively affect the production of phenolic and triterpenoid compounds in Lingonberry.[49] Plants from higher altitudes have more effective antibacterial activity.[50] Similar results were observed in the antibacterial activity of its endophytic fungi. In general, endophytic fungi from location 1 with cooler environmental conditions, higher humidity, and higher altitude showed more effective antibacterial activity than those from location 2 with hotter conditions and lower altitude.

Endophytic fungi isolated from Moringa leaves from both locations showed inhibition activity against Gram-positive and Gram-negative bacteria. Eleven isolates from location 1 had inhibitory activity against *E. coli, S. aureus, S. typhi,* and *B. subtilis* bacteria, while from location 2, there were six isolates that showed similar properties. The endophytic fungi from location 1 showed higher inhibitory activity than those from location 2. The endophytic fungi isolate EN5 from location 1 showed the best activity of all isolates based on the clear zone formed. EN5 isolates had very strong inhibitory activity against *E. coli, S. aureus,* and *S. typhi* and potent inhibition of *B. subtilis*. The endophytic fungus isolate J14 from location 2 showed the best antibacterial activity of all isolates based on the clear zone formed. Isolate J14 showed very strong inhibition on *E. coli* and *S. typhi* bacteria and on *S. aureus* and *B. subtilis* bacteria (Table 3). Differences in the ability of endophytic fungi isolates probably come from differences in metabolite compounds produced by the host. Based on this, it is known that geographical location and climate differences affect the bioactivity of Moringa leaves as the host and

their colonizing endophytic fungi. The inhibitory ability of Moringa leaf extracts from different locations is consistent with the inhibitory ability of endophytic fungi. From these results, it can be inferred that endophytes are not only capable of producing compounds similar to their host, but further research indicates that the bioactivity of compounds produced by the host plant and endophytic fungi mutually

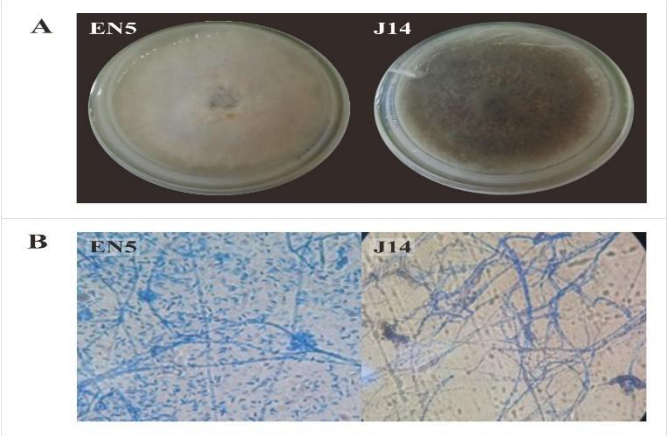

Figure 1. Colonies of the endophytic fungus of *M. oleifera* leaves (A) Macroscopic characteristics (B) Microscopic characteristics of isolates EN5 and J14.

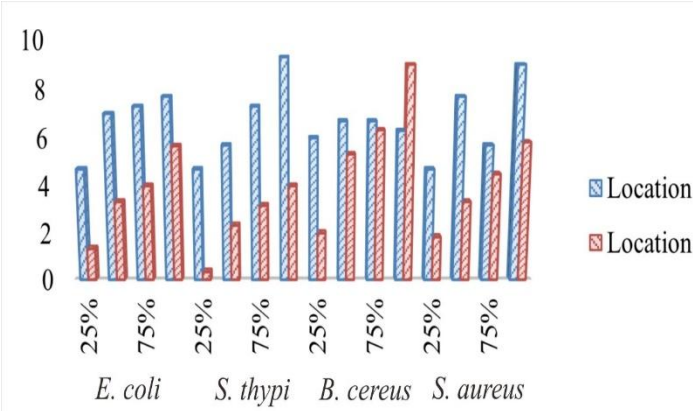

Figure 2. Antibacterial activity of methanol extract of *M. oleifera* against pathogenic bacteria

influence each other, possibly because they share metabolic pathways as a consequence of their symbiosis. This is consistent with the statement that Endophytes could influence plant metabolites' type, quantity, and quality. Furthermore, it may also influence the quality of these metabolites through microbial degradation or conjugation between plant and microbial metabolites.[51]

The ability of *M. oleifera* and their endophytic fungi isolates to inhibit bacterial growth comes from the secondary metabolites they produce. The endophytes of *C. gloeosporioides* have been reported before produce compounds with antibacterial properties in the form of phenol derivatives, colletotrin, 9-octadecenamide, hexadecanamide, diethyl phthalate, 2-methyl-3-methyl-3-hexene, 3-ethyl-2,4-dimethyi-pentane, Aureonitol, protocatechuic acid, and glucobrassicin and showed inhibition on Gram positive and Gram-negative bacteria.[52,53] The ability of *C. siamense* endophytes to inhibit the growth of human pathogenic bacteria has been reported before.[54,55,56,57] Based on endophytic phytochemical tests, *C. siamense,* can produce compounds from the class of flavonoids, alkaloids, tannins, saponins, and terpenoids.[58]

FTIR and GC-MS analysis were carried out to investigate further the compounds produced in moringa leaves and the endophytic fungi. The FTIR of Moringa leaves analysis results of the two extracts showed different spectral patterns, indicating that the two samples contained qualitatively different compounds (Figures 3 and 4). Generally, the absorption of endophytic fungi was greater than leaf extract. This shows that endophytes have more compounds than the leaves as the host. Although in this study, no in-depth investigation has been carried out on this matter, it is likely because endophytes produce more compounds to carry out their metabolism as individuals and produce compounds to stimulate the production of compounds from plants for various physiological and biochemical functions.

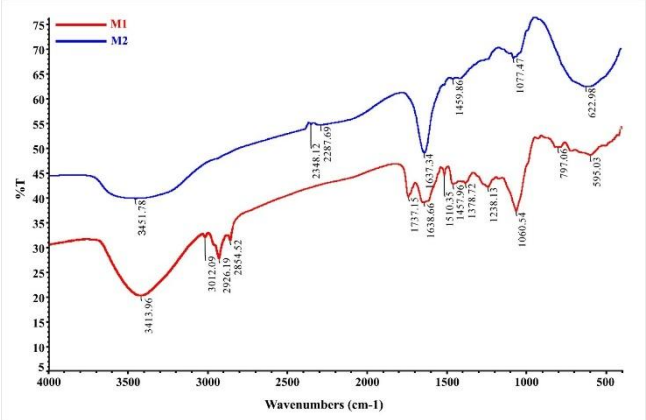

Figure 3. FTIR Spectra results of methanol extract of *M. oleifera* leaves in the 4000-400 cm^{-1} area

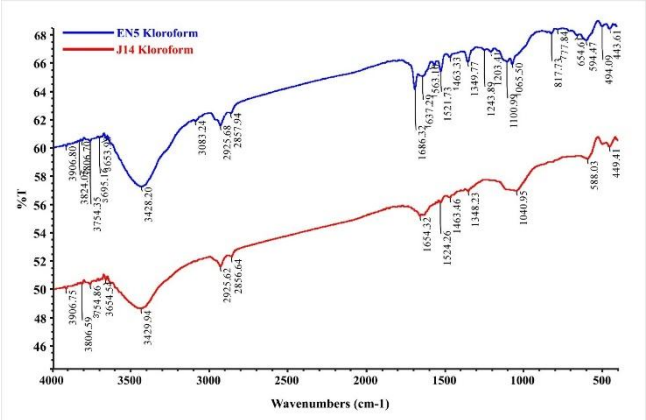

Figure 4. FTIR Spectra results of chloroform extract of *M. oleifera* leaves endophytic fungi in the 4000-400 cm^{-1} area

As a whole, the Sample in location 1 shows a varied fingerprint area compared to location 2, which indicates that the compounds produced in sample 1 were more diverse. This reveals an environmental influence on the compound content of *M. oleifera* leaves and their endophytic fungi from different locations. However, both extract samples showed the presence of the OH group at wave number 3413.96 cm^{-1} in sample location 1 and 3451.78 cm^{-1} in location 2. The region 3400–3200 cm^{-1} shows the polymer hydroxyl groups (-OH) H-bond stretching, characteristic of polyphenolic compounds. The existence of this wave number indicates that both samples contain phenolic compounds but at different concentrations, which are marked by differences in absorption intensity. The absorption at 1378.72 cm^{-1} indicates -OH bending for phenol compounds. phenolic C–O stretching was observed at ~ 1200 cm^{-1} in sample location 2. This stretching is typical of flavonoid C-rings. The presence of the C=O carbonyl group was also detected at wave number 1737.15 cm^{-1} in Moringa leaves at location 1, C=C aromatic ring at wave number 1510 -1450 cm^{-1}. CH bending aromatic compound at 1638.66 cm^{-1}. Aliphatic CH was detected at wave numbers 2926.19 and 2854.52 cm^{-1}. In Moringa leaves at location 2, absorption at 1459.86 cm−1 was detected as a C = C aromatic double bond belonging to the flavonoid compound. CH bending aromatic compound was detected at absorption 1637.34 cm^{-1}. The presence of the O=C=O strain was detected in absorption 2348.12 cm^{-1}. Terpenoids were detected by absorption in the wave number range 1459.86 cm^{-1}. The wave number

1490-1400 cm^{-1} is the absorption for OH and RCO_2H, indicating terpenoids' presence.

The components contained in Moringa leaves and their endophytic fungi at location 1 and location 2 were identified using GC-MS. The dominant compounds found in the Moringa leaves at location 1 sample were Levoglucosan (13.6%), Benzeneacetonitrile, 4-hydroxy (12.24%), and 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid (11.61%). While the dominant compound found in Moringa leaves at location 2 was 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (16.38%), Quinic acid (12.63%), and n-Hexadecanoic acid (11.22%) (Figure 5). Meanwhile, in endophytic fungi chloroform extract at location 1 the most dominant compound was 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (29.34%), followed by Phenol, 2,4-bis(1,1-dimethylethyl) (8.94%) and Benzene, 1,3-bis(1,1-dimethylethyl) (6.68%). At location 2, the endophytic fungi dominant compound was Bis(2-ethylhexyl) phthalate (26.46 %), phenol, 2,4-bis(1,1-dimethylethyl)- (6.96%), 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (5.00%) (Figure 6).

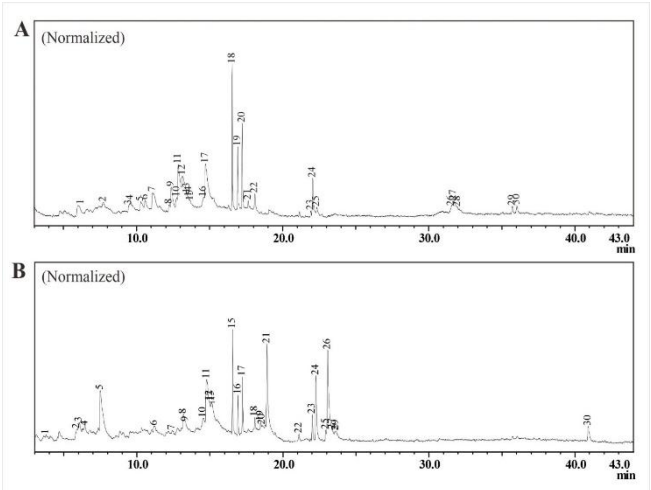

Figure 5. GC-MS chromatogram results of methanol extract of *M.. oleifera* leaves from location 1 (A) and location 2 (B)

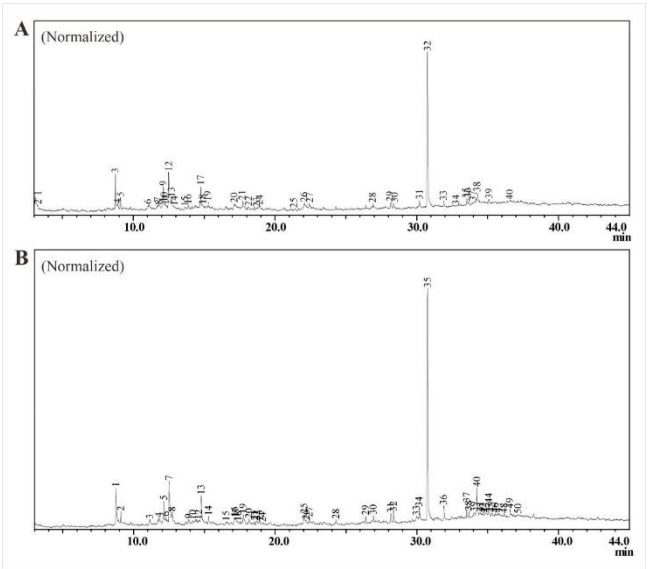

Figure 6. GC-MS chromatogram results of chloroform extract of *Moringa oleifera* leaves endophytic fungi from location 1 (A) and location 2 (B)

The differences in compounds *Moringa oleifera* leaves and endophytic fungi produce result from the response to their environment. In different environments, plants produce different compounds to adapt to specific environments. The metabolite compounds produced allow plants to survive biotic and abiotic stress. Internal and external factors influence the phytochemical content in plants. Internal factors such as genes and external factors include light, temperature, humidity, pH, nutrient content in the soil, and altitude of the location. As symbiont organisms that inhabit host tissue, the endophytic fungi also showed different metabolites produced by the abiotic factor that impacts plants' metabolites. The endophytic fungi produce specific metabolites that plants need to adapt to environmental habitat.

The results of molecular identification of isolates with the best antibacterial activity from both locations using primers Internal Transcribe Sequences 1 and 4 (ITS1 and ITS4) indicated that both endophytic fungi isolate belonged to the genus *Colletotrichum*. Isolates from location 1, namely *Colletotrichum gloeosporioides* and endophytic fungi isolated from location 2, were identified as *Colletotrichum siamense* (Figure 7).

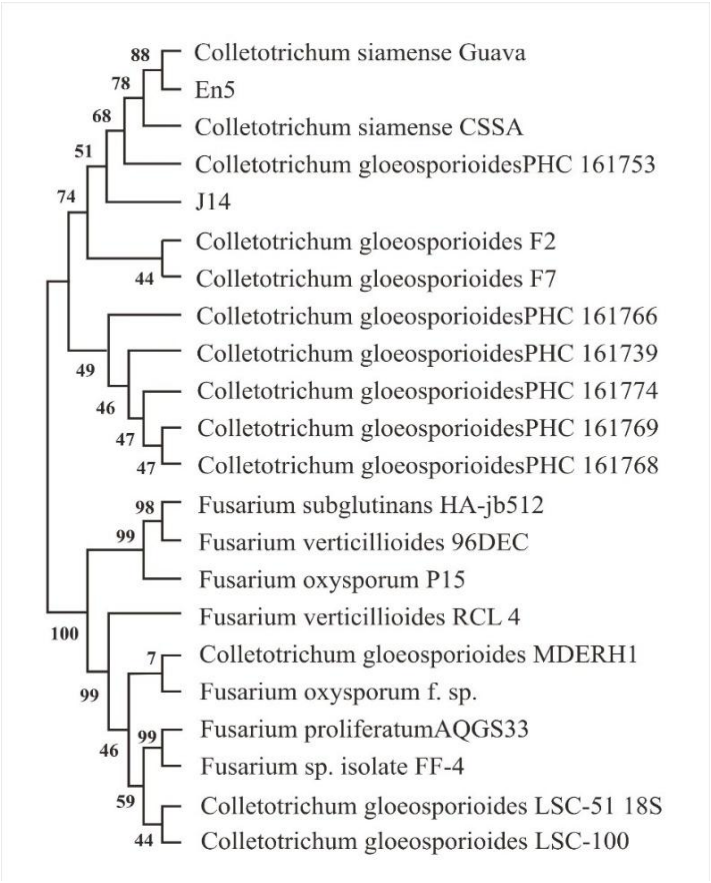

Figure 7. Phylogenic tree of isolate EN5 and Isolate J14 using MEGA 11 with the neighbor-joining method

Colletotrichum species have been reported to have a broad geographic and host distribution.[59,60] This study supports that the genus *Colletotrichum* was found at both locations with different environmental conditions. *Colletotrichum* is a common fungus found in pathogens, saprobes, and endophytes.[61] As an endophyte in plant tissues, *Colletotrichum* forms mutualistic interactions with plants by providing resistance to disease by producing antimicrobial compounds.[62,63] *Colletotrichum* has been found previously as an endophyte in *M.* oleifera.[64] They are also found in *Dendrobium spp*[65], *Huperzia serrata* (Thunb. ex Murray) Trev[66], *Palicurea corymbifera*[67], and other plants. Although *C. gloeosporioides* and *C. siamense* are plant pathogenic fungi, they show an endophytic lifestyle under other conditions. The lifestyle exchange of fungi as endophytes and pathogens is a complex mechanism. Fungi that live as endophytes on one host plant can become pathogens on other plants. The mechanism can be in the form of differences in the expression of fungal genes in plant responses or differences in the ability of plants to respond to

fungi. Under conditions of living as an endophyte, *C. gloeosporioides* expresses genes that code for glycoproteins that resemble plant cell wall proteins. These proteins coat fungal hyphae so plants do not recognise them as aliens.[68] The fact that a fungus can exhibit an endophytic lifestyle under certain conditions and transform into a pathogen under other circumstances demonstrates the complexity of biological reality.[69]

Table 3. Antibacterial activity of the endophytic fungus of *M. oleifera* against pathogenic bacteria

Isolate	Zone of Inhibition Diameter (mm) (mean±SD)			
	E. coli	*S. aureus*	*S. typhi*	*B. subtilis*
EN1	18.5±0.7**	17.5±0.7**	11.5±0.7**	0
EN2	16.5±0.7**	0	14±2.8**	6.5±0.7*
EN3	0	16±1.4**	20±2.1***	8.5±2.1*
EN4	21±1.4***	23±2.8***	19.5±0.7*	9±1.4*
EN5	30.5±0.7 ***	33.5±1.7***	30.5±0.7***	15.3±1.4**
EN6	0	0	0	0
EN7	6±1.4*	0	0	0
EN8	0	0	0	0
EN9	0	17±1.4**	21±1.4***	10.5±0.7**
EN10	16±1.4**	0	22±1.4***	5.5±0.7*
EN11	25.5±2.1***	22.5±0.7***	16.5±0.7**	11.5±2.1**
EN12	20±1.4***	16.5±0.7**	19±2.8**	6.5±2.1*
EN13	21±1.4***	16.5±0.7**	21.5±0.7***	10±1.4**
EN14	18.5±0.7**	0	24±1.4***	10±1.4**
EN15	21±1.4***	17.5±2.1**	23.5±2.1***	11±0.4**
EN16	25±1.4***	22±2.8***	0	12.5±2.1**
EN17	17±1.4**	17±2.8**	18±2.8**	6.5±0.7*
EN18	13.5±0.7**	18±1.4**	20.5±0.7***	11.5±0.7**
EN19	18.5±2.12**	21.5±0.7***	23±2.8***	6.5±0.7*
J1	14±0.7**	10±0**	15.5±6.3**	7±0*
J2	17.5±0.7**	15.5±0.7**	23.5±1.4***	12.5±0.7**
J3	0	0	0	0
J4	19±0.7**	18±2.8**	16.5±2.1**	9±2.8*
J5	21.5±0.7***	0	0	9±1.4*
J6	0	15.5±0.7**	15.5±2.1**	0
J7	0	0	0	7±0*
J8	7.5±0.7**	14±0**	18±0**	0
J9	13.5±0.7**	0	11±0**	8.5±2.1*
J10	13±0.8**	0	11±0**	11±0**
J11	0	14.5±1.4**	20±0***	10±1.4*
J12	0	0	18±4.2**	6.5±0.7*
J13	13±0**	17±0.1**	20±0***	6±0*
J14	20±0***	19±4.2**	20±0***	11.5±0.7**
J15	0	18±0**	20±0***	10.5±2.1**
Control	28.7±2.3***	26.3±1.5***	25.6±1.5***	23±0***

> 20 mm = Very strong*** , 10-20 mm = Strong**, 5-10 mm =Medium* , >5 mm =No response[28].

Conclusion

This study has shown that the *M. oleifera* leaves extract and their endophytic fungus from location 1 with high altitude, cooler

temperature, and high humidity showed higher antibacterial activity than endophytes from location 2 with lowland, hotter temperature, and lower humidity. Based on this, it was concluded that geographical location and climate differences affect the bioactivity of *Moringa* leaves as the host and their colonizing endophytic fungi. This suggests that endophytic fungi can produce compounds similar to their hosts. The identification results showed that the isolates with the best antibacterial activity from each location were *C. gloeosporioides* and *C. siamense*. The results of this study can be used to select host locations for endophytic fungi as a source of bioactive pharmacological compounds. For further research, it is necessary to analyze the differences in soil nutrients in the two locations as one of the factors that influence the production of metabolites.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original, and any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The author would like to thank Hasyimuddin M.Si for supporting sample collection and the Microbiology Lab Team for facilitating this research.

References

1. Paikra BK, Dhongade HKJ, Gidwani B. Phytochemistry and Pharmacology of *Moringa oleifera* Lam. J Pharmacopuncture. 2017;20(3):194–200.
2. Al-husnan LA, Alkahtani MDF. Impact of Moringa aqueous extract on pathogenic bacteria and fungi in vitro. Ann Agric Sci. 2016;61(2):247–250.
3. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. Int J Mol Sci. 2015;16(6):12791–12835.
4. Nizioł-Łukaszewska Z, Furman-Toczek D, Bujak T, Wasilewski T, Hordyjewicz-Baran Z. *Moringa oleifera L*. Extracts as Bioactive Ingredients That Increase Safety of Body Wash Cosmetics. Dermatol Res Pract. 2020;1:1-14.
5. Ade FY, Supratman U, Sianipar NF, Gunadi JW, Radhiyanti PT, Lesmana, R. A Review of the Phytochemical, Usability Component, and Molecular Mechanisms of *Moringa oleifera*. Trop J Nat Prod Res. 2022;6(12): 1906–1913.
6. Rani NZA, Husain K, Kumolosasi E. Moringa genus: A review of phytochemistry and pharmacology. Front Pharmacol. 2018;9(108):1–26.
7. Anzano A, de Falco B, Ammar M, Ricciardelli A, Grauso L, Sabbah M, Troselj V, Lanzotti V. Chemical Analysis and Antimicrobial Activity of *Moringa oleifera* Lam. Leaves and Seeds. Molecules. 2022;27(24):1–12.
8. Ervianingsih, Mursyid M, Annisa RN, Zahran I, Langkong J, Kamaruddin I. Antimicrobial activity of moringa leaf (*Moringa oleifera* L.) extract against the growth of Staphylococcus epidermidis. IOP Conf Ser Earth Environ Sci. 2019;343(1):6–10.
9. Oladeji OS, Odelade KA, Oloke JK. Phytochemical screening and antimicrobial investigation of *Moringa oleifera* leaf extracts. African J Sci Technol Innov Dev. 2020;12(1):79–84.
10. Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol Biochem. 2020;148:80–89.
11. Verma N, Shukla S. Impact of various factors responsible for fluctuation in plant secondary metabolites. J Appl Res Med Aromat Plants. 2015;2(4):105–13.
12. Barakat H, Ghazal GA. Physicochemical Properties of *Moringa oleifera* Seeds and Their Edible Oil Cultivated at Different Regions in Egypt. Food Nutr Sci. 2016;07(06):472–484.
13. Touré D, Kouamé BKFP, Bedi G, Joseph A, Guessennd N, Oussou R, Chalchat JR, Dosso M, Tonzibo F. Effect of geographical location and antibacterial activities of essential oils from Ivoirian Chromolaena odorata (L) R. M. King & Robinson (Asteraceae). J Pharmacogn Phyther. 2014;6(6):70–78.
14. Ukwueze CK, Okogwu OI, Ebem EC, Nwonumara GN, Nwodo JN. Evaluation of the Influence of Geographical Location on Phytochemical Composition of *Moringa oleifera* Seeds. World Appl Sci J. 2019;37(3):196–201.
15. Mubarak K, Natsir H, Wahab AW, Satrimafitrah P. Analysis of α-Tokopherol (Vitamin E) Extracted from Moringa Leaves (*Moringa oleifera* Lam) Collected from Seashore and Highland Areas and Its Potencyl as Antioxidant. Kovalen. 2017;3(1):78-88.
16. Tiwari P, Bae H. Endophytic Fungi: Key Insights, Emerging Prospects, and Challenges in Natural Product Drug Discovery. Microorganisms. 2022;10(360):1-43.
17. Zheng R, Li S, Zhang X, Zhao C. Biological activities of some new secondary metabolites isolated from endophytic fungi: A review study. Int J Mol Sci. 2021;22(2):1–75.
18. Rustamova N, Bozorov K, Efferth T, Yili A. Novel secondary metabolites from endophytic fungi: synthesis and biological properties. Phytochem Rev. 2020;19:425–448.
19. Odelade KA, Babalola OO. Bacteria, fungi, and archaea domains in rhizospheric soil and their effects in enhancing agricultural productivity. Int J Environ Res Public Health. 2019;16(20):3873.
20. Igiehon NO, Babalola OO, Cheseto X, Torto B. Effects of rhizobia and *Arbuscular mycorrhizal* fungi on yield, size distribution and fatty acid of soybean seeds grown under drought stress. Microbiol Res. 2021;242(126640):1-16.
21. Huang Y-L. Effect of Host, Environment and Fungal Growth on Fungal Leaf Endophyte Communities in Taiwan. J Fungi. 2020;6(4):244.
22. Jin J, Zhao Q, Zhang XM, Li WJ. Research progress on bioactive products from endophytes. J Microbiol. 2018;38:103–113.
23. Mosquera WG, Criado LY, Guerra BE. Antimicrobial activity of endophytic fungi from the medicinal plants *Mammea americana* (Calophyllaceae) and *Moringa oleifera* (Moringaceae). Biomedica. 2020;40(1):55–71.
24. Silva, Pereira D, Cardoso, Silva M, Macedo, Jose A. Endophytic Fungi as a Source of Antibacterial Compounds—A Focus on Gram-Negative Bacteria. Antibiotics. 2022;11(1509):1-45.
25. Li Z, Wen W, Qin M, He Y, Xu D, Li L. Biosynthetic Mechanisms of Secondary Metabolites Promoted by the Interaction Between Endophytes and Plant Hosts. Front Microbiol. 2022;13(928037):1-15.
26. Hamzah TNT, Lee SY, Hidayat A, Terhem R, Faridah-Hanum I, Mohamed R. 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. 2018;9:1–17.
27. El-Barnossi A, Moussaid F, Iraqi HA. Antifungal activity of Bacillussp. Gn-A11-18 isolated from decomposing solid green household waste in water and soil against *Candida albicans* and *Aspergillus Niger*. E3S Web Conf. 2020;150:02003.
28. Fadhila G.S AA, Darwis W, Wibowo RH, Sipriyadi S, Supriati R. Antibacterial Activity of the Ethanolic Extract of Sembung Rambat (*Mikania micrantha* Kunth) Leaves Against *Bacillus subtilis*. Nat Sci J Sci Technol.

2021;10(1):06–11.
29. Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin PL. A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. Front Microbiol. 2016;9(7):906.
30. Jiang S, Duan J, Tao J-H, Yan H, Zheng JB. Ecological distribution and elicitor activities of endophytic fungi in *Changium smyrnioides*. 2010;1(41):121–5.
31. Lyons KG, Mann M, Lenihan M, Roybal O, Carroll K, Reynoso K, Kivlin SN, Taylor DL, Rudgers JA. Culturable root endophyte communities are shaped by both warming and plant host identity in the Rocky Mountains, USA. Fungal Ecol. 2021;49:101002.
32. Bowman EA, Arnold AE. Distributions of ectomycorrhizal and foliar endophytic fungal communities associated with *Pinus ponderosa* along a spatially constrained elevation gradient. Am J Bot. 2018;105(4):687–699.
33. Cobian GM, Egan CP, Amend AS. Plant-microbe specificity varies as a function of elevation. ISME J. 2019;13(11):2778–2788.
34. Afkhami ME, Rudgers JA, Stachowicz JJ. Multiple mutualist effects: conflict and synergy in multispecies mutualisms. Ecology. 2014;95(4):833–844.
35. Kivlin SN, Lynn JS, Kazenel MR, Beals KK, Rudgers JA. Biogeography of plant-associated fungal symbionts in mountain ecosystems: A meta-analysis. Divers Distrib. 2017;23(9):1067–1077.
36. Crandall SG, Gilbert GS. Meteorological factors associated with abundance of airborne fungal spores over natural vegetation. Atmos Environ. 2017;162:87–99.
37. DeMers M, May G. Habitat-scale heterogeneity maintains fungal endophyte diversity in two native prairie legumes. Mycologia. 2021;113(1):20–32.
38. Aleynova OA, Nityagovsky NN, Suprun AR, Ananev AA, Dubrovina AS, Kiselev KV. The Diversity of Fungal Endophytes from Wild Grape *Vitis amurensis* Rupr. Plants. 2022;11(21):2897.
39. Del Olmo-Ruiz M, Arnold AE. Community structure of fern-affiliated endophytes in three neotropical forests. J Trop Ecol. 2017;33(1):60–73.
40. Mishra S, Priyanka, Sharma S. Metabolomic Insights Into Endophyte-Derived Bioactive Compounds. Front Microbiol. 2022;13:835931.
41. Tiwari A, Mahadik KR, Gabhe SY. Piperine: A comprehensive review of methods of isolation, purification, and biological properties. Med Drug Discov. 2020;7:100027.
42. Zhao J, Zhou L, Wang J, Shan T, Zhong L, Liu X, Gao X. Endophytic fungi for producing bioactive compounds originally from their host plants. Curr Res, Technol Educ Trop Appl Microbiol Microbial Biotechnol. 2010;1:567–576.
43. Ervianingsih, Mursyid M, Annisa RN, Zahran I, Langkong J, Kamaruddin I. Antimicrobial activity of moringa leaf (*Moringa oleifera* L.) extract against the growth of *Staphylococcus epidermidis*. IOP Conf Ser Earth Environ Sci. 2019;343(1):012145.
44. Fouad EA, Abu EASM, Kandil MM. Antibacterial efficacy of *Moringa oleifera* leaf extract against pyogenic bacteria isolated from a dromedary camel (*Camelus dromedarius*) abscess. Vet world. 2019;12(6):802–808.
45. Enerijiofi KE, Akapo FH, Erhabor JO. GC–MS analysis and antibacterial activities of *Moringa oleifera* leaf extracts on selected clinical bacterial isolates. Bull Natl Res Cent. 2021;45(179)1-10.
46. Eremwanarue OA, Shittu HO. Antimicrobial activity of *Moringa oleifera* leaf extracts on multiple drug-resistant bacterial isolates from urine samples in Benin City. Niger J Biotechnol. 2019;35(2):16-26.
47. Ibrahim IA, Jabbour AA, Abdulmajeed AM, Elhady ME, Almaroai YA, Hashim AM. Adaptive Responses of Four Medicinal Plants to High Altitude Oxidative Stresses through the Regulation of Antioxidants and Secondary Metabolites. Agronomy. 2022;12:3032.
48. Adeosun, Bamise W, Bodede O, Prinsloo G. Effect of Different Climatic Regions and Seasonal Variation on the Antibacterial and Antifungal Activity and Chemical Profile. Metabolites. 2022;12:758.
49. Vilkickyte G, Raudone L. Phenological and Geographical Effects on Phenolic and Triterpenoid Content in *Vaccinium vitis-idaea* L. Leaves. Plants. 2021;10(10):1986.
50. Naseem U. Impact of geographical locations on *Mentha spicata* antibacterial activities. J Med Plants Res. 2012;6(7).
51. Kumari P, Deepa N, Trivedi PK, Singh BK, Srivastava V, Singh A. Plants, and endophytes interaction: a "secret wedlock" for sustainable biosynthesis of pharmaceutically important secondary metabolites. Microb Cell Fact. 2023;22(1):1–19.
52. Packiaraj R, Jeyakumar S, Ayyappan N, Adhirajan N, Premkumar G, Rajarathinam K, Muthuramkumar S. Antimicrobial and

cytotoxic activities of endophytic fungus *Colletotrichum gloeosporioides* isolated from endemic tree *Cinnamomum malabatrum*. Stud Fungi. 2016;1(1):104–13.
53. Abonyi DO, Eze PM, Abba CC, Chukwunwejim CR, Ejikeugwu CP, Okoye FBC, Esimone CO. Metabolites of endophytic *Colletotrichum gloeosporioides* isolated from leaves of *Carica papaya*. Am J Essent Oils Nat Prod. 2019;7(1):39–46.
54. Nangmo KP, Akone SH, Tsamo TA, Zhen L, Müller WEG, Proksch P, Nkengfack EN. Colletotrin: a sesquiterpene lactone from the endophytic fungus *Colletotrichum gloeosporioides* associated with *Trichilia monadelpha*. Z Naturforsch. 2017;72(10):697–703.
55. Lutfia A, Munir E, Yurnaliza Y, Basyuni M. Antagonistic activity of endophytic fungi isolated from *Globba patens* miq. Rhizome against human pathogenic bacteria. J Pure Appl Microbiol. 2021;15(1):232–9.
56. Talukdar R, Tayung K. Antimicrobial Activities Of Endophytic Fungi Isolated From Three Ethno-Medicinal Plants Of Assam. J Bio Innov. 2020;9(6):93–7.
57. Moron LS, Lim Y-W, Dela Cruz TEE. Antimicrobial activities of crude culture extract from mangrove fungal endophytes collected in Luzon Island, Philippines. Philipp Sci Lett. 2018;11(28):28-36.
58. Hastuti US, Sulisetijono S, Thoyibah C, Pratiwi SH, Khotimah K. Histological Observation, Identification, and Secondary Metabolites Content in Endophytic Fungi of Mahogany Tree (*Swietenia mahagoni* Jacq) . Proc 7th Int Conf Biol Sci (ICBS 2021). 2022;22:156–64.
59. Jayawardena RS, Bhunjun CS, Hyde KD, Gentekaki E, Itthayakorn P. Colletotrichum: lifestyles, biology, morpho-species, species complexes and accepted species. Mycosphere. 2021;12(1):519–669.
60. Jayawardena RS, Hyde KD, Chen YJ, Papp V, Palla B, Papp D, Bhunjun CS, Hurdeal DL, Senwanna C, Manawasinghe IS, Harischandra DL, Gautam AK, Avasthi S, Chuankid B, Goonasekara ID, Hongsanang S, Zeng XY, Liyanage KK, Liu NG, Karunarathna A, Hapuarachchi KK, Lungharn T, Raspe O, Brahmanage R, Doilom M, Lee HB, Mei L, Jeewon R, Huanraleuk N, Chaiwan N, Stadler M, Wang Y. One stop shop IV: taxonomic update with molecular phylogeny for important phytopathogenic genera: 76–100. Fungal Diversity. 2020;103: 87–218.
61. Zheng H, Yu Z, Jiang X, Fang L, Qiao M. Endophytic Colletotrichum Species from Aquatic Plants in Southwest China. J Fungi. 2022;8(87):1-29.
62. Jayawardena RS, Hyde KD, Damm U, Cai L, Liu M, Li XH, Zhang W, Zhao WS, Yan JY. Notes on currently accepted species of *Colletotrichum*. Mycosphere. 2016;7(8):1192–260.
63. Fontana DC, de Paula S, Torres AG, de Souza VHM, Pascholati SF, Schmidt D, Neto DD. Endophytic fungi:

Biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens. 2021;10(5):1–28.
64. Ramadhani I, Rohadi H, Yuliani Y, Ilyas M. Study on Endophytic Fungi Associated with *Moringa oleifera* Lam. Collected from Lombok Island, West Nusa Tenggara. Ann Bogor. 2021;24(2):66.
65. Ma X, Nontachaiyapoom S, Jayawardena RS, Hyde KD, Gentekaki E, Zhou S, Qian Y, Wen T, Kang J. Endophytic *Colletotrichum* species from *Dendrobium* spp. In China and northern Thailand. Myco Keys. 2018;43:23–57.
66. Lei HM, Ma N, Wang T, Zhao PJ. Metabolites from the endophytic fungus *Colletotrichum* sp. F168. Nat Prod Res. 2021;35(7):1077–83.
67. Silva D. Evaluation of the phytotoxic effect of extracts from endophytic fungi *Colletotrichum dianesei* and *Xylaria* sp. isolated from *Palicourea corymbifera* (Rubiaceae). Agrociencia Uruguay. 2020;24(2):1–9.
68. Pillai TG. Pathogen to Endophytic Transmission in Fungi- A Proteomics Approach. SOJ Microbiol Infect Dis. 2017;5(3):1–5.
69. Collinge DB, Jensen B, Jørgensen HJL. Fungal endophytes in plants and their relationship to plant disease. Curr Opin Microbiol. 2022;69:102177.

