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Original article

Evaluation of Antibacterial Potency of Endophytic Fungi Isolated from *Mentha piperita*

Lawal Garba [a, *], Abdulhamid Abdulfatah [a], Adamu M.T. [a],
Ismail H. [b], Yusuf I. [c] & Dahiru A.S. [d]

[a] Department of Microbiology, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria
[b] Department of Biological Sciences, Faculty of Science, Bauchi State University, P.M.B. 65 Gadau, Bauchi State, Nigeria
[c] Department of Microbiology, Faculty of Life Sciences, Bayero University Kano, Nigeria
[d] Department of Integrated Science, School of Science Education, Federal College of Education Technical Gombe, P.M.B. 60, Ashaka Road, Gombe

Abstract

The *Mentha piperita* is an aromatic perennial herb, a member of the family *Lamiaceae* (*Labiatae*) that produces creeping stolons, growing in the range of 45 to 80 cm tall. Fungal endophytes reside in the healthy plant tissues to produce several metabolic products such as plants growth hormones, anti-phagocytes to biological feeding, medicinal ingredients, and many products of biological activities. Hence, they are regarded as a reservoir of active metabolites for the development of novel drugs. Although, many endophytic fungi have been reported from different plants, reports on fungal endophytes from *M. piperita* are very limited. In this study, fungal endophytes from the leaf and stem of *M. piperita* were successfully evaluated for their potential antibacterial properties. Healthy leaves of the peppermint were prepared and cultured on potato dextrose agar (PDA) plates for 5 to 7 days at 28 °C until fungal colonies appeared. Fifteen (15) fungal isolates were identified based on cultural and morphological characteristics and had six (6) rapid growing species, namely *Aspergillus fumigatus, Rhizopus arrhizus, Aspergillus flavus, Fusarium oxysporum, Aspergillus niger, Alternaria alternate* which were selected and evaluated their crude metabolites against *c* using agar well diffusion method. The susceptibility study showed a remarkable *in vitro* antibacterial activity of the fungal crude metabolites against all the test bacteria which increased with an increased incubation time of 7, 14 and 21 days incubation. However, the fungi displayed maximal zone of growth inhibition after 21 days of incubation. In conclusion, fungal endophytes were isolated from *M. piperita* and evaluated *in vitro* antibacterial activity of their crude metabolites against the test bacterial isolates.

Keywords: Antibacterial potential, Endophytic Fungi, *Mentha piperita*, Test bacteria, Bioassay.

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* **Corresponding author:**

Lawal Garba, Department of Microbiology, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria.
Email: lagarpak@gmail.com

INTRODUCTION

Plants of medicinal values such as mint species have been exploited by man in folk medicine for several years (Saharkhiz et al., 2012). To date, over 80% of the world population rely on folk medicine as well as extracts or essential oil from medicinal plants to fulfil their prime health requirements (Loolaie et al., 2017). *Mentha piperita* which is otherwise known as peppermint or simply mint is naturally cultivated as hybrid of spearmint (*Mentha spicata* L.) and water mint (*Mentha aquatic* L.) (Khalil et al., 2015; Loolaie et al., 2017). Even though, this plant is a native to Mediterranean region, it is being grown all over the world for several uses such as fragrance, flavour, medicinal, as well as pharmaceutical significance (Bellassoued et al., 2018; Heywood et al., 1993). Moreover, the plant has been used in herbal tea preparations, food, confectioneries and medicinal applications such as anti-inflammatory, carminative, antispasmodic, diaphoretic, antiemetic, analgesic, emmenagogue, stimulant, anticatharrhal applications and several other uses (Bellassoued et al., 2018; Cowan, 1999). Generally, plants have remained the key sources of several biologically active compounds for the search of natural products. For instance, medicinal plants have been confirmed to harbour certain fungal species referred to as endophytic fungi, which are known to produce active metabolites (Marcellano et al., 2017).

Endophytic fungi are types of fungi that reside within the plant tissues without causing any clear plant diseases (Garba et al., 2020; Inuwa et al., 2017; Jia et al., 2016). Both the fungi and their plant hosts have resorted to a very multifaceted relationship. Some fungal endophytes are able to produce hormones which promote plant growth, anti-phagocytes to biological feeding, produce medicinal ingredients, and many products of biological activities (Du et al., 2020; Jia et al., 2016). Natural products derived from endophytic fungi that have antimicrobial property may compensate for the deficiency of plant resources, limited length of regeneration cycle as well as application of large scale fermentation to yield naturally active compounds for a sizable production at cheaper cost and less pollution (Yuan et al., 2017).

Several researchers have reported some novel structures and the antimicrobial properties of metabolites from endophytic fungi. An endophytic fungus, *Campylocarpon* sp. HDN13-307 which was isolated from a mangrove tree has produced a novel family of 4-hydroxy-2-pyridone (Zhu et al., 2016). In another study, a monoterpenoid indole alkaloid called Vincamine discovered from an oleander endophytic fungus *Geomyces* sp. CH1 has inhibited acetylcholinesterase activity (Na et al., 2016). Kalyanasundaram, Nagamuthu, & Muthukumaraswamy, (2015) evaluated the antibacterial activity of endophytic fungi isolated from the leaves and stems of *Suaeda maritime* and *Suaeda monoica*. The crude extracts of these fungi showed high degree of inhibitory activity against *Salmonella typhi* and *Trichophyton rubrum* with inhibitory zones of 1s1.6 mm and 8.3 mm, respectively. In another study, twelve (12) fungal endophytes isolated from the bark of *Cinnamomum mercadoi* demonstrated antibacterial activity against at least one of the test bacterial pathogens including, *Bacillus cereus*,

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Escherichia coli* (Marcellano et al., 2017) while a similar report by Handayani, Rivai, Hutabarat, & Rasyid, 2017 established the antibacterial activity of endophytic fungal extracts isolated from leaf, bark and root Mangrove *Sonneratia grifithii* Kurz against *Staphylococcus aureus* and *Escherichia coli*. Many other reports confirmed the anti-plant pathogens or antimicrobial potential of fungal endophytes isolated from various plants such as *Mentha piperita* (Chowdhary & Kaushik, 2018), *Sceletium tortuosum* (Manganyi et al., 2019), and *Securinega suffruticosa* (Du et al., 2020).

MATERIALS and METHODS

Collection and Authentication of plant samples

Healthy leaves of peppermint were collected in sterile plastic bags from Gombe State University Botanical Garden, authenticated at the herbarium section of the department of Biological Sciences and finally transported to Microbiology laboratory of the same University for processing.

Preparation of all media and reagents

The media and reagents used in this research were prepared in accordance with the manufacturers recommendations.

Isolation of fungal endophytes from the leaves and stems

The fungal endophytes were isolated from the leaves and stems of the plant according to the procedure adopted by Garba et al., (2020). Firstly, the two samples were rinsed in running tap water to remove dust and debris. The samples were surface sterilized for 1 minute using 70% ethanol (Subala Labchem, India) and then immersed in 1% mercuric chloride solution (Alpha Chemika, India) for 30 seconds to 1 minute. Again, the samples were rinsed for 1 minute in sterile distilled water and then allowed the surface to dry on filter paper. The samples were cut into small pieces using a sterile blade to expose the internal tissues. Four (4) pieces of each sample were placed on prepared potato dextrose agar (TM Media, India) plate supplemented with chloramphenicol (30µg, Elan Pharm, USA). The plates were incubated for 5 to 7 days at 28 ^{O}C until colonies appearance. Individual fungal colonies were cultured on potato dextrose agar (TM Media, India) slant under the same condition and preserved at 4 ^{O}C until the next experiment.

Morphological Identification of Endophytic fungi

The fungi were identified on the basis of cultural and morphological characteristics such as shape, size, texture, colour, pattern and arrangement of mycelium, conidial arrangement and type of spore as described in fungal Atlas (Watanabe, 2002). Prior to microscopic examination, a fungal smear was prepared on a clean grease-free microscopic slide according to Leck, (1999). Briefly, a fungal sample

was placed into 1-2 drops of lactophenol cotton blue stain (FirmTech Bioscience, Nigeria), covered with a cover slip and examined under a light microscope (Motic Scientific, China).

Source and confirmation of test bacterial organisms

The test bacteria (*Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Klebsiella pneumoniae*) were clinical bacterial isolates obtained from Gombe Specialist Hospital and confirmed using standard microbiological techniques according to Cheesbrough, 2005 as described below:

Gram staining

The bacteria were cultured on nutrient agar (Sigma-Aldrich) plates at 37 ^{o}C for 24 hours. A single colony was emulsified in a drop of 0.9% normal saline (Fidson Aventra) on a clean microscopic slide and allowed to air-dry to form a smear. The smear was fixed over a Bunsen burner flame, stained with 1% (w/v) crystal violet stain (Sigma-Aldrich) for 1 min and washed with clean tap water. A few drops of Lugol's iodine solution (Sigma-Aldrich) were added for 1 min and washed with tap water. The smear was decolorized with 95% ethanol (Subala Labchem, India) for 1 to 2 seconds and washed immediately with tap water. A few drops of safranin solution (Sigma-Aldrich) were added for 2 min and washed with tap water. The smear was allowed to air-dry and examined using X 100 objective of a light microscope (Trinocular, Olympus BX40).

Coagulase test

A colony of the test bacteria was picked from an overnight culture on nutrient agar (Sigma-Aldrich) plate and emulsified in a drop of distilled water. A drop of blood plasma was added to the bacterial suspension, gently mixed and observed for clumping within ten (10) seconds.

Catalase test

A single colony of each isolate from an overnight culture on nutrient agar (Sigma-Aldrich) plate was emulsified in a drop of normal saline on microscopic slides. Two drops of 3% hydrogen peroxide (H_2O_2,) (Sigma-Aldrich) were added and observed immediately.

Oxidase test

A single colony of each isolate from an overnight culture on nutrient agar (Sigma-Aldrich) plate was rubbed onto the surface of an oxidase strip (Sigma-Aldrich, USA) and observed within five (5) seconds.

Urease test

A single colony of each isolate from an overnight culture on nutrient agar (Sigma-Aldrich, USA) plate was inoculated at the slant part of urease agar medium (Merck, Germany) and incubated at 15 ^{o}C for 24 to 48 h.

Indole test

A single colony of each isolate from an overnight culture on nutrient agar (Sigma-Aldrich, USA) plate was inoculated into a Bijou bottle containing about 3 ml of sterile peptone water (TM Media, India). The inoculated peptone water was then incubated for 24 hours at 37 ^{0}C.

Test for indole was achieved by the addition of about 0.5 ml of Kovac's regent (Fisher Scientific), shaken gently and observed within 10 minutes.

Citrate test

A single colony of each isolate from an overnight culture on nutrient agar plate was inoculated at the butt and slant parts of Simmons citrate agar (Oxoid, England) in a universal bottle and incubated at 37 ^{o}C for 24 hours.

Triple Sugar Ion test

A single colony of each isolate from an overnight culture on nutrient agar plate was cultured at the butt and slant parts of TSI agar (Hardy Diagnostic, USA) and incubated at 37 ^{o}C for 24 hours.

***In vitro* Antibacterial Potential of Fungal Endophytes**

Each test bacterial isolate was cultured on Brain Heart Infusion agar (TM Media, India) and incubated for 24 hours at 37 ^{o}C. Individual colonies of the test bacterial isolates were picked from an overnight culture using a sterilized wire loop and emulsified in 3-4 ml of sterile normal saline (Fidson Aventra). Turbidity of the suspension was compared to 0.5 McFaland standards (Cheesbrough, 2005; Garba et al., 2019).

Bioassay procedure

Agar well diffusion method was used to investigate the antibacterial potential of the endophytic fungi against the standardized inocula of the test bacteria. Six (6) fungal species, namely, *A. alternate*, *A. niger, A. flavus, A. fumigitus, R. arrhizus,* and *F. oxysporum* cultured in three batches of bijou bottles, each containing 10 ml of potatoes dextrose broth (TM Media, India). The bottles were incubated for 7, 14, and 21 days at 28 °C. Following the incubation, the respective fungal broth cultures were filtered using a whatman no.1 filter paper (GE Healthcare, UK) to separate the mycelial mat from the culture filtrate (Mishra et al., 2017). Wells of 6 mm diameter were aseptically punched using a sterile cork borer on Mueller Hinton agar (TM Media, India) plates seeded with the test organisms. Aliquot of 100 µl of the culture filtrate containing the crude metabolites of each fungal isolate was poured into the wells and incubated for 24hrs at 37 °C (Garba et al., 2021).

RESULTS

Fungal Endophytes isolated and identified from *Mentha piperita*

Table 1 shows the endophytic fungi isolated from the leaves and stems of *Mentha piperita*. A total of 15 fungal endophytes were isolated and identified as *Alternaria alternate, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus arrhizus, Epidermophytum floccosum, Fusarium solani, Fusarium oxysp orum, Aspergillus nomius, Penicillium crustosum, Phoma saccardo, Alternaria solani, Curvularia trifolii, Epicoccum nigrum,* and *Fusarium equiseti* based on cultural characteristics on PDA and/or SDA and microscopic appearance.

Table 1. Endophytic fungi associated with the leaves and stems of Mentha piperita

S/No	Source of isolation	Fungal Isolate
1	Leaf	*Alternaria alternate*
2	Leaf	*Aspergillus niger*
3	Leaf	*Aspergillus flavus*
4	Leaf	*Aspergillus fumigatus*
5	Leaf	*Rhizopus arrhizus*
6	Leaf	*Epidermophytum floccosum*
7	Leaf	*Fusarium solani*
8	Leaf	*Fusarium oxysporum*
9	Leaf	*Aspergillus nomius*
10	Stem	*Penicillium crustosum*
11	Stem	*Phoma saccardo*
12	Stem	*Alternaria solani*
13	Stem	*Curvularia trifolii*
14	Stem	*Epicoccum nigrum*
15	Stem	*Fusarium equiseti*

Confirmation of clinical bacterial isolates

Table 2 shows results for the confirmatory test of *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Klebsiella pnuemoniae*. The bacteria were confirmed using Gram staining and biochemical tests.

Table 2. Confirmed clinical bacterial isolates

S/No	Test bacteria	Gxr	Biochemical tests										
			Ca	Co	Ci	In	Ox	Ur	TSI				
									L	S	G	GS	HS
1	*E. coli*	-	+	-	-	+	-	-	+	+	+	+	-
2	*S. typhi*	-	+	-	-	-	-	-	-	-	+	-	+
3	*S. aureus*	+	+	+	+	-	-	+	+	+	+	-	-
4	*K. pneumoniae*	-	+	-	+	-	-	+	+	+	+	+	-

Key: Gxr: Gram reaction, Ca:Catalase test, Co:Coagulase test, Ci:Citrate utilization test, In:Indole test, Ox:Oxidase test, Ur: Urease test, L:Lactose fermentation, S:Sucrose fermentation, G:Glucose fermentation, GS:Gas production, HS: Hydrogen sulphide production.

In vitro Antibacterial Properties of the Endophytic Fungi

The Six (6) fungal species including *A. alternate*, *A. niger*, *A. flavus*, *A. fumigatus*, *R. arrhizus*, and *F. oxysporum* were investigated for their potential antibacterial activity against four (4) clinical bacterial isolates as presented in Figures 1, 2, and 3. The antibacterial activity of the fungal crude metabolites in the 7, 14, and 21 day-old culture filtrates was determined using agar well diffusion method. One interesting thing is that, all the test bacteria were found to be sensitive to the fungal culture crude metabolites. However, the crude metabolites provided maximum zone of inhibition after 21 days of incubation. *A. fumigatus* showed maximum zone of inhibition against *E. coli* (25mm), *S. aureus* (24mm), *K. pneumoniae* (23mm) and *S. typhi* (22mm). Similarly, *R. arrhizus* showed higher zone of inhibition against, *S. aureus* (21mm), *S. typhi* (20mm), but least activity against *E. coli* (19 mm) and *K. pneumoniae* (18mm). Likewise, *A. flavus* showed good activity against *E. coli* (18mm) and *K. pneumoniae* (15mm), *S. aureus* (13mm) and minimum activity against *S. typhi* (8mm). *F. oxysporum* produced some level of inhibition against *S. typhi* (25mm), *S. aureus* (24mm), *E. coli* (20mm), and *K.pneumoniae* (19mm). *A. niger* showed maximum activity against *K. pneumoniae* (23mm), *S. typhi* (20mm), *S. aureus* (19mm), and least activity against *E. coli* (12mm). *A. alternate* showed antibacterial activity against *S. aureus* (25mm), K. *pneumoniae* (22mm), *S. thphi* (19mm), *E. coli* (18mm).

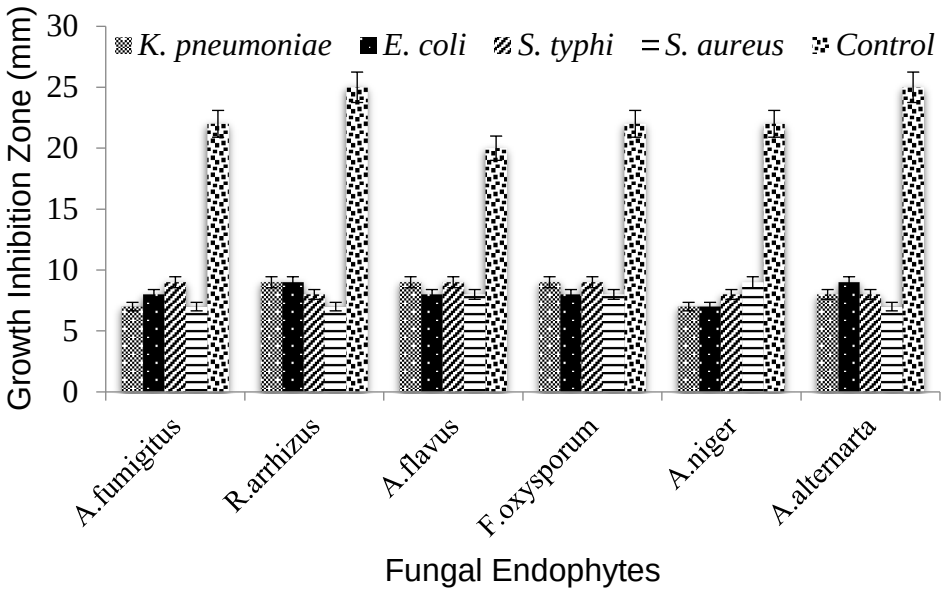

Figure 1. Antibacterial activity of crude endophytic fungal metabolites in 7day-old culture.

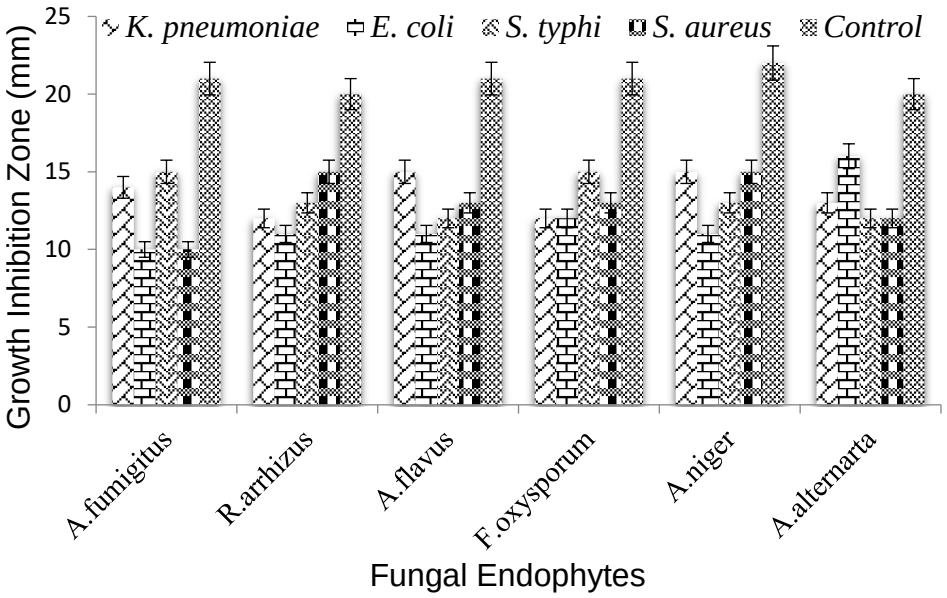

Figure 2. Antibacterial activity of crude endophytic fungal metabolites in 14-day old culture.

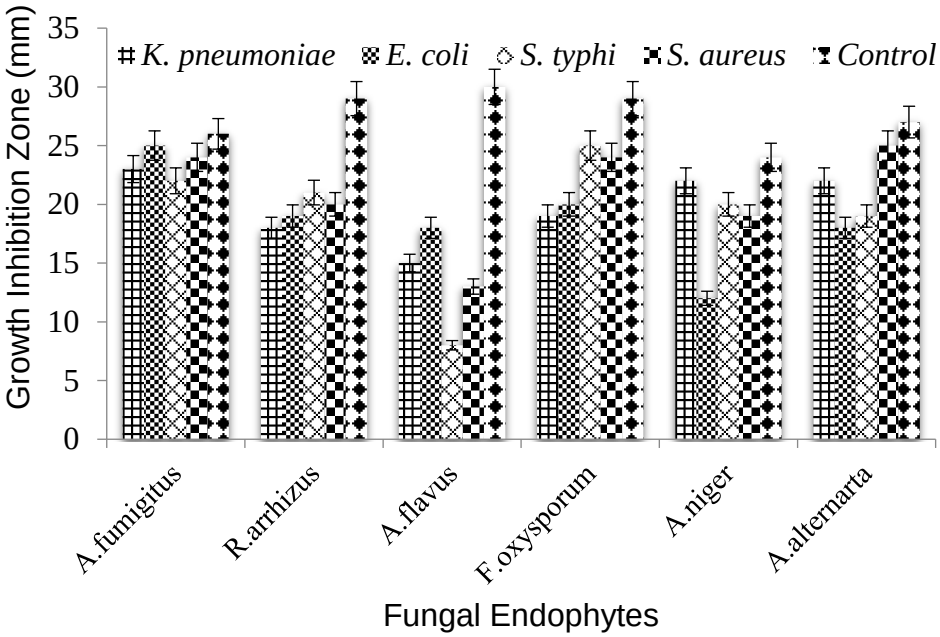

Figure 3. Antibacterial activity of crude endophytic fungal metabolites in 21-day old culture.

DISCUSSION

Resistance to potent antimicrobial agents remains a key health problem and threat to the global health care system (Ferri et al., 2017). Several reports have indicated that microorganisms develop antibiotics resistance through different mechanisms such as alteration of drugs as well as inhibition of drug access to target sites (Blair et al., 2015; Garba et al., 2018). This global issue has led to an increased research for alternative compounds that could alleviate the problem of drug resistance such as active

compounds from endophytic fungi, especially those identified from medicinal plants to serve as potential source of novel and potent antibiotics (Liang et al., 2012; Marcellano et al., 2017).

Several reports have established the abundance of bioactive compounds in medicinal plants' extracts as well as the isolation and characterization of secondary metabolites from endophytic fungi. These pave the way to researching the potentially important and biological active secondary metabolites which may be effective against a range of drug resistant Gram-positive and Gram-negative bacteria (Manganyi et al., 2019).

In this study, a total of 15 fungal species were isolated from the leaves and stems of *M. piperita*, out of which seven (7) species belonged to Ascomycetes (*A. alternate, A. niger, A. flavus, A. fumigatus, A. nomius, A. solani, C. trifolii)*, four (4) to Hyphomycetes (*F. solani, F. oxysporum, P. crustosum, F. equiseti)*, two (2) species to Dothideomycetes (*P. saccardo, E. nigrum)*, one (1) species to Zygomycetes (*R. arrhizus)*, and one (1) species to Eurotiomycetes (*E. floccosum)*. Six (6) species of these fungi isolated from the leaves and belonging to three (3) different classes, namely Ascomycetes, Hyphomycetes and Zygomycetes have been selected and evaluated for the *in vitro* antibacterial potential of their culture crude metabolites against clinical isolates of *E. coli, S. aureus, S. typhi,* and *K. pneumoniae* after seven (7), fourteen (14) and twenty one (21) days of incubation as presented in Figures 1, 2 and 3, respectively. Interestingly, all the fungal isolates displayed various levels of inhibitory activity against the test bacteria, which increased with an increased number of incubation days most likely as a result of accumulation of more active metabolites released into the growth medium. In line with our findings, previous studies have confirmed the antibacterial activity of endophytic fungi isolated from several plants such as mangrove *Sonneratia grifithii* (Handayani et al., 2017), *Securinega suffruticosa* (Du et al., 2020), *Mentha piperita* (Chowdhary & Kaushik, 2018), *Sceletium tortuosum* (Manganyi et al., 2019), and *Psidium guajava* (Garba et al., 2020). Moreover, *S. aureus, E. coli* and *S. typhi* which are amongst the test bacteria inhibited by the isolated endophytic fungi in this study, have been reported to be sensitive to fungal endophytes in another studies (Handayani et al., 2017; Kalyanasundaram et al., 2015). Contrary to our findings in which the maximal inhibitory activity was observed after twenty four (24) days incubation of the fungal endophytes (Figure 3), a similar report showed that the endophytes were found to be more active against the test bacteria following seven (7) days of incubation (Sandhu et al., 2014). The antibacterial potencies of the fungal crude metabolites observed in this study corroborated well with the previous reports like that of Mwanga, Mvungi, & Tibuhwa, 2019, where qualitative phytochemical analysis of extracts from fungal endophytes isolated from *Moringa oleifera* revealed the presence of secondary metabolites of alkaloids, phenols, flavonoids, tannins, terpenoids, and saponins all of which were believed to possess antimicrobial activity. Moreover, other reports show that fungal endophytes generally produce secondary metabolites such as 4-

hydroxybenzoic acid, indole-3-acetic acid (IAA) and gibepyrone D which are known to possess antimicrobial properties (Bogner et al., 2017; Deshmukh et al., 2014).

Conclusion

In this study, fifteen (15) endophytic fungi have been successfully isolated from the leaves and stems of peppermint (*Mentha piperita*). The isolated fungi have revealed a strong *in vitro* antibacterial activity against the test bacteria, serving more preliminary information about the antagonistic potentials of fungal endophytes from medicinal plants towards controlling drug resistant organisms.

Conflict of Interest

The authors declare that they have no any conflict of interest.

REFERENCES

Bellassoued, K., Hsouna, A. B., Athmouni, K., van Pelt, J., Ayadi, F. M., Rebai, T., & Elfeki, A. (2018). Protective effects of *Mentha piperita* L. leaf essential oil against CCl 4 induced hepatic oxidative damage and renal failure in rats. *Lipids in Health and Disease*, *17*(1), 1–14.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, *13*(1), 42–51.

Bogner, C. W., Kamdem, R. S., Sichtermann, G., Matthäus, C., Hölscher, D., Popp, J., Proksch, P., Grundler, F. M., & Schouten, A. (2017). Bioactive secondary metabolites with multiple activities from a fungal endophyte. *Microbial Biotechnology*, *10*(1), 175–188.

Cheesbrough, M. (2005). *District laboratory practice in tropical countries, part 2*. Cambridge university press.

Chowdhary, K., & Kaushik, N. (2018). Biodiversity study and potential of fungal endophytes of peppermint and effect of their extract on chickpea rot pathogens. *Archives of Phytopathology and Plant Protection*, *51*(3–4), 139–155.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4), 564–582.

Deshmukh, S. K., Verekar, S. A., & Bhave, S. V. (2014). *Endophytic fungi: a reservoir of antibacterials. Front Microbiol 5: 715*.

Du, W., Yao, Z., Li, J., Sun, C., Xia, J., Wang, B., Shi, D., & Ren, L. (2020). Diversity and antimicrobial activity of endophytic fungi isolated from *Securinega suffruticosa* in the Yellow River Delta. *PloS One*, *15*(3), e0229589.

Ferri, M., Ranucci, E., Romagnoli, P., & Giaccone, V. (2017). Antimicrobial resistance: a global emerging threat to public health systems. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2857–2876.

Garba, L., Abba, H., Adamu, Z. Y., Muhammed, I., Adamu, M. T., Aisami, A., Yusha'u, M., & Tahir, F. (2020). Isolation and Evaluation of In vitro Antibacterial Potential of Endophytic Fungi from the leaves of *Psidium guajava* (Guava). *Bayero Journal of Pure and Applied Sciences*, *13*(1), 20–24.

Garba, L., Lawan, H. S., Puma, H. U., Abdullahi, M. M., Yusuf, I., & Mukhtar, M. D. (2019). Phytochemical Screening and in vitro Bacteriostatic Effects of *Syzigium aromaticum* (Clove) Extracts on Clinical Bacterial Isolates. *Journal of Biochemistry, Microbiology and Biotechnology*, *7*(1), 5–9.

Garba, L., Muhammad, M. B., Adamu, M. T., Isa, S., Abdullahi, M. M., & Yarma, A. A. (2021). Potential *in vitro* Inhibitory effects of *Moringa oleifera* Leaf extracts on Extended-Spectrum β-Lactamase-producing Bacteria. *Journal of Fundamental and Applied Sciences*, *13*(1), 137–150.

Garba, L., Yusha'u, M., Abdullahi, M. M., Abubakar, M. U., Inuwa, A. B., Isa, S., & Adamu, M. T. (2018). Effectiveness of Double Discs Synergy Test in the Confirmation of Extended Spectrum β-lactamase (ESβL) Production. *Journal of Biochemistry, Microbiology and Biotechnology*, *6*(2), 15–18.

Handayani, D., Rivai, H., Hutabarat, M., & Rasyid, R. (2017). Antibacterial activity of endophytic fungi isolated from mangrove plant *Sonneratia griffithii* Kurz. *Journal of Applied Pharmaceutical Science*, *7*(04), 209–212.

Heywood, V. H., Moore, D. M., Richardson, I. B. K., & Stearn, W. T. (1993). *Flowering plants of the world*. Oxford university press.

Inuwa, A. B., Maryam, Y. A., Arzai, A. H., Hafsat, Y. B., Kawo, A. H., Usman, A. U., Ama, S. J., & Ibrahim, K. H. (2017). Distribution of culturable endophytic bacteria in lemon grass (*Cymbopogon citratus*). *Bayero Journal of Pure and Applied Sciences*, *10*(1), 95–98.

Jia, M., Chen, L., Xin, H.-L., Zheng, C.-J., Rahman, K., Han, T., & Qin, L.-P. (2016). A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Frontiers in Microbiology*, *7*, 906.

Kalyanasundaram, I., Nagamuthu, J., & Muthukumaraswamy, S. (2015). Antimicrobial activity of endophytic fungi isolated and identified from salt marsh plant in Vellar Estuary. *Journal of Microbiology and Antimicrobials*, *7*(2), 13–20.

Khalil, A. F., Elkatry, H. O., & El Mehairy, H. F. (2015). Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats. *Annals of Agricultural Sciences*, *60*(2), 353–359.

Leck, A. (1999). Preparation of lactophenol cotton blue slide mounts. *Community Eye Health*, *12*(30), 24.

Liang, H., Xing, Y., Chen, J., Zhang, D., Guo, S., & Wang, C. (2012). Antimicrobial activities of endophytic fungi isolated from *Ophiopogon japonicus* (Liliaceae). *BMC Complementary and Alternative Medicine*, *12*(1), 1–6.

Loolaie, M., Moasefi, N., Rasouli, H., & Adibi, H. (2017). Peppermint and its functionality: A review. *Arch Clin Microbiol*, *8*(4), 54.

Manganyi, M. C., Regnier, T., Tchatchouang, C.-D. K., Bezuidenhout, C. C., & Ateba, C. N. (2019). Antibacterial activity of endophytic fungi isolated from *Sceletium tortuosum* L.(Kougoed). *Annals of Microbiology*, *69*(6), 659–663.

Marcellano, J. P., Collanto, A. S., & Fuentes, R. G. (2017). Antibacterial activity of endophytic fungi isolated from the bark of *Cinnamomum mercadoi*. *Pharmacognosy Journal*, *9*(3).

Mishra, V. K., Passari, A. K., Chandra, P., Leo, V. V., Kumar, B., Uthandi, S., Thankappan, S., Gupta, V. K., & Singh, B. P. (2017). Determination and production of antimicrobial compounds by *Aspergillus clavatonanicus* strain MJ31, an endophytic fungus from *Mirabilis jalapa* L. using UPLC-ESI-MS/MS and TD-GC-MS analysis. *PloS One*, *12*(10), e0186234.

Mwanga, Z., Mvungi, E., & Tibuhwa, D. (2019). Antimicrobial activities of endophytic fungi secondary metabolites from *Moringa oleifera* (Lam). *Tanzania Journal of Science*, *45*(3), 463–476.

Na, R., Jiajia, L., Dongliang, Y., Yingzi, P., Juan, H., Xiong, L., Nana, Z., Jing, Z., & Yitian, L. (2016). Indentification of vincamine indole alkaloids producing endophytic fungi isolated from *Nerium indicum*, Apocynaceae. *Microbiological Research*, *192*, 114–121.

Saharkhiz, M. J., Motamedi, M., Zomorodian, K., Pakshir, K., Miri, R., & Hemyari, K. (2012). Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. *International Scholarly Research Notices*, *2012*.

Sandhu, S. S., Kumar, S., & Aharwal, R. P. (2014). Isolation and identification of endophytic fungi from *Ricinus communis* Linn. and their antibacterial activity. *Int. J. Res. Pharm. Chem*, *4*(3), 611–618.

Watanabe, T. (2002). Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC press.

Yuan, Y., Feng, H., Wang, L., Li, Z., Shi, Y., Zhao, L., Feng, Z., & Zhu, H. (2017). Potential of endophytic fungi isolated from cotton roots for biological control against verticillium wilt disease. *PLoS One*, *12*(1), e0170557.

Zhu, M., Zhang, X., Feng, H., Che, Q., Zhu, T., Gu, Q., & Li, D. (2016). Campyridones A–D, pyridone alkaloids from a mangrove endophytic fungus Campylocarpon sp. HDN13-307. *Tetrahedron*, *72*(37), 5679–5683.