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Bioassay-guided isolation of constituents from the endophytic fungi *Fusarium solani* inhabiting *Rhizophora apiculata* Blume

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ABSTRACT: Endophytic fungi (EF) are excellent sources of diverse and pharmacologically relevant molecules for drug development. The mangrove genus *Rhizophora* have been identified to contain fungal isolates. In this study, the EF *Fusarium solani* was isolated and characterized from *R. apiculata*. Disk diffusion assay of the crude ethyl acetate extract of *F. solani* on the ESKAPE clinical isolate pathogens showed inhibitory activities against *Escherichia coli* 5218S (ZOI 12.20 mm), methicillin-resistant *Staphylococcus aureus* 2729B (ZOI 8.90 mm), *Klebsiella pneumoniae* 4830WD (ZOI 12.58 mm), *Acinetobacter baumannii* 3975B (ZOI 12.85 mm), and *Enterobacter cloacae* 5276S (ZOI 15.53 mm). Bioassay guided isolation and spectroscopic analysis led to the identification of benzoic acid (**1**) and *bis*(3-methylhexyl) phthalate (**2**). Microtiter plate antimicrobial assay gave minimum inhibitory concentration (MIC) of 125 µg/mL for compound **1** and 250 µg/mL for compound **2**. To the best of our knowledge, this is the first report on the isolation of *F. solani* from *R. apiculata* and the isolation of compounds **1** and **2** from the genus *Fusarium*. Hence, this study validated the importance of the EF as sources of bioactive molecules.

KEYWORDS: *Rhizophora*; *Fusarium solani*; Benzoic acid; ESKAPE; *Enterobacter cloacae*

1. INTRODUCTION

Endophytic fungi (EF) constitute a diverse group of microorganisms inhabiting the plant tissues and occupying various niches and ecosystems [1]. EF are capable of metabolic and physiological mechanism development. This has allowed the EF to synthesize secondary metabolites that closely mimic or match those naturally produced by their host [2]. This unique characteristic has sparked extensive research on EF natural products recognizing their potential for pharmacological applications and sources of diverse and biologically active compounds [3]. EF are also more sustainable as sources of pharmacologically active compounds as they offer the advantage of being cultured and kept for future use [4].

Mangrove species are recognized as remarkable reservoir of EF capable of producing diverse compounds with significant medicinal properties [5]. The mangrove-related EF exhibit unique morphological structures and physiological mechanisms due to their tolerance of various stressors such as high salt environment, high temperature and humidity, tidal cycles, among others [6,7]. A notable mangrove genus is *Rhizophora* of the family Rhizophoraceae. Previously, sixty-six EF were isolated from various *Rhizophora* mangrove species [8]. More than 195 secondary metabolites were identified from these EF encompassing alkaloids, flavonoids, terpenoids, steroids, and phenolic natural products with diverse pharmacological properties [8]. In the Philippines, the *Rhizophora apiculata* Blume, a true mangrove species, is widely distributed in coastal saltwater zones. *R. apiculata* is highly regarded due its medicinal properties including antimicrobial, antioxidant, anticancer, antimicrobial, and antiviral properties [9,10]. *R. apiculata* collected from Indonesia produced EF from the genera *Aspergillus*, *Cunninghamella*, and *Humicola* [11]. Twenty fungal isolates belonging to *Trichoderma*, *Nigrospora*, *Lasiodiplodia*, *Scopulariopsis*, *Curvularia*, *Fusarium*, *Neopestaliopsis*, *Pestaliopsis*, *Aspergillus*, *Penicillium*, and two unidentified species were isolated from *R. apiculata* collected in three location sites in Indonesia [12].

In our interest of exploring the EF of Philippine *R. apiculata*, we herein report the isolation of *Fusarium solani* and the bioassay-guided isolation of its secondary metabolites.

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2. RESULTS AND DISCUSSION

2.1 Isolation and identification of *Fusarium solani*

The endophytic fungus *Fusarium solani* was isolated from the leaf segments of the Philippine mangrove *R. apiculata*. Morphological characterization (Figure 1A) exhibited white colony with abundance of aerial mycelia. As shown in Figure 1B, the microscopic characteristic exhibited septate hyphae, hyaline simple conidiophores, and branched phialides clustered in a brush-like arrangement at the tips of conidiophores. Figure 1C showed the septate macroconidia exhibiting a slightly curved shape. These characteristics are in agreement with *F. solani* as previously described [13]. The isolated fungal species was assigned the taxonomy ID 169388 from the NCBI taxonomy database [14]. To the best of our knowledge, this is the first report on the isolation of *F. solani* from the mangrove species *R. apiculata*.

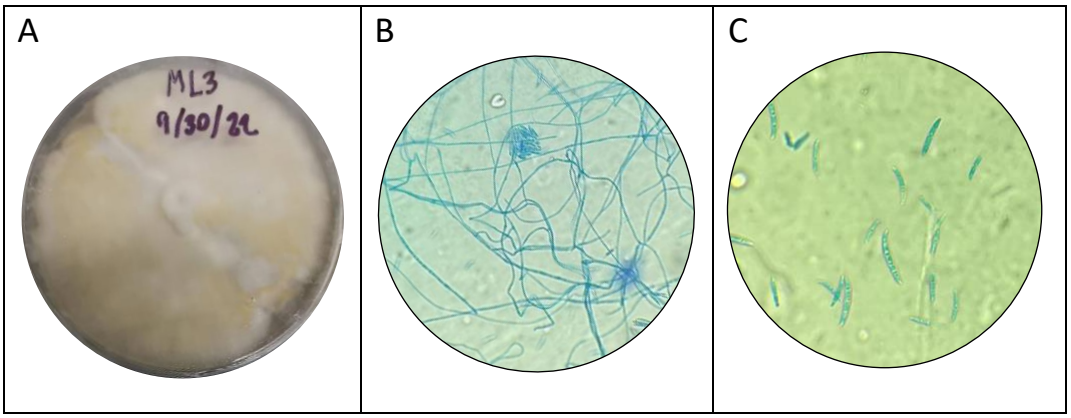

Figure 1. Morphology (A) and microscopic (B and C) characteristics of the isolated endophytic fungus *Fusarium solani* from *Rhizophora apiculata*.

2.2 Isolation of constituents from the crude extract of *Fusarium solani*

Antibacterial assay (Table 1) of the crude extract using the ESKAPE pathogens showed the highest inhibition on the growth of *E. cloacae* (ZOI 15.53 mm). Weak ZOI were also observed in *E. coli*, *K. pneumoniae*, and *A. baumanii*. Hence, for the bioassay guided isolation of the chromatographic extracts of *F. solani* using TLC bioautography, the *E. cloacae* clinical isolate was used to determine the extracts for further purification. A series of chromatographic purification on Fs2 yielded benzoic acid (**1**) and bis(3-methylhexyl) phthalate (**2**) (Fig. 2). The structures were determined based on NMR analyses. The NMR spectra of compounds **1** and **2** are deposited in the Supplementary Material. Previous reports have also identified the isolation of phthalates from EF which regarded them as natural products formed by enzymatic esterification or trans-esterification in the shikimic acid pathway [15]. Extensive literature also indicated the first identification of compounds **1** and **2** from the genus *Fusarium*.

2.3 Antimicrobial activity of Compounds 1 and 2

The microtiter plate assay method was used to evaluate the minimum inhibitory concentration (MIC) of benzoic acid (**1**) and *bis*(3-methylhexyl) phthalate (**2**) against *E. cloacae*. An initial concentration of 500 µg/mL was added in a 96-well plate and serially diluted up to 1.9 µg/mL. Results showed that compound **1** gave an MIC of 125 µg/mL and compound **2** showed 250 µg/mL. Benzoic acid was earlier reported to be antimicrobial showing broad spectrum antibacterial and antifungal effects [16-19]. Moreover, the isolated phthalates from nature possessed biological activities including their antimicrobial, cytotoxic and antioxidant activity [15, 20, 21].

Table 1. Disk-diffusion antibacterial assay of the *Fusarium solani* crude extract.

ESKAPE Pathogens Clinical Isolates	Zone of Inhibition (Mean (mm) ± SD, n=3)		
	Fusarium solani Crude extract	Positive control (penicillin)	Negative control* (EtOAc)
Escherichia coli 5218S	12.20 ± 0.95	6.00 ± 0.0	6.00 ± 0.0
Methicillin-resistant *Staphylococcus aureus* 2729B	8.90 ± 0.70	22.15 ± 0.10	6.00 ± 0.0
Klebsiella pneumoniae 4830WD	12.58 ± 1.03	6.00 ± 0.0	6.00 ± 0.0
Acinetobacter baumannii 3975B	12.85 ± 0.80	6.00 ± 0.0	6.00 ± 0.0
Pseudomonas aeruginosa 5220U	6.00 ± 0.0	6.00 ± 0.0	6.00 ± 0.0
Enterobacter cloacae 5276S	15.53 ± 4.13	6.00 ± 0.0	6.00 ± 0.0

*A 6.00-mm disk diameter was used in the assay, indicating a "no inhibition" for ZOI of 6.00 mm.

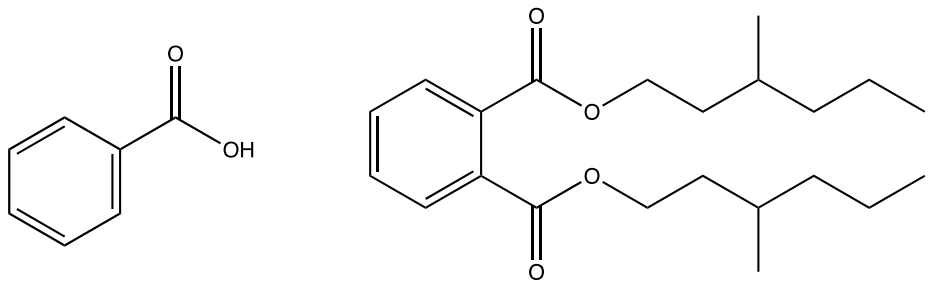

Figure 2. Isolated compounds from the ethyl acetate extract of *Fusarium solani*.

The rapid emergence and spread of multidrug-resistant bacteria have become one of the biggest threats to our society. This impending crisis could potentially render progress in medical science obsolete and ineffective, thus threatening our ability to effectively treat common infections. The rising multi-drug resistance and indiscriminate use of existing antibiotics outpace the discovery of novel antibiotics.

One of the promising ways of discovering lead molecules for drug discovery is through the exploration of microbes from understudied and specialized niches. Fungal endophytes have garnered increasing attention due to their ability to produce pharmacologically significant metabolites. These endophytes have been found to be a rich source of bioactive compounds with diverse biological activities. Despite the vast number of fungal endophytes estimated to exceed 1 million, only a small fraction of them have been investigated for their biosynthetic potential [3]. Hence, fungal endophytes currently represent an understudied resource for the discovery of novel bioactive molecules.

Difficulty in synthetic reproduction of secondary metabolites led to a global upsurge in the overexploitation of plants as a major source of complex bioactive molecules of pharmaceutical interest [22]. Overharvesting and habitat destruction threaten the extinction of 15,000 plant species used for medicinal purposes [23]. Exhaustion of these resources would lead to an accelerated loss of species that creates a huge disturbance in the ecological balance. It is crucial to ensure the sustainable extraction and utilization of species for bioactive compounds to prevent substantial changes in the natural ecosystem. Fungal endophytes produced these compounds in limited varying quantities and concentrations across different plant organs.

Therefore, the adoption of *in vitro* culturing techniques for the mass production of secondary metabolites from EF is essential for their conservation and sustainable use. These techniques have minimal ecological impact and help maintain the ecological equilibrium.

These issues have been addressed in this study. *R. apiculata*, a true mangrove species, has been shown to contain biologically active EF. Using in vitro culturing techniques, we were able to report the first isolation of *F. solani* from the *R. apiculata*. Moreover, two potentially antimicrobial compounds against clinical isolate E. cloacae were firstly identified from the EF *F. solani* and the genus *Fusarium*.

3. CONCLUSION

We have validated the potential of EF as an excellent source of bioactive natural products. Our results highlight the first isolation of the EF *F. solani* from the mangrove *R. apiculata* and the first isolation of benzoic acid (**1**) and bis(3-methylhexyl) phthalate (**2**) from the genus *Fusarium*. The ethyl acetate crude EF extract showed growth inhibitions against ESKAPE clinical isolates. Benzoic acid (**1**) and bis(3-methylhexyl) phthalate (**2**) also showed MICs of 125 µg/mL and 250 µg/mL, respectively, against *E. cloacae*. The Philippine mangrove *R. apiculata* holds an array of EF which are potentially attractive in medicinal chemistry. Thus, further exploration of these important microorganisms and their chemical constituents is highly encouraged.

4. MATERIALS AND METHODS

4.1 General considerations

^{1}H- (600 MHz) and ^{13}C- (150 MHz) NMR were measured in a JEOL ECZR 600 spectrometer using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts were reported as ppm (δ) and J values in Hz. Merck silica gel 7734 or silica gel 9385 were used for column chromatography. Aluminum-backed thin-layer chromatography (TLC) plates coated with Si gel F254 was used for TLC. UV_{254} followed by vanillin-sulfuric acid with heating were used for visualization. All solvents used for extraction and chromatography were analytical grade.

The succeeding sections summarizes the workflow on the isolation of bioactive compounds. The process involved the collection of the leaves of *R. apiculata*, collection, identification, and cultivation of the EF, extraction of the EF, purification using chromatography, structure elucidation of the pure metabolites, and biological testing.

4.2 Mangrove material and isolation of endophytic fungi

Matured leaves of *R. apiculata* were collected from Cagraray Island, Albay, Philippines in July 2022 with GPS coordinates 13°18′26.8″N and 123°54′32.4″E. The mangrove was authenticated by Dr. Cecilia Moran, Curator of the University of Santo Tomas Herbarium (USTH). A voucher specimen (USTH 017168) was deposited at the UST Herbarium.

4.3 Isolation of the endophytic fungi

The leaves were thoroughly cleaned under running tap water and cut into 6 mm fragments. The leaves were surface sterilized using 75% EtOH for 1 min and by 5% NaHClO for 3 min. The leaves were then immersed in 75% EtOH for 30 sec and were blot-dried and plated on a 2% malt extract agar (MEA). The plates were incubated for 5 days at RT. The fungal hyphae growing from the inoculum were isolated and purified. A pure fungal colony was sub-cultured into MEA plates for morphological identification and into PDB slant tubes for mass production. Samples of *R. apiculata* were stored at the Mycology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas.

4.4 Morphological and microscopic identification of the EF

Fresh culture of the EF grown on MEA plate was processed for morphological characterization by observing colony pigmentation, texture, color and shape of spores, septation of hyphae, and shape of conidiophores using a microscope with 4x magnification. Identification of the EF was done by comparison of

morphological characteristics with identification keys of the EF of interest with species morphology in literature [13,24]. Taxonomy ID of the EF was assigned using the NCBI taxonomy browser.

4.5 Fermentation and extraction of the endophytic fungi

Several purified EF strains were inoculated in an autoclaved 3L nutrient media (26.5 g potato dextrose broth (PDB) / L water). After a 7-day growth period, 5.0 mL distilled water was added. The slant culture was then poured into several bottles containing PDB and sealed with sterile cotton. The culture suspension was left under stationary conditions at room temperature for a duration of three weeks. Both mycelia and culture were extracted with equal volume of ethyl acetate and left overnight. The mixture was then filtered, and the liquid mixtures were separated in a separatory funnel. The combined ethyl acetate layers were concentrated under reduced pressure to obtain the crude EF extract. The extract was kept at 4 ^{o}C until further use.

4.6 Clinical isolates

The ESKAPE clinical isolates (*Escherichia coli* 5218S, methicillin-resistant *Staphylococcus aureus* (MRSA) 2729B, *Klebsiella pneumoniae* 4830WD, *Acinetobacter baumannii* 3975B, *Pseudomonas aeruginosa* 5220U, and *Enterobacter cloacae* 5276S) were a gift from the Region 2 Trauma and Medical Center, Philippines.

4.7 Screening of the crude EF extract

The antibacterial activity of the crude EF extract was determined using clinical ESKAPE isolates using the Kirby-Bauer paper disk diffusion method. A 1000 µg/mL crude extract in ethyl acetate was used. The zone of inhibition (ZOI) was determined after 24 h incubation at RT using a Vernier caliper. Ethyl acetate was used as the negative control while penicillin was used as the positive control.

4.8 Isolation of compounds from the crude EF extract

The crude EF extract (Fs, 810 mg) was subjected to gravity column chromatography via gradient elution using hexane/$CHCl_3$ (1:1), neat $CHCl_3$, $CHCl_3$/acetone (9:1), $CHCl_3$/MeOH (9:1, 4:1, 1:1) and neat MeOH as eluents. TLC of the eluates yielded six pooled fractions, Fs1 – Fs6. All fractions were subjected to bioautography obtaining Fs2 for further purification. Silica gel CC of Fs2 (176 mg) using hexane/$CHCl_3$ gradient elution followed by TLC gave nine pooled fractions, Fs2A – Fs2I. Upon bioautography of the Fs2 fractions, Fs2C and Fs2I were further purified. Isocratic elution CC of Fs2C (42 mg) using hexane/$CHCl_3$ (1:2) gave benzoic acid (1, 18.6 mg). Isocratic silica gel CC using hexane/EtOAc (1:1) of Fs2I afforded bis(3-methylhexyl) phthalate (2, 1.0 mg).

Benzoic acid (**1**, white solid): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (2H, dd, J=8.1, 1.0 Hz, H_2-3), 7.48 (2H, dd, J=10.9, 4.6 Hz, H_2-4), 7.63 (1H, m, H-5); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8 (C-1), 129.4 (C-2), 130.4 (C-3), 128.6 (C-4), 133.9 (C-5).

Bis(3-methylhexyl) phthalate (**2**, colorless oil): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (2H, dd, J=5.6, 3.4 Hz), 7.53 (2H, dd, J=5.8, 3.2 Hz), 4.21 (2H, qd, J=10.8, 6.0 Hz), 1.68 (4H, m), 1.63 (4H, m), 1.41 (4H, m), 1.30 (4H, m), 0.92 (6H, t, J=7.6 Hz), 0.89 (6H, d, J=7.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0 (C-1), 132.5 (C-2), 128.9 (C-3), 131.1 (C-4), 68.3 (C-1′), 38.8 (C-2′), 30.5 (C-3′), 29.5 (C-4′), 23.8 (C-5′), 11.1 (C-6′), 14.2 (C-7′).

4.9 General procedure for TLC bioautography

Clinical isolate *E. cloacae* 5276S was used for TLC bioautography. *E. cloacae* was swabbed onto a Mueller Hinton Agar plate. A TLC plate spotted with the pooled fractions was placed on the surface of the agar plate and was cooled for 2 h. The TLC plate was then removed, and the agar plate was sealed and incubated for 24 h. After incubation, the bioactivity of each fraction was assessed by determining the ZOI.

4.10 Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of the isolated compounds were evaluated using the clinical isolate *E. cloacae* following previously published procedure on the microtiter plate antimicrobial assay [25]. A 500 µg/mL concentration of compounds in DMSO was prepared and serially diluted up to 1.9 µg/mL in a 96-well plate. Gentamycin

was used as the positive control with MIC of 15.65 µg/mL and DMSO was utilized as the negative control with MIC of >500 µg/mL.

4.11 Statistical analysis

The ZOIs were reported as mean ± standard deviation of triplicate trials. A Vernier caliper was used to measure the ZOI.

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Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Wen J, Okyere S, Wang S, Wang J, Xie L, Ran Y, Hu Y. Endophytic fungi: An effective alternative source of plant-derived bioactive compounds for pharmacological studies. J Fungi. 2022; 8(2):205. https://doi.org/10.3390/jof8020205

[2] 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):959. https://doi.org/10.3390/ijms22020959

[3] Gakuubi M, Munusamy M, Liang Z-X, Ng B-S. Fungal endophytes: A promising frontier for discovery of novel bioactive compounds. J Fungi. 2021; 7(10):786. https://doi.org/10.3390/jof7100786

[4] Verma A, Shameem N, Singh Jatav H, Sathyanarayana E, Parray J, Poczai P, Sayyed R. Fungal endophytes to combat biotic and abiotic stresses for climate-smart and sustainable agriculture. Front Plant Sci. 2022; 13:953836. https://doi.org/10.3389/fpls.2022.953836

[5] Nicoletti R, Salvatore M, Andolfi A. Secondary metabolites of mangrove-associated strains of Talaromyces. Mar Drugs. 2018; 16(1):12. https://doi.org/10.3390/md16010012

[6] Cadamuro R, da Silveira Bastos I, Silva I, da Cruz A, Robl D, Sandjo L, Alves S, Lorenzo J, Rodriguez-Lazaro D, Treichel H, Steindel M, Fongaro G. Bioactive compounds from mangrove endophytic fungus and their uses for microorganism control. J Fungi. 2021; 7(6):455. https://doi.org/10.3390/jof7060455

[7] Xu J. Biomolecules produced by mangrove-associated microbes. Curr Med Chem. 2011; 18(34):5224–5266. https://doi.org/10.2174/092986711798184307

[8] Zhou J, Feng Z, Zhang W, Xu J. Evaluation of the antimicrobial and cytotoxic potential of endophytic fungi extracts from mangrove plants *Rhizophora stylosa* and *R. mucronata*. Sci Rep. 2022; 12:2733. https://doi.org/10.1038/s41598-022-06711-9

[9] Ramalingam V, Rajaram R. Enhanced antimicrobial, antioxidant and anticancer activity of *Rhizophora apiculata*: An experimental report. 3 Biotech 2018; 8(4):200. https://doi.org/10.1007/s13205-018-1222-2.

[10] Baishya S, Banik SK, Choudhury MD, Talukdar DD, Talukdar AD. Chapter 11- Therapeutic potentials of littoral vegetation: an antifungal perspective. In: *Biotechnological Utilization of Mangrove Resources*. Eds. Patra JK, Mishra RR, Thatoi H. Academic Press, 2020; pp. 275–292. https://doi.org/10.1016/b978-0-12-819532-1.00011-1.

[11] Elfiati D, Delvian, Syafitri M, Hidayat A. Exploring endophytic fungi from Rhizophora spp leaves for improving plant growth. IOP Conf Ser: Earth Environ Sci. 2022; 977:012018. https://doi.org/10.1088/1755-1315/977/1/012018.

[12] Shara M, Basyuni M, Hasanuddin. Potential of phylloplane fungi from mangrove plant (*Rhizophora apiculata* Blume) as biological control agents against *Fusarium oxysporum* f. sp. *cubense* in banana plant (*Musa acuminata* L.). Forests. 2023; 14(2):167. https://doi.org/10.3390/f14020167

[13] Kiti H, Munga C, Odalo J, Guyo P, Kibiti C. Diversity of mangrove fungal endophytes from selected mangrove species of coastal Kenya. WIO J Mar Sci. 2021; 20(1):125–136. https://doi.org/10.4314/wiojms.v20i1.11

[14] National Library of Medicine., National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/taxonomy/?term=169388 (accessed on 10 July 2023).

[15] Huang L, Zhu X, Zhou S, Cheng Z, Shi K, Zhang C, Shao H. Phtalic acid esters: Natural sources and biological activities. Toxins. 2021; 13(7):495. https://doi.org/10.3390/toxins13070495

[16] Amborabé B, Fleurat-Lessard P, Chollet J, Roblin G. Antifungal effects of salicylic acid and other benzoic acid derivatives towards *Eutypa lata*: structure-activity relationship. Plant Physiol Biochem. 2002; 40(12):1051–1060. https://doi.org/10.1016/S0981-9428(02)01470-5

[17] Amin D, Abolmaaty A. Efficacy assessment of various natural and organic antimicrobials against *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*. Bull Natl Res Cent. 2020; 44:172. https://doi.org/10.1186/s42269-020-00423-8

[18] Peh E, Kittler S, Reich F, Kehrenberg C. Antimicrobial activity of organic acids against Campylobacter spp. and development of combinations—A synergistic effect? PLoS One 2020; 15(9):e0239312. https://doi.org/10.1371/journal. pone.0239312

[19] Synowiec A, Zyla K, Gniewosz M, Kieliszek M. An effect of positional isomerism of benzoic acid derivatives on antibacterial activity against *Escherichia coli*. Open Life Sci. 2021; 16:594–601. https://doi.org/10.1515/biol-2021-0060

[20] Aboobaker Z, Viljoen A, Chen W, Crous P, Maharaj V, van Vuuren S. Endophytic fungi isolated from *Pelargonium sidoides* DC: Antimicrobial interaction and isolation of a bioactive compound. S Afr J Bot. 2019; 122:535–542. https://doi.org/10.1016/j.sajb.2019.01.011

[21] Louis M, Tahrioui A, Verdon J, David A, Rodrigues S, Barreau M, Manac'h M, Thiroux A, Luton B, Dupont C, Le Calve M, Bazire A, Crepin A, Clabaut M, Portier E, Taupin L, Defontaine F, Clamens T, Bouffartigues E, Cornelis P, Feuilloley M, Caillon J, Dufour A, Berjeaud J, Lesouhaitier O, Chevalier S. Effect of phthalates and their substitutes on the physiology of *Pseudomonas aeruginosa*. Microorganisms. 2022; 10(9):1788. https://doi.org/10.3390/microorganisms10091788

[22] Ochatt S, Alan A, Bhattacharya A, Hano C, Kiselev K, Marconi P, Otoni W, Park S, Tang K, Weathers P. Secondary metabolites: a boon from plants, the best chemist in nature: preface from the editors. Plant Cell Tissue Organ Cult. 2022; 149:1–6. https://doi.org/10.1007/s11240-022-02289-2

[23] Chen S, Yu H, Luo H, Wu Q, Li C, Steinmetz A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese Med. 2016; 11:37. https://doi.org/10.1186/s13020-016-0108-7

[24] Hamzah T, Lee S, 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; 9:1707. https://doi.org/10.3389/fmicb.2018.01707

[25] Tan M, Castro S, Oliva P, Yap P, Nakayama A, Magpantay H, dela Cruz T. Biodiscovery of antibacterial constituents from the endolichenic fungi isolated from *Parmotrema rampoddense*. 3 Biotech. 2020; 10:212. https://doi.org/10.1007/s13205-020-02213-5