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Evaluation of Antibacterial Activity, Total Phenolic and Flavonoid Contents of Extracts of Endophytic Fungi Associated with *Tinospora crispa* (L.) Hook. f. & Thomson

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Abstract*— In the preliminary study, endophytic fungi associated with *Tinospora crispa* have been reported as antibacterial that assayed by TLC-bioautography. While more comprehensive studies for antibacterial activity using microdilution, total phenolic, flavonoid contents, and their relationship of extracts of fungal endophytes from this plant have never been investigated yet. This research aims to assess antibacterial activity, total phenolic, total flavonoid, and their relationship of fungal extracts associated with *T. crispa*. Based on morphological identification, this study revealed that endophytic *Phomopsis* sp. is the most isolated fungi (35% of fungal isolate composition). Based on the microdilution method, morphological and molecular identification showed that the fungal extracts performing a vigorous antibacterial activity (MIC value: <64 µg.ml^{-1}) against *S. aureus* InaCC-B4 were three extracts i.e., *Colletotrichum brevisporum* TcDn1Bd-01, and *Diaporthe passifloricola* TcBt2Bo-03, and *Alternaria alstroemeriae* TcTd2Bo-07. While one extracts, *Phomopsis* sp. TcBt1Bo-06, have potent bacterial growth inhibition toward *E. coli* InaCC-B5 (MIC value: <64 µg.ml^{-1}). The highest of both total phenolic content (TPC) and total flavonoid content (TFC) values of the extract is *A. alstroemeriae* TcTd2Bo-07 which are 166.210 ± 0.000 GAE/extract (mg/g) and 339.991 ± 0.136 QE/extract (mg/g), respectively. There is a negative and significantly very high Pearson's correlation TPC values toward the MIC value of antibacterial against *S. aureus* and *E. coli* (r = -0.671 and -0.969, respectively, P<0.01). The results suggest that the extracts of endophytic fungi can be used as antibacterial sources. Evaluation of chemical structure and antibacterial activity of pure compound need to be solved.

***Keywords*— Antibacterial; microdilution method; TPC; TFC; endophytic fungi; *Tinospora crispa*.**

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I. INTRODUCTION

In recent decades, endophytic fungi associated with plants have been interesting to study both biologically and chemically for pharmaceutical use as a potential producer of bioactive compounds [1], [2]. They can be a great source of active substances and have a large repository of varied substances such as antifungal, antibacterial, anticancer, anti-inflammatory, antiviral, immunosuppressive, plant growth-stimulating, pesticide, antioxidant, antiparasitic, antidiabetic, and antimalarial [1]–[4]. Plants coexist with endophytic microorganisms present inside plant tissues and produce valuable metabolites for protecting plants [5] .

Fungal endophytic associated with plants have been well proven for their host plant valuables attributes, such as mobilization of different nutrients and helping the uptake of these nutrients, production of phytohormones for plant growth, and induction of abiotic stress tolerance in plants [6], [7]. Endophytic fungi are microorganisms symbiotically related to living plant tissue that causes asymptomatic disease [3] in their host and nonhost-specific [8]. This microorganism keeps balancing the fungal community to protect plants from the pathogenic causing disease [9].

Fungal endophytes are also the reservoir of natural bioactive substances inside plants because they can synthesize active compounds such as anti-microbial compounds that can be used for their host plant protection against pathogenic fungi, bacteria, and abiotic pressure [5], [10]. Previous studies reported that fungal endophyte extracts associated with T .crispa collected from Pamempeuk and Ogan Ilir produced bioactive metabolites [11], [12].

T. crispa is a medicinal plant that belongs to the Menispermaceae family. This plant can be found in the forests in Asia and Africa and have been used for traditional remedy with various pharmacological properties due to the richness of its chemical constituents [13]. Moreover, fungal endophytes metabolites associated with Menispermaceae family plants possessed wide-ranging pharmacological activities as antibacterial, antifungal, and anti-hyperuricemic [14], [15].

In a previous study, fungal endophytes isolated from the medicinal plant *T. crispa* have been reported to inhibit bacterial growth that was applied by bioautography with the TLC method [16]. While research corresponding to antibacterial activity using microdilution method, total phenolic, total flavonoid and their relationship of these extracts isolated from T. crispa collected from several areas has not yet been informed. Work process depicted in the diagram below (Fig. 1). This study aimed to investigate the antibacterial activity, total phenolic, total flavonoid, and their relationship of fungal extracts associated with *T. crispa.*

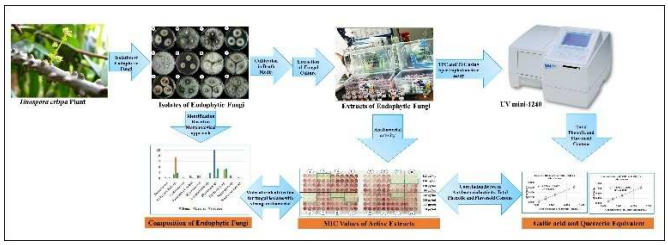

Fig. 1 Work Diagram Illustrations.

II. Materials and Methods

A. Chemicals and Instruments

All chemicals were purchased from commercial resources. Standard antibiotics were used as the positive control for antibacterial, i.e., vancomycin (Sigma-Aldrich), erythromycin (Sigma-Aldrich), and amoxicillin (Sigma-Aldrich) were dissolved in dimethyl sulfoxide (DMSO, Merck). Iodonitrotetrazolium p-violet (INT, Sigma) for antibacterial activity using a microdilution assay.

Sodium carbonate (Merck), Folin-Ciocalteau (Merck), and gallic acid (Sigma-Aldrich) were used for total phenolic contents. Total flavonoid contents were quercetin (Sigma-Aldrich), ethanol p.a (Merck), $NaNO_2$ (Merck), $AlCl_3$ (Merck), NaOH (Merck). Total phenolic and flavonoid contents were determined by a colorimetric assay using UV-VIS spectrophotometry (UV mini-1240, Shimadzu).

B. Materials

Samples of healthy and fresh leaves, stems, and petioles of *T. crispa* plants were taken from Bandung, Bekasi, and Bogor, West Java Province, Indonesia. The specimens were identified and deposited at Herbarium Bogoriense, Botany Division, RC for Biology.

C. Bacterial Strains for Antibacterial Testing

Gram-negative and positive bacteria were used for testing of antibacterial activity, i.e., *S. aureus* strain InaCC - B4 and *E. coli* strain InaCC - B5, respectively. Both bacterial strains were obtained from the Indonesian Culture Collection (InaCC), Microbiology Division, RC for Biology.

D. Isolation of Fungal Endophytes

Isolation of fungal endophytes was performed by sterilization on the surface of plant parts, according to Praptiwi et al. [17]. The fungi isolates were stored in 10 % (v/v) glycerol, added 5 % (w/v) trehalose at a temperature of -80 ºC at InaCC, Microbiology Division, RC for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

E. Identification of Endophytic Fungi: Morphological analysis

Fungal identification was conducted based on a morphological approach, according to Ilyas et al. [18]. The morphological identification approach was conducted by observing the macroscopic and microscopic phenotypes. Identification using macroscopic characterizations was observed, including the color, surface, texture, colony shape, exudate drop, and inverted colors. In comparison, fungal mycelia are placed in one drop of 1% lactophenol cotton blue stain (LCB, Hardy Diagnostic) solution for microscopic observation. The characterization was performed under a light microscope (Olympus BX43) by observing hyphae, spores, septate, clamp connections, hyphae pigmentation, and other reproductive structures.

F. Preparation of Active Extracts and Standard Antibiotic

The previous study did cultivation, extraction, and initial study to provide active extracts as antibacterial of fungal extracts [16]. The active extracts as antibacterial or selected extracts and standard antibiotics were used as a positive control consisting of vancomycin (Sigma-Aldrich), erythromycin (Sigma-Aldrich), and amoxicillin (Sigma-Aldrich). Preparation of the active extracts and standard antibiotic as the stock solution, i.e., 20480 and 1280 µg/ml respectively, was dissolved in dimethyl sulfoxide (DMSO, Merck).

G. Antibacterial Activity Assay: Determination MIC Value

The MIC values were determined by serial microdilution in a 96-well microplate (Corning), according to Pessini et al. [19]. The MIC value is the minimum concentration that occurs in clear wells, indicating the lack of bacterial growth. The growth of bacterial occurs in the negative control chamber without active antibacterial compounds, INT (yellow color) reduced to formazan (purple color) due to mitochondrial dehydrogenase in the bacterial cell.

H. Determination of TPC Value

The TPC of the fungal extract was determined by the Folin-Ciocalteau spectrophotometric method, according to Ismail et al. [20]. It was performed in triplicate, and the TPC value was stated as Gallic Acid Equivalent (GAE) per extract (mg / g).

I. Determination of TFC Value

The TFC was investigated using a colorimetric assay, according to Zou et al. [21]. It was performed in triplicate. The TFC value was stated as Quercetin Equivalent (QE) per extract (mg / g).

J. Statistical Analysis

A statistical analysis of the variance of both TPC and TFC values was performed by multiple ranges of Duncan tests using SPSS 16.0. The experiment was performed in triplicate. It was stated as mean ± standard deviation.

K. Correlation Test between Antibacterial Activity and Total Phenolic Contents

The correlation test of antibacterial, TPC, and TFC was performed by Pearson's correlation ($P<0.01$).

L. Identification of Selected Endophytic Fungi: Molecular and phylogenetic analysis

The purified fungal isolates were identified based on both genotypic and phenotypic (morphology) characters. Characteristics based on morphology were identified both macroscopically and microscopically under a light microscope. Genotypic identification was conducted based on an analysis of the sequence of the internal transcribed spacer (ITS) regions, including 5.8S rDNA.

1) Extraction of DNA and amplification of polymerase chain reaction (PCR): Nucleon PhytoPure kit was used to isolate fungal DNA, and extraction kits (GE Healthcare) were used to extract fungal DNA. Amplification using PCR for ITS 1 and ITS 2 regions, including 5.8S rDNA, and the D1 and D2 domains of LSU rDNA. The primer set is 5`-TCCTCCGCTTATTGATATGC-3` and 5`-GGAAGTAAAAGTCGTAACAAGG-3` for ITS4 and ITS5, respectively. They were used to amplify ITS1 and ITS2, including 5.8S rDNA [22]. Amplification was performed in a TaKaRa PCR (TaKaRa BIO Inc.,); program amplification according to Napitupulu et al. [23]. The product was purified using the PEG precipitation method [24].

2) Sequencing Reaction: Sequencing reactions were performed using a Reaction Kit (Applied Biosystems). The primers used are ITS4 and ITS5 [22]. The reaction was performed in a TaKaRa PCR (TaKaRa BIO Inc.); reaction program, purification, and analysis according to Napitupulu et al. [23].

3) Phylogenetic Analyses: BioEdit program was used for trimming and assembling the sequence [25]. Download from DNA Data Bank of Japan for alignment for the assembled sequences by using the Clustal X 1.83 package. The phylogenetic analysis was done by maximum-parsimony and neighbor-joining methods using Phylogenetic Analyses Using Parsimony (PAUP) v. 4.0b8 program. The quality for every branch was assessed by clustering with 1000 resampling in PAUP v. 4.0b8.

III. Results and Discussion

A. Identification of Endophytic Fungi: Morphological Analysis

Eighty fungal isolates have been successfully collected and identified. Morphological identification revealed that the most endophytic fungi obtained from *T. crispa* were *Phomopsis* sp. (28 isolates; 35% of the composition; found dominantly in the stem) (Table 1). They are also the predominantly endophytic fungi found in the stems and petioles of *T. crispa* plant. According to Ilyas et al., [18], the endophytic fungi strains were obtained from several medicinal plants, also dominated by the genera *Phomopsis*. Another study reported that endophytic fungi *Phomopsis* sp. are commonly found in many host plants [26]. Endophytic *Phomopsis* also the genus recovered mainly from tropical plants [27].

Besides Phomopsis sp., *Colletotrichum* sp. is the leaf's dominant endophytic fungi (15 isolates). While *Phyllosticta* sp. were the most frequently obtained in the petioles (7 isolates) (Fig. 2). Several representatives of fungi are shown in Fig.3. The fungal composition is affected by the species of their host and the types of tissues, and several fungal endophytes indicated preference tissue and significant host [28]. Several studies explained that the colonization rate, composition of community, and diversity of fungal isolates were influenced by their host species, genotypic, types of tissue, geographical position, and abiotic causes [29]–[32].

TABLE I
IDENTIFICATION OF FUNGAL ISOLATES.

Sample No.	Isolate Name	Strain Code	Sample No	Isolate Name	Strain Code
1	*Phomopsis* sp.	TcBt1Bd-2	41	*Phyllosticta* sp.	TcTd2Be-4
2	*Phomopsis* sp.	TcBt1Bd-5	42	*Phomopsis* sp.	TcBt1Bo-1
3	*Phomopsis* sp.	TcBt1Bd-7	43	*Phomopsis* sp.	TcBt1Bo-2
4	*Phomopsis* sp.	TcBt1Bd-8	44	*Colletotrichum* sp.	TcBt1Bo-3
5	*Phomopsis* sp.	TcBt1Bd-9	45	*Phomopsis* sp.	TcBt1Bo-4
6	*Phomopsis* sp.	TcBt1Bd-10	46	*Phomopsis* sp.	TcBt1Bo-5
7	*Fusarium* cf. *solani*	TcBt2Bd-1	**47**	*Phomopsis* sp.	TcBt1Bo-6
8	*Phomopsis* sp.	TcBt2Bd-3	48	*Phomopsis* sp.	TcBt1Bo-7
9	*Phomopsis* sp.	TcBt2Bd-4	49	Dematiaceae	TcBt1Bo-8
10	Hypomycetes	TcBt2Bd-6	50	*Phomopsis* sp.	TcBt1Bo-9
11	*Colletotrichum* sp.	TcDn1Bd-1	51	*Phomopsis* sp.	TcBt1Bo-10
12	*Colletotrichum* sp.	TcDn1Bd-2	52	*Neofusicoccum* sp.	TcBt2Bo-1
13	*Fusarium* cf. *solani*	TcDn1Bd-3	53	*Fusarium* cf. *solani*	TcBt2Bo-2
14	*Colletotrichum* sp.	TcDn2Bd-1	54	*Phomopsis* sp.	TcBt2Bo-3
15	*Colletotrichum* sp.	TcDn2Bd-2	55	*Phomopsis* sp.	TcBt2Bo-4
16	Coelomycetes	TcDn2Bd-3	56	*Phyllosticta* sp.	TcDn1Bo-1
17	*Colletotrichum* sp.	TcDn2Bd-4	**57**	Hypomycetes	TcDn1Bo-2

18	*Colletotrichum* sp.	TcTd1Bd-1	58	*Phyllosticta* sp.	TcDn1Bo-3
19	*Phomopsis* sp.	TcTd1Bd-2	59	Hypomycetes	TcDn1Bo-4
20	*Lasiodiplodia* sp.	TcTd1Bd-3	60	*Phyllosticta* sp.	TcDn1Bo-5
21	*Phomopsis* sp.	TcTd2Bd-1	61	*Colletotrichum* sp.	TCDn2Bo-1
22	*Phyllosticta* sp.	TcTd2Bd-2	62	*Colletotrichum* sp.	TcDn2Bo-2A
23	*Lasiodiplodia* sp.	TcTd2Bd-3	63	*Phyllosticta* sp.	TCDn2Bo-3
24	*Phomopsis* sp.	TcTd2Bd-4	64	*Colletotrichum* sp.	TCDn2Bo-4
25	*Colletotrichum* sp.	TcTd2Bd-5	65	*Colletotrichum* sp.	TCDn2Bo-5
26	*Lasiodiplodia* sp.	TcTd2Bd-6	66	*Colletotrichum* sp.	TcDn2Bo-6
27	Hypomycetes	TcBt1Be-1	67	*Colletotrichum* sp.	TcDn2Bo-7
28	*Nigrospora* sp.	TcBt1Be-3	68	*Phomopsis* sp.	TcDn2Bo-8A
29	*Phomopsis* sp.	TcBt2Be-1	69	*Colletotrichum* sp.	TcDn2Bo-9
30	*Colletotrichum* sp.	TcBt2Be-5	70	*Phyllosticta* sp.	TcTd1Bo-1
31	*Phomopsis* sp.	TcBt2Be-6	71	*Phyllosticta* sp.	TcTd1Bo-2
32	*Colletotrichum* sp.	TcBt2Be-7	72	*Phyllosticta* sp.	TcTd1Bo-3
33	*Colletotrichum* sp.	TcDn1Be-1	73	*Colletotrichum* sp.	TcTd1Bo-4
34	*Colletotrichum* sp.	TcDn2Be-1	74	*Phyllosticta* sp.	TcTd2Bo-1A
35	*Phyllosticta* sp.	TcDn2Be-2	75	*Phomopsis* sp.	TcTd2Bo-2
36	*Colletotrichum* sp.	TcDn2Be-3	76	*Lasiodiplodia* sp.	TcTd2Bo-3
37	*Phyllosticta* sp.	TcDn2Be-4	77	*Phomopsis* sp.	TcTd2Bo-4
38	*Nigrospora* sp.	TcBt1Be-2	78	*Phomopsis* sp.	TcTd2Bo-5
39	*Phyllosticta* sp.	TcTd2Be-2	79	*Phomopsis* sp.	TcTd2Bo-6
40	*Colletotrichum* sp.	TcTd2Be-3	**80**	Dematiaceae	TcTd2Bo-7

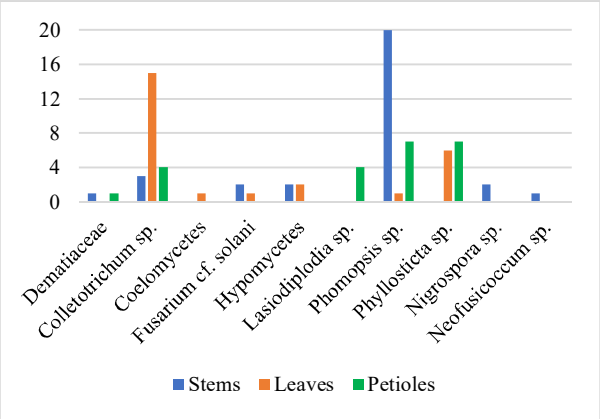

Fig. 2 The composition of fungal endophytes isolated from *T. crispa*.

B. Antibacterial Activity Assay: Determination of MIC value

The MIC value of active extracts was evaluated against *S. aureus* and *E. coli*. A representative of the antibacterial test of several endophytic fungi extracts against *S. aureus* (Fig. 4). The result showed that some fungal extracts have a range of MIC values of <16 and 512 µg.ml^{-1} (Table 2). According to a previous study, the MIC value of extracts lower than 100 µg.ml^{-1} were classified as strong inhibit of bacterial growth, while extracts with MIC values between 100-512 µg.ml^{-1} were categorized as moderate action [19]. This study revealed that three extracts of endophytic fungi performed vigorous antibacterial activity with MIC values of <64 µg/ml against *S. aureus*, i.e., extract No. 11 (*Colletotrichum brevisporum* TcDn1Bd-01), 54 (*Diaporthe passifloricola* TcBt2Bo-03), and 80 (*Alternaria alstroemeriae* TcTd2Bo-07). While one extract, extract No.47 (*Phomopsis* sp. TcBt1Bo-06), showed strong antibacterial activity (MIC values of <64 µg/ml) against *E.coli* (Table 2).

In other studies, endophytic fungi *Phomopsis* genera produced potent antibacterial compounds such as against *Pseudomonas syringae* and *were* reported as the source of bioactive metabolites with various biological action [33], [34]. While endophytic fungi Dematiaceae family was reported to be excellent antibacterial against *S. aureus* bacteria [35]. The endophytic fungi Dematiaceae family, such as *Alternaria,* also exhibited various biological activities such as anti-nematode [36], cytotoxic[36][37][38], antimicrobial [36], [37], anti-viral [39], anti-parasitic [40], enzyme inhibitors [38], [41], [42].

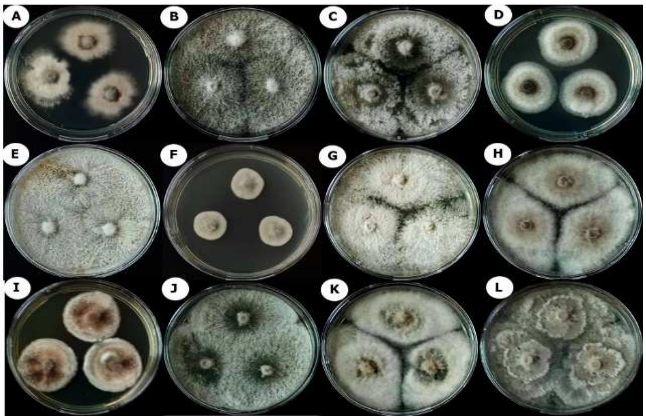

Fig. 3 Representatives of Fungal Isolates from *T.crispa* (cultivated in 20 ml PDA medium in Petri dishes for seven days). A: *Nigrospora* sp. TcBt1Be-2, B: *Phomopsis* sp. TcBt1Bo-10, C: *Phomopsis* sp. TcDn2Bo-8A, D: *Colletotrichum* sp. TcDn1Bd-1, E: *Phomopsis* sp. TcBt2Bo-3, F: Hyphomycetes TcDn1Bo-4, G: *Phomopsis* sp. TcBt1Bd-10, H: *Neofusicoccum* sp. TcBt2Bo-1, I: Dematiaceae TcTd2Bo-7, J: *Phomopsis* sp. TcBt1Bo-1, K: *Lasiodiplodia* sp. TcTd2Bo-3, L: *Phomopsis* sp. TcTd2Bo-4.

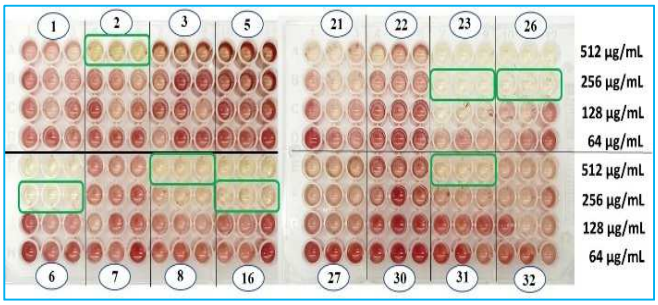

Fig. 4. Antibacterial activity using microdilution method of several endophytic fungi extracts against *S.aureus* (Sample No. 1, 2, 3, 5, 6, 7, 8, 16, 21, 22, 23, 26, 27, 30, 31, and 32). The MIC value is less drug or minimum concentration of the sample extract required for inhibiting the organism growth at which no growth is observed (marked with green color).

C. TPC and TFC Values and the Correlation for Antibacterial Activity

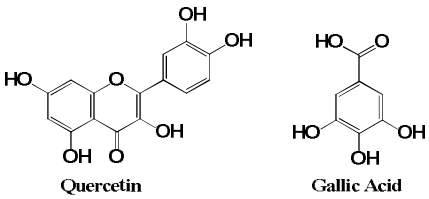

Fig. 5 Quercetin (Flavonoid) and Gallic Acid (Phenolic) as Standard for TFC and TPC.

The chemical structure of quercetin (flavonoid) and gallic acid (phenolic) is standard (Fig.5). The standard curve of quercetin (flavonoid) and gallic acid (Phenolic) obtained the linear equation y=1.2718x-0.0097 (R^2=0.9991) and y=4.9299+0.0385 (R^2=0.9992), respectively (Fig.6). TPC and TFC of the extracts obtained were stated as gallic acid equivalent (mg GAE.g^{-1}) and quercetin equivalent (mg QE.g^{-1}), respectively, using the linear equation based on the standard calibration curve. TPC and TFC of the extracts are displayed in Table 2. This study revealed that endophytic fungus Dematiaceae, *Alternaria alstroemeriae* TcTd2Bo-07 (isolate No.80) showed the highest both TPC and TFC values (166.210 ± 0.000 milligrams of GAE per gram extract and 339.991 ± 0.136 milligrams QE per gram extract, respectively) and also had a strong antibacterial action against *S. aureus* (Gram-positive bacteria). At the same time, endophytic fungus No. 47 (*Phomopsis* sp. TcBt1Bo-06), which has a moderate total phenolic content, showed excellent activity against Gram-negative bacteria.

Previous studies have indicated that flavonoids and phenolics have the potential to inhibit the Gram-positive and negative bacteria [43]–[46]. While another two extracts, *Colletotrichum brevisporum* (No.11) and *Diaporthe passifloricola* (No. 54), although TPC and TFC are low, they also are excellent to inhibit Gram-positive bacteria.

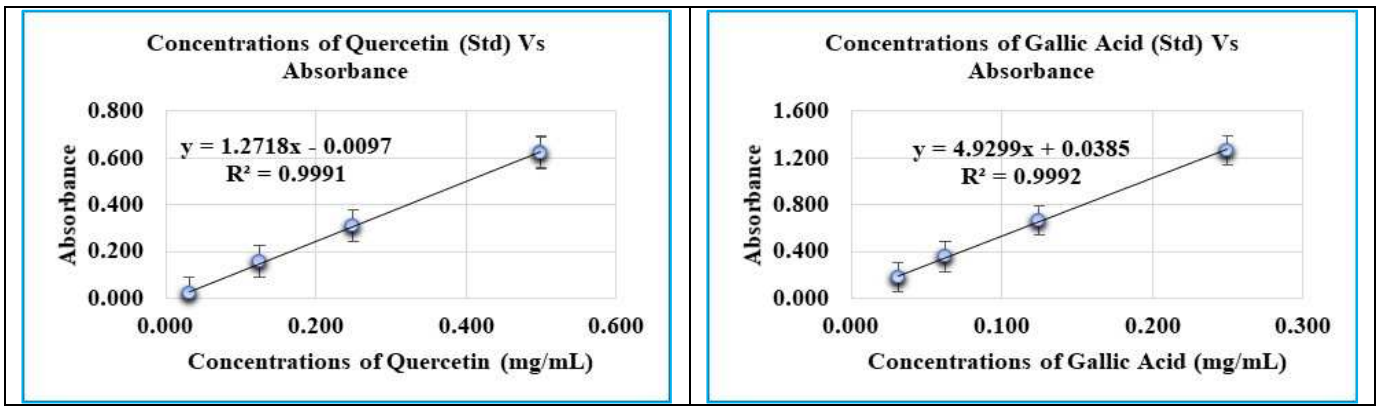

Fig. 6 Standard Calibration Curve of Quercetin (Flavonoid; Up) and Gallic Acid (Phenolic; Down)

TABLE II
ANTIBACTERIAL ACTIVITY, TPC, AND TFC VALUES OF THE ACTIVE EXTRACTS OF ENDOPHYTIC FUNGI

No. Sample	TPC (mg GAE.g^{-1} extract)	TFC (mg QE.g^{-1} extract)	*MIC S.aureus* (µg.ml^{-1})	Category of Antibacterial against *S.aureus*	*MIC E.coli* (µg.ml^{-1})	Category of Antibacterial against *E.coli*
2	5.051^{r} ± 0.000	40.651^{j} ± 0.079	512	**Moderate**	> 512	Weak
3	< 0.020	12.214^{w} ± 0.318	512	**Moderate**	> 512	Weak
5	< 0.020	10.169^{x} ± 0.045	512	**Moderate**	> 512	Weak
6	< 0.020	< 7.627	256	**Moderate**	> 512	Weak
7	1.197^{y} ± 0.041	**94.617^{d} ± 0.045**	512	**Moderate**	> 512	Weak
8	< 0.020	< 7.627	256	**Moderate**	> 512	Weak
10	< 0.020	< 7.627	128	**Moderate**	NT	
11	< 0.020	23.327^{p} ± 0.120	**< 64**	**Strong**	NT	
14	< 0.020	< 7.627	512	**Moderate**	NT	
16	4.341^{s} ± 0.000	42.591^{j} ± 0.091	256	**Moderate**	> 512	Weak
17	< 0.020	23.877^{o} ± 0.403	128	**Moderate**	NT	
19	< 0.020	< 7.627	256	**Moderate**	NT	
20	17.756^{k} ± 0.104	**85.967^{e} ± 0.318**	256	**Moderate**	NT	
23	< 0.020	13.603^{v} ± 0.208	128	**Moderate**	> 512	Weak
26	< 0.020	25.214^{n} ± 0.569	256	**Moderate**	> 512	Weak
28	**76.067^{c} ± 0.000**	**130.130^{c} ± 0.000**	256	**Moderate**	> 512	Weak
31	< 0.020	< 7.627	512	**Moderate**	> 512	Weak
35	< 0.020	< 7.627	512	**Moderate**	> 512	Weak
38	46.363^{f} ± 0.012	< 7.627	256	**Moderate**	> 512	Weak
39	**56.154^{e} ± 0.234**	< 7.627	256	**Moderate**	NT	
42	5.666^{q} ± 0.012	< 7.627	512	**Moderate**	512	**Moderate**
45	< 0.020	< 7.627	256	**Moderate**	NT	
47	**56.249^{d} ± 0.000**	9.829^{y} ± 0.000	256	**Moderate**	**< 64**	**Strong**
51	2.055^{w} ± 0.031	20.889^{q} ± 0.091	512	**Moderate**	512	**Moderate**
52	20.650^{j} ± 0.000	**63.191^{f} ± 0.045**	128	**Moderate**	256	**Moderate**
53	< 0.020	< 7.627	512	**Moderate**	> 512	Weak
54	< 0.020	< 7.627	**< 64**	**Strong**	> 512	Weak
59	**117.142^{b} ± 0.000**	19.893^{r} ± 0.208	256	**Moderate**	> 512	Weak

60	< 0.020	< 7.627	256	**Moderate**	NT	
63	< 0.020	< 7.627	256	**Moderate**	NT	
67	$42.029^{g} \pm 0.000$	$\mathbf{158.568^{b} \pm 0.045}$	512	**Moderate**	> 512	Weak
68	< 0.020	$18.504^{s} \pm 0.091$	512	**Moderate**	> 512	Weak
77	$2.826^{u} \pm 0.012$	$16.486^{t} \pm 0.227$	512	**Moderate**	512	**Moderate**
78	< 0.020	< 7.627	128	**Moderate**	128	**Moderate**
79	$3.922^{t} \pm 0.023$	$14.468^{u} \pm 0.000$	512	**Moderate**	> 512	Weak
80	$\mathbf{166.210^{a} \pm 0.000}$	$\mathbf{339.991^{a} \pm 0.136}$	**< 64**	**Strong**	> 512	Weak
Amox	NT	NT	<0.5	**Strong**	8	**Strong**
Eryth	NT	NT	<0.5	**Strong**	16	**Strong**
Vanco	NT	NT	<0.5	**Strong**	>32	**Moderate-Strong**

Remark: NT: not tested. Analysis of variance was performed using Duncan's multiple ranges for TPC and TFC values. The experiment was carried out in triplicate. It is stated as mean ± standard deviation. Values in each column with the distinctive letters are significantly different (P<0.05). Statistical Criteria of MIC values for extracts as follows: weak < 625 µg/ml < moderate < 100 µg/ml < strong [47].

TABLE III
PEARSON CORRELATIONS COEFFICIENT (R) BETWEEN TPC, TFC VALUES, AND ANTIBACTERIAL ACTIVITY

Variables	Pearson correlations coefficient (r)	
	MIC value against *S.aureus*	MIC value against *E.coli*
TPC value	-0.671*	-0.969*
TFC value	-0.276	-0.073

Remark: (*): Correlation is significant at P< 0.01.

The correlation between antibacterial activity, TPC, and TFC values is shown in Table 3. This research showed that correlation is negative and significantly high in Pearson's correlation TPC values toward the MIC value of antibacterial against both Gram-positive and negative bacteria with the r-value of -0.671 (strong correlation) and -0.969 (robust correlation), respectively (P<0.01). The higher TPC in the extract, the lower value of MIC (the higher effectivity of antibacterial).

In contrast, the correlation between TFC and the MIC values was not significantly high in Pearson's correlation. However, in this case of endophytic fungus extract of *Alternaria alstroemeriae* TcTd2Bo-07 (family Dematiaceae), the extract with the highest TFC value as well as the TPC values have strong as an antibacterial against *S. aureus*. The results indicated that total phenolic contents of fungal endophytic extracts had a high correlation to antibacterial activity.

According to da Silva et al. [48] a moderate correlation between anti-microbial activity and TPC value. While [49] reported the extract of the endophytic fungus *Alternaria alternata* with lower TPC value, it showed moderate as antioxidant and good as anti-microbial agents.

In another study, Borges et al. [50] reported that phenolic compounds, such as gallic acid, led to irreversible changes in membrane properties, including charge, physicochemical, and permeability of extracellular and intracellular properties. The change of membrane properties due to a decrease of negative surface charge, change of hydrophobicity, and leakage of the cellular membrane caused pore formation in the cell membranes [50]. The results suggest that the fungal endophytic extracts can be utilized as natural antibacterial sources.

D. Identification of Selected Endophytic Fungi: Molecular and Phylogenetic Analysis

Molecular identification for three selected endophytic fungi which have antibacterial (strong against S.aureus) using the ITS rDNA analysis and NCBI BLAST is shown in Table 4. C. brevisporum TcDn1Bd-1, D. passifloricola TcBt2Bo-3, and A. alstroemeriae TcTd2Bo-7 have the opportunity to be explored and characterized antibacterial as source bioactive compounds for future research.

TABLE IV
THE BLAST RESULT OF SELECTED STRAINS OF ENDOPHYTIC FUNGI ASSOCIATED WITH *T. CRISPA*

Sample No	Strain	Highest similarity based on NCBI BLAST (https://blast.ncbi.nlm.nih.gov)	Accession number	Similarity
11	TcDn1 Bd-1	*Colletotrichum brevisporum*	NR111637	98.08%
54	TcBt2 Bo-3	*Diaporthe passifloricola*	NR147595	99.29%
80	TcTd2 Bo-7	*Alternaria alstroemeriae*	NR163686	99.65%

Colletotrichum is endophytic and plant pathogenic [51]–[53]. It is distributed throughout tropical and subtropical regions worldwide [54], [55]. Colletotric acid is an anti-microbial compound from Colletotrichum gloeosporioides isolated from *Artemisia mongolica*. It potent as antibacterial agent against *S. aureus*, *B. subtilis*, and *Sarcina lutea* [56].

The genus *Diaporthe* is a sexual state of *Phomopsis* that has interaction with the host as saproic, pathogenic, endophytic, and biocontrol with distribution in temperate and tropical areas [57][58][26]. Antibacterial compounds from other endophytic fungi with great power that isolated from fungi *Diaporthe* sp. GNBP-10 (associated with *Uncaria gambier*) is bisanthraquinone, (+) - 2,2'-episitoskirin A. This compound has strong antibacterial properties against *S. aureus* BCC 1452 which is stronger than the positive controls (chlorampenicol and erythromycin) [59]. Coumarin derivative from *Diaporthe* sp. has antibacterial potency against *Bacillus subtilis* [60]. Bioactive compounds such as the derivative of α-pyrone produced by *Diaporthe* [61] have a wide spectrum of biological performance as antifungal, antiinsect, and cytotoxic [61], [62].

Genus *Alternaria* fungi, belonging to the family Dematiaceae of the order Hyphomycetes in the Fungi Imperfecti, are widely distributed in Nature [63]. Antibacterial compounds, derivative of chromenes from endophytic fungal isolated from *Dasymaschalon rostratum* [64]. A fungal endophyte, *A. alternata* obtained from leaves of *Catharanthus roseus* produce *p*-coumaric acid that potent as anti-microbial [65]. The derivative compounds of polyketides isolated from *A. alternata* inhibit bacteria growth

of plant pathogenic and also have the ability as antiplatelet agent [66], [67].

IV. CONCLUSION

The study of evaluation of antibacterial activity, TPC, TFC, and their relationship of fungal endophytes associated with *T.crispa* plants can be summarized as three extracts showed excellent antibacterial testing against *S.aureus*, while one extracts have high inhibition potency against *E.coli.* Fungi *Phomopsis* genera are the most endophytic fungi isolated from this plant. The relationship is a negative and significantly high Pearson's correlation between TPC values and the MIC value of antibacterial against both positive and negative bacteria (TPC value contributes to increasing the antibacterial activity) ($P<0.01$). The results suggest that the extracts of endophytic fungi can be used as antibacterial sources. The bioactive metabolite(s) isolation is responsible for the anti-microbial activity of *A. alstroemeriae* TcTd2Bo-07 and other fungal extracts (*Phomopsis* sp. TcBt1Bo-06, *C. brevisporum* TcDn1Bd-1, and *D. passifloricola* TcBt2Bo-3) which have strong antibacterial activity needs to be done.

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AUTHOR CONTRIBUTIONS

We state that all authors contributed to this paper and worked equally as primary contributors.

CONFLICT OF INTEREST

We state that there are no conflicts of interest in this paper.

REFERENCES

[1] N. Rai *et al.*, "Plant associated fungal endophytes as a source of natural bioactive compounds," *Mycology*, pp. 1–21, Jan. 2021, doi: 10.1080/21501203.2020.1870579.

[2] G. Strobel, "The Emergence of Endophytic Microbes and Their Biological Promise," *J. fungi*, vol. 4, no. 2, p. 57, May 2018, doi: 10.3390/jof4020057.

[3] G. Caruso, M. Abdelhamid, A. Kalisz, and A. Sekara, "Linking Endophytic Fungi to Medicinal Plants Therapeutic Activity. A Case Study on Asteraceae," *Agriculture*, vol. 10, no. 7, p. 286, 2020, doi: 10.3390/agriculture10070286.

[4] B. S. Adeleke and O. O. Babalola, "Pharmacological potential of fungal endophytes associated with medicinal plants: A review," *J. Fungi*, vol. 7, no. 2, pp. 1–16, 2021, doi: 10.3390/jof7020147.

[5] A. E. Fadiji and O. O. Babalola, "Elucidating Mechanisms of Endophytes Used in Plant Protection and Other Bioactivities With Multifunctional Prospects," *Front. Bioeng. Biotechnol.*, vol. 8, no. May, pp. 1–20, 2020, doi: 10.3389/fbioe.2020.00467.

[6] K. L. Rana *et al.*, "Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability," *Antonie Van Leeuwenhoek*, vol. 113, no. 8, pp. 1075–1107, 2020, doi: 10.1007/s10482-020-01429-y.

[7] R. Lata, S. Chowdhury, S. Gond, and Jj. White, "Induction of abiotic stress tolerance in plants by endophytic microbes," *Lett Appl Microbiol*, vol. 66, pp. 268–276, 2018.

[8] B. U. Furtado, S. Szymańska, and K. Hrynkiewicz, "A window into fungal endophytism in Salicornia europaea: deciphering fungal characteristics as plant growth promoting agents," *Plant Soil*, vol. 445, no. 1, pp. 577–594, 2019, doi: 10.1007/s11104-019-04315-3.

[9] A. Manzotti *et al.*, "Insights into the community structure and lifestyle of the fungal root endophytes of tomato by combining amplicon sequencing and isolation approaches with phytohormone profiling," *FEMS Microbiol. Ecol.*, vol. 96, no. 5, May 2020, doi: 10.1093/femsec/fiaa052.

[10] C. An, S. Ma, X. Shi, W. Xue, C. Liu, and H. Ding, "Isolation, diversity, and antimicrobial activity of fungal endophytes from Rohdea chinensis (Baker) N.Tanaka (synonym Tupistra chinensis Baker) of Qinling Mountains, China," *PeerJ*, vol. 8, p. e9342, 2020.

[11] Praptiwi, Y. Jamal, A. Fathoni, A. Nurkanto, and A. Agusta, "3-Acetyl-2,5,7-Trihydroxy-1,4-Naphtalenedione, An Antimicrobial Metabolite from The Culture of Endophytic Fungus Coelomycetes TCBP4 from Tinospora crispa," *Media Heal. Res. Dev.*, vol. 23, no. 3, pp. 95–101, 2013, doi: 10.22435/mpk.v23i3.3278.95-101.

[12] Elfita, Munawar, Muharni, and M. Sudrajat, "Identification of New Lactone Derivatives Isolated from Trichoderma sp., An Endophytic Fungus of Brotowali (Tinaspora crispa)," *HAYATI J. Biosci.*, vol. 21, no. 1, pp. 15–20, 2014, doi: 10.4308/hjb.21.1.15.

[13] W. Ahmad, I. Jantan, and S. N. A. Bukhari, "Tinospora crispa (L.) Hook. f. & Thomson: A review of its ethnobotanical, phytochemical, and pharmacological aspects," *Front. Pharmacol.*, vol. 7, pp. 1–19, 2016, doi: 10.3389/fphar.2016.00059.

[14] D. Wulansari, Y. Jamal, P. Praptiwi, and A. Agusta, "Pachybasin, a Major Metabolite from Culture Broth of Endophytic Coelomyceteous AFKR-18 Fungus isolated from a Yellow Moonsheed Plant, Arcangelisia flava (L.) Merr.," *HAYATI J. Biosci.*, vol. 21, no. 2, pp. 95–100, 2014, doi: 10.4308/hjb.21.2.95.

[15] N. Kapoor and S. Saxena, "Endophytic fungi of Tinospora cordifolia with anti-gout properties," *3 Biotech*, vol. 8, no. 6, p. 264, 2018.

[16] A. Fathoni, S. Hudiyono, E. Budianto, A. H. Cahyana, and A. Agusta, " Metabolite Detection and Antibacterial Activity of Fungal Endophytic Extracts Isolated from Brotowali (Tinospora crispa) Plants using TLC-Bioautography Assay ," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 1011, p. 012041, 2021, doi: 10.1088/1757-899x/1011/1/012041.

[17] Praptiwi, M. Raunsai, D. Wulansari, A. Fathoni, and A. Agusta, "Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi," *J. Appl. Pharm. Sci.*, vol. 8, no. 8, pp. 69–74, 2018, doi: 10.7324/JAPS.2018.8811.

[18] M. Ilyas, Praptiwi, D. Wulansari, A. Fathoni, and A. Agusta, "An assemblages of fungal endophytes isolated from medicinal plants collected from Toba and Samosir, North Sumatra," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 308, no. 1, 2019, doi: 10.1088/1755-1315/308/1/012070.

[19] G. Pessini, B. Dias-Filho, C. Nakamura, and D. Cortez, "Antibacterial activity of extracts and neolignans of Piper regnellii (Miq.) C.DC. var pallescens (C.DC)," *Yunck Mem I Oswalso Cruz*, vol. 98, pp. 1115–1120, 2003.

[20] J. Ismail, M. Runtuwene, and F. Fatimah, "Penentuan total fenolik dan uji aktivitas antioksidan pada biji dan kulit buah pinang Yaki (Areca vestiaria Giseke)," *J. Ilm. Sains*, vol. 12, no. 2, pp. 84–88, 2012.

[21] Y. Zou, Y. Lu, and D. Wei, "Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro," *J. Agric. Food Chem.*, vol. 52, no. 16, pp. 5032–5039, 2004, doi: 10.1021/jf049571r.

[22] T. J. White, T. D. Bruns, S. B. Lee, and J. W. Taylor, "Amplification and direct sequencing of fungal RNA genes for phylogenetics," in *PCR protocols*, M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, Eds. San Diego: Academic Press, Inc., 1990.

[23] T. P. Napitupulu, M. Ilyas, A. Kanti, and I. M. Sudiana, "In vitro evaluation of Trichoderma harzianum strains for the control of Fusarium oxysporum f.sp. cubense," *Plant Pathol. Quar.*, vol. 9, no. 1, pp. 152–159, 2019.

[24] A. Hiraishi, Y. Kamagata, and N. Nakamura, "Polymerase chain reaction amplification and restriction fragment length polymorphism analysis of 16S rRNA genes from methanogens.," *Journals Ferment. Bioeng.*, vol. 79, pp. 523–529, 1995.

[25] T.A. Hall, "BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT," *Nucleic Acids Symp Ser.*, vol. 41, pp. 95-98, 1999.

[26] C. Chepkirui and M. Stadler, "The genus Diaporthe: a rich source of diverse and bioactive metabolites," *Mycol Prog.*, vol. 16, pp. 477–494, 2017.

[27] T. S. Murali, T. S. Suryanarayanan, and R. Geeta, "Endophytic Phomopsis species: Host range and implications for diversity estimates," *Can. J. Microbiol.*, vol. 52, no. 7, pp. 673–680, 2006, doi: 10.1139/W06-020.

[28] J. L. Li, X. Sun, Y. Zheng, P. P. Lü, Y. L. Wang, and L. D. Guo, "Diversity and community of culturable endophytic fungi from stems and roots of desert halophytes in northwest China," *MycoKeys*, vol. 62, pp. 75–95, 2020, doi: 10.3897/mycokeys.62.38923.

[29] K. Fang *et al.*, "Tissue-Specific and Geographical Variation in Endophytic Fungi of Ageratina adenophora and Fungal Associations With the Environment," *Front. Microbiol.*, vol. 10, p. 2919, Dec. 2019, doi: 10.3389/fmicb.2019.02919.

[30] C. Santos, B. N. S. da Silva, A. F. T. A. F. E Ferreira, C. Santos, N. Lima, and J. L. da S. Bentes, "Fungal endophytic community associated with guarana (Paullinia cupana var. sorbilis): Diversity driver by genotypes in the centre of origin," *J. Fungi*, vol. 6, no. 3, pp. 1–20, 2020, doi: 10.3390/jof6030123.

[31] F. Silva *et al.*, "Diversity of cultivable fungal endophytes in Paullinia cupana (Mart.) Ducke and bioactivity of their secondary metabolites," *PLoS One.*, vol. 13, no. 4, p. e0195874, 2018.

[32] M. J. Guevara-Araya, C. Vilo, A. Urzúa, and M. González-Teuber, "Differences in community composition of endophytic fungi between above- and below-ground tissues of Aristolochia chilensis in an arid ecosystem," *Rev. Chil. Hist. Nat.*, vol. 93, no. 1, p. 3, 2020, doi: 10.1186/s40693-020-00091-y.

[33] H. R. Qu *et al.*, "Antibacterial bisabolane sesquiterpenoids and isocoumarin derivatives from the endophytic fungus Phomopsis prunorum," *Phytochem. Lett.*, vol. 37, pp. 1–4, 2020, doi: https://doi.org/10.1016/j.phytol.2020.03.003.

[34] T. C. Xu *et al.*, "Bioactive secondary metabolites of the genus diaporthe and anamorph phomopsis from terrestrial and marine habitats and endophytes: 2010–2019," *Microorganisms*, vol. 9, no. 2, pp. 1–49, 2021, doi: 10.3390/microorganisms9020217.

[35] Praptiwi, K. Palupi, A. Fathoni, D. Wulansari, M. Ilyas, and A. Agusta, "Evaluation of antibacterial and antioxidant activity of extracts of endophytic fungi isolated from Indonesian Zingiberaceous plants," *Nusant. Biosci.*, vol. 8, no. 2, pp. 306–311, 2016, doi: 10.13057/nusbiosci/n080228.

[36] J. Tian *et al.*, "Dibenzo-α-pyrones from the endophytic fungus Alternaria sp. Samif01: isolation, structure elucidation, and their antibacterial and antioxidant activities," *Nat Prod Res*, vol. 31, no. 4, pp. 387–396, 2017.

[37] A. A. Al Mousa, H. Mohamed, A. M. A. Hassane, and N. F. Abo-Dahab, "Antimicrobial and cytotoxic potential of an endophytic fungus Alternaria tenuissima AUMC14342 isolated from Artemisia judaica L. growing in Saudi Arabia," *J. King Saud Univ. - Sci.*, vol. 33, no. 5, p. 101462, 2021, doi: https://doi.org/10.1016/j.jksus.2021.101462.

[38] J. T. Wang *et al.*, "Chemical constituents from plant endophytic fungus Alternaria alternata," *Nat Prod Res*, vol. 35, no. 7, pp. 1199–1206, 2021.

[39] L. A. El-Kassem, U. W. Hawas, S. El-Souda, E. F. Ahmed, W. El-Khateeb, and W. Fayad, "Anti-HCV protease potential of endophytic fungi and cytotoxic activity," *Biocatal Agric Biotechnol*, vol. 19, p. 101170, 2019, doi: 10.1016/j.bcab.2019.101170.

[40] Y. N. Shi *et al.*, "(±)-Alternarlactones A and B, Two Antiparasitic Alternariol-like Dimers from the Fungus Alternaria alternata P1210 Isolated from the Halophyte Salicornia sp," *J Org Chem*, vol. 84, no. 17, pp. 11203–11209, 20219.

[41] R. Song *et al.*, "The study of metabolites from fermentation culture of Alternaria oxytropis," *BMC Microbiol*, vol. 19, no. 1, p. 35, 2019.

[42] N. Ranganathan and G. Mahalingam, "Secondary metabolite as therapeutic agent from endophytic fungi Alternaria longipes strain VITN14G of mangrove plant Avicennia officinalis," *J Cell Biochem*, vol. 120, no. 3, pp. 4021–4031, 2019.

[43] I. Górniak, R. Bartoszewski, and J. Króliczewski, "Comprehensive review of antimicrobial activities of plant flavonoids," *Phytochem. Rev.*, vol. 18, no. 1, pp. 241–272, 2019, doi: 10.1007/s11101-018-9591-z.

[44] S. M. Mandal, R. O. Dias, and O. L. Franco, "Phenolic Compounds in Antimicrobial Therapy," *J. Med. Food*, vol. 20, no. 10, pp. 1031–1038, 2017, doi: 10.1089/jmf.2017.0017.

[45] F. Farhadi, B. Khameneh, M. Iranshahi, and M. Iranshahy, "Antibacterial activity of flavonoids and their structure–activity relationship: An update review," *Phyther. Res.*, vol. 33, no. 1, pp. 13–40, 2019, doi: 10.1002/ptr.6208.

[46] L. Bouarab-Chibane *et al.*, "Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure-Activity Relationship) Models," *Front. Microbiol.*, vol. 10, p. 829, Apr. 2019, doi: 10.3389/fmicb.2019.00829.

[47] V. Kuete, "Potential of Cameroonian plants and derived products against microbial infections: A review," *Planta Med.*, vol. 76, no. 14, pp. 1479–1491, 2010, doi: 10.1055/s-0030-1250027.

[48] J. F. M. da Silva, M. C. de Souza, S. R. Matta, M. R. de Andrade, and F. V. N. Vidal, "Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities," *Food Chem.*, vol. 99, no. 3, pp. 431–435, 2006, doi: https://doi.org/10.1016/j.foodchem.2005.07.055.

[49] M. D. R. V. Fernandes *et al.*, "Biological activities of the fermentation extract of the endophytic fungus Alternaria alternata isolated from Coffea arabica L.," *Brazilian J. Pharm. Sci.*, vol. 45, no. 4, pp. 677–685, 2009, doi: 10.1590/S1984-82502009000400010.

[50] A. Borges, C. Ferreira, M. J. Saavedra, and M. Simões, "Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria," *Microb Drug Resist*, vol. 19, no. 4, pp. 256–265, 2013.

[51] S. Zhou *et al.*, "Two new endophytic Colletotrichum species from Nothapodytes pittosporoides in China," *MycoKeys*, vol. 49, pp. 1–14, 2019.

[52] V. Guarnaccia, I. Martino, G. Gilardi, A. Garibaldi, and M. L. Gullino, "Colletotrichum spp. causing anthracnose on ornamental plants in northern Italy," *J. Plant Pathol.*, vol. 103, no. 1, pp. 127–137, 2021, doi: 10.1007/s42161-020-00684-2.

[53] L. L. da Silva, H. L. A. Moreno, H. L. N. Correia, M. F. Santana, and M. V. de Queiroz, "Colletotrichum: species complexes, lifestyle, and peculiarities of some sources of genetic variability," *Appl Microbiol Biotechnol*, vol. 104, pp. 1891–1904, 2020.

[54] P. C. Chung *et al.*, "Diversity and pathogenicity of Colletotrichum species causing strawberry anthracnose in Taiwan and description of a new species, Colletotrichum miaoliense sp. nov.," *Sci. Rep.*, vol. 10, no. 1, pp. 1–16, 2020, doi: 10.1038/s41598-020-70878-2.

[55] A. Morkeliūnė, N. Rasiukevičiūtė, and A. Valiuškaitė, "Meteorological conditions in a temperate climate for colletotrichum acutatum, strawberry pathogen distribution and susceptibility of different cultivars to anthracnose," *Agric.*, vol. 11, no. 1, pp. 1–13, 2021, doi: 10.3390/agriculture11010080.

[56] W. Zou *et al.*, "Metabolites of Colletotrichum gloeosporioides, an endophytic fungus in Artemisia mongolica," *J Nat Prod.*, vol. 63, no. 11, pp. 1529–1530, 2000.

[57] Z. Dong *et al.*, "Endophytic Diaporthe Associated With Citrus grandis cv. Tomentosa in China ," *Frontiers in Microbiology* , vol. 11. p. 3621, 2021.

[58] S. Huang, J. Xia, X. Zhang, and W. X. Sun, "Morphological and phylogenetic analyses reveal three new species of Diaporthe from Yunnan, China," *MycoKeys*, vol. 78, pp. 49–77, 2021.

[59] A. Agusta, D. Wulansari, Y. Jamal, A. Nurkanto, Praptiwi, and A. Fathoni, "Antibacterial Activity and Mode of Action of (+)-2,2'-Epicytoskyrin A," *Microbiol. Indones.*, vol. 9, no. 1, pp. 35–43, 2015, doi: https://doi.org/10.5454/mi.9.1.5.

[60] L. Guo, S. Niu, S. Chen, and L. Liu, "Diaporone A, a new antibacterial secondary metabolite from the plant endophytic fungus Diaporthe sp.," *J. Antibiot*, vol. 73, no. 2, pp. 116–119, 2020, doi: 10.1038/s41429-019-0251-3.

[61] Q. Zhang *et al.*, "α-pyrone derivatives from endophytic fungus Diaporthe sp. RJ-41," *Biochem. Syst. Ecol.*, vol. 94, no. August 2020, p. 104198, 2021, doi: 10.1016/j.bse.2020.104198.

[62] X. N. Sang *et al.*, "α-Pyrone derivatives with cytotoxic activities, from the endophytic fungus Phoma sp. YN02-P-3," *Biochem. Syst. Ecol.*, vol. 27, no. 16, pp. 3723–3725, 2017, doi: 10.1016/j.bmcl.2017.06.079.

[63] J. Lou, L. Fu, Y. Peng, and L. Zhou, "Metabolites from Alternaria fungi and their bioactivities," *Molecules*, vol. 18, no. 5, pp. 5891–5935, 2013, doi: 10.3390/molecules18055891.

[64] X. M. Song *et al.*, "A New Chromene Derivative from Alternaria sp. ZG22," *Chem. Nat. Compd.*, vol. 56, no. 3, pp. 409–411, 2020, doi: 10.1007/s10600-020-03049-4.

[65] T. N. Sudharshana, H. N. Venkatesh, B. Nayana, K. Manjunath, and D. C. Mohana, "Anti-microbial and anti-mycotoxigenic activities of endophytic Alternaria alternata isolated from Catharanthus roseus (L.) G. Don.: molecular characterisation and bioactive compound isolation," *Mycology*, vol. 10, no. 1, pp. 40–48, 2019, doi: 10.1080/21501203.2018.1541933.

[66] S. Zhao *et al.*, "Novel metabolites from the Cercis chinensis derived endophytic fungus Alternaria alternata ZHJG5 and their antibacterial activities," *Pest Manag. Sci.*, Jan. 2021, doi: https://doi.org/10.1002/ps.6251.

[67] H. Yang *et al.*, "Polyketides from Alternaria alternata MT-47, an endophytic fungus isolated from Huperzia serrata," *Fitoterapia*, vol. 137, p. 104282, 2019, doi: https://doi.org/10.1016/j.fitote.2019.104282.