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1 **Bioguided isolation of alternariol derivatives from *Ficus*-derived**
2 **endophyte *Alternaria alternata***

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1 **Research Article**

2 **Running title: Bioactive metabolites of *Alternaria alternata* endophyte**

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4 **Bioguided Isolation of Alternariol Derivatives from *Ficus*-derived Endophyte**

5 ***Alternaria alternata***

6 **ABSTRACT**

7 Background: Endophytes are a rich source of bioactive natural products and suggested to
8 contribute to the biological or defense activities of their host plants. Following our research
9 on the discovery of bioactive metabolites from endophytes, *Alternaria alternata* was isolated
10 from the leaves of *Ficus carica* L. fam *Moraceae*. Materials and methods: Large scale
11 cultivation of the endophytic strain was carried out and the obtained extract was subjected
12 to preliminary screening of antifungal and cytotoxic activities. Results: Promising antifungal
13 and cytotoxic activities were obtained for the extract. Bio-guided fractionation resulted in
14 the isolation and identification of four alternariol derivatives (alternariol, alternariol-5-O-
15 sulphate, alternariol-5-O-methyl ether, alternariol-5-O-methyl ether-4'-O-sulphate). The
16 isolated compounds were tested for antifungal and cytotoxic effects. Results revealed highest
17 antifungal activity for alternariol against *A. terreus* (MIC= 2.64 $\mu\text{g mL}^{-1}$) and *F. oxysporum*
18 (MIC= 36 $\mu\text{g mL}^{-1}$) while alternariol-5-O-methyl ether exhibited the highest cytotoxicity
19 against K-562 (CC₅₀= 3.72 $\mu\text{g mL}^{-1}$) and HUVEC (CC₅₀= 2.06 $\mu\text{g mL}^{-1}$) cell lines.
20 Conclusion: All alternariol derivatives showed potent cytotoxic and antifungal activities
21 against *A. terreus* suggesting the contribution of this endophyte in the known antimicrobial
22 and anticancer activities of the host plant.

23 **Key words:** Endophyte, Alternariol, *Alternaria alternata*, Anticancer, *Ficus carica*.

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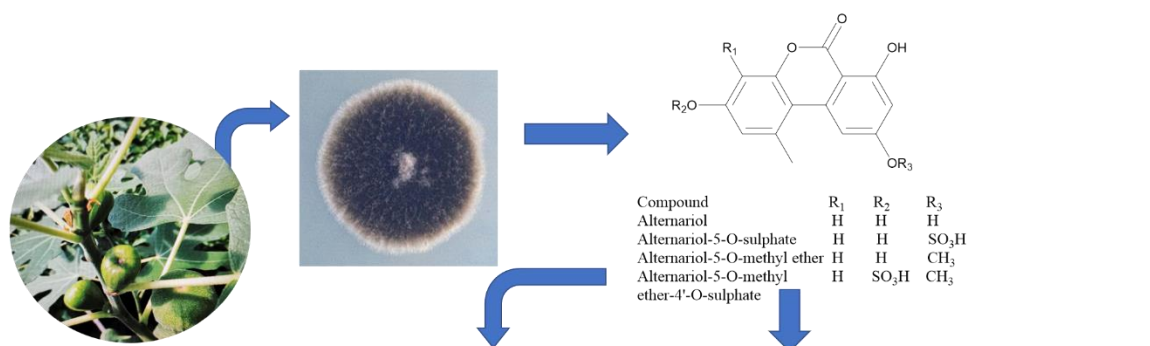
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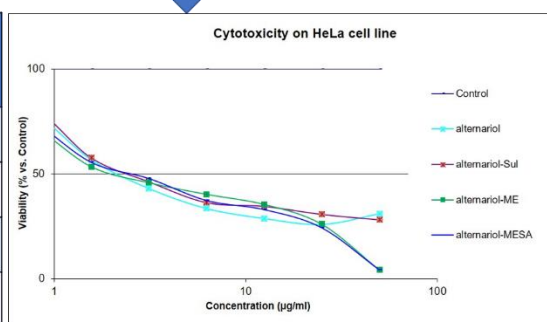
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30 **Pictorial Abstract**

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Compounds	alternariol	alternariol-5-O-sulphate	alternariol-5-O-methyl ether	alternariol-5-O-methyl ether-4'-O-sulphate
MIC against <i>A. terreus</i>	2.64 µg mL ⁻¹	3.67 µg mL ⁻¹	7.73 µg mL ⁻¹	8.52 µg mL ⁻¹
MIC against <i>F. oxysporum</i>	36 µg mL ⁻¹	44 µg mL ⁻¹	-----	-----
MIC against <i>P. notatum</i>	3.54 µg mL ⁻¹	4.45 µg mL ⁻¹	9.05 µg mL ⁻¹	10.67 µg mL ⁻¹
MIC against <i>P. chrysogenum</i>	4.26 µg mL ⁻¹	5.62 µg mL ⁻¹	10.31 µg mL ⁻¹	11.98 µg mL ⁻¹



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46 INTRODUCTION

47 Plants and plant-derived microbial endophytes represent rich sources of natural products
48 with different chemical classes and diverse biological activities ¹. Biological activity and
49 growth conditions are important criteria in the adequate selection of a host plant for
50 endophyte investigation ². *Ficus* species were employed for the treatment of many diseases
51 such as gastrointestinal, cardiovascular, respiratory disorders and cancers ³. Studies
52 performed on *Ficus carica* L. extract revealed its antioxidant, cancer suppressive and
53 antiviral effects ^{4,5}. Antimicrobial activity of *F. carica* extract was reported against several
54 bacterial strains with MIC values ranging from 0.3-5mg/mL ⁶. Additionally, antifungal
55 activity of *F. carica* against both *Microsporum canis* (MIC 75 µg/mL) and *C. albicans* (MIC
56 500 µg/mL) was also proven ⁷. The anticancer activity of the plant leaves extract was
57 reported against Huh7it liver cancer cells with an IC₅₀ of 653 µg/mL ⁸. Taking the reported
58 biological activities ⁵ and the hot and dry growth conditions in Makkah, Saudi Arabia into
59 consideration, *F. carica* L. was chosen as a host plant for endophyte study ⁹. Previous studies
60 suggested endophytes' contribution in the biological effects of host plants ¹⁰. Additionally,
61 investigation of *Ficus* spp. mainly focused on the plant itself. These two facts encouraged us
62 to investigate the chemical profile of the endophyte *Alternaria alternata* recovered from *F.*
63 *carica* leaves in addition to its anticancer and antimicrobial effects.

64

65 MATERIALS AND METHODS

66 Plant collection and endophyte isolation

67 The medicinal plant *Ficus carica* L. fam. *Moraceae* was collected from Makkah (Wadi
68 Fatima), KSA. Plant identification was carried out by Dr. Hany (Pharmacognosy
69 Department, college of Pharmacy, Najran University). A voucher specimen of the plant
70 (UQU-2019-1) is available at the herbarium of the college of Pharmacy (Department of
71 Pharmacognosy), UQU, Makkah, KSA. Collected plant material was decreased in size,
72 washed and its surface sterilized followed by drying under laminar flow. By the aid of a
73 sterile scalpel outer plant tissues were removed, and internal tissues were cut under aseptic
74 conditions. Endophyte isolation and cultivation was performed as previously published ¹¹,
75 ¹².

76 **Endophyte identification**

77 Identification of the fungal endophyte was carried out as previously described in our study
78 on all endophytes isolated from *F. carica*¹³. The standard protocol based on the cultural
79 and microscopic properties of the endophyte¹⁴ was first employed for identification and
80 afterwards it was confirmed using molecular biological techniques through DNA extraction
81 followed by amplification using Polymerase chain reaction (PCR), and finally sequencing was
82 performed using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-
83 TCCTCCGCTTATTGATATGC-3') primers as previously published^{13,14}.

84 **Fermentation and fractionation of the endophytic extract**

85 Cultivation of the isolated fungal endophyte was carried out in potato dextrose agar for two
86 weeks at 23°C. Formed mycelia were employed for inoculation of Erlenmeyer flasks each
87 containing 250 mL of the MPG-medium that consisted of malt extract (20 g/L), soybean
88 flour (2 g/L), glucose (10 g/L), KH₂PO₄ (1g/L), MgSO₄.7H₂O (0.5 g/L) and yeast extract
89 (1g/L). A stationary culture (40 L) was incubated at 23 °C for three weeks. After the
90 incubation period, culture filtrate and mycelium of each flask were mixed homogenously
91 followed by maceration in 200 mL ethyl acetate (EtOAc) for 24 h and afterwards decantation
92 and collection of the supernatant. The collected EtOAc extract was evaporated and defatted
93 with *n*-hexane. Using the agar diffusion assay, the antimicrobial activity of the fungal extract
94 was tested and found to be effective against several bacterial and fungal strains which
95 encouraged us to subject it to bioactivity guided chromatographic fractionation for
96 determination of the active metabolites. Accordingly, Silica gel was used as a stationary
97 phase and a mixture of methanol and chloroform (1:9) as a mobile phase in the first
98 bioguided chromatographic fractionation step of the extract. Polarity of the mobile phase
99 was gradually increased till 100% methanol was used as the last eluent. Further purification
100 was performed on Sephadex LH-20 using methanol as an eluent. Isolation of the bioactive
101 metabolite from the active fraction was finally achieved using preparative HPLC using a
102 gradient mobile phase composed of 25% acetonitrile in H₂O till 100 % acetonitrile over 45
103 min and a flow rate of 10 mL
104 min⁻¹. This resulted in the isolation of four metabolites; alternariol (5 mg), alternariol-5-O-

105 sulphate (5.5 mg), Alternariol-5-O-methyl ether (5.8 mg) and alternariol-5-O-methyl ether-
106 4'-O-sulphate (4.8 mg) (Fig 1) which were identified by different spectroscopic analyses.

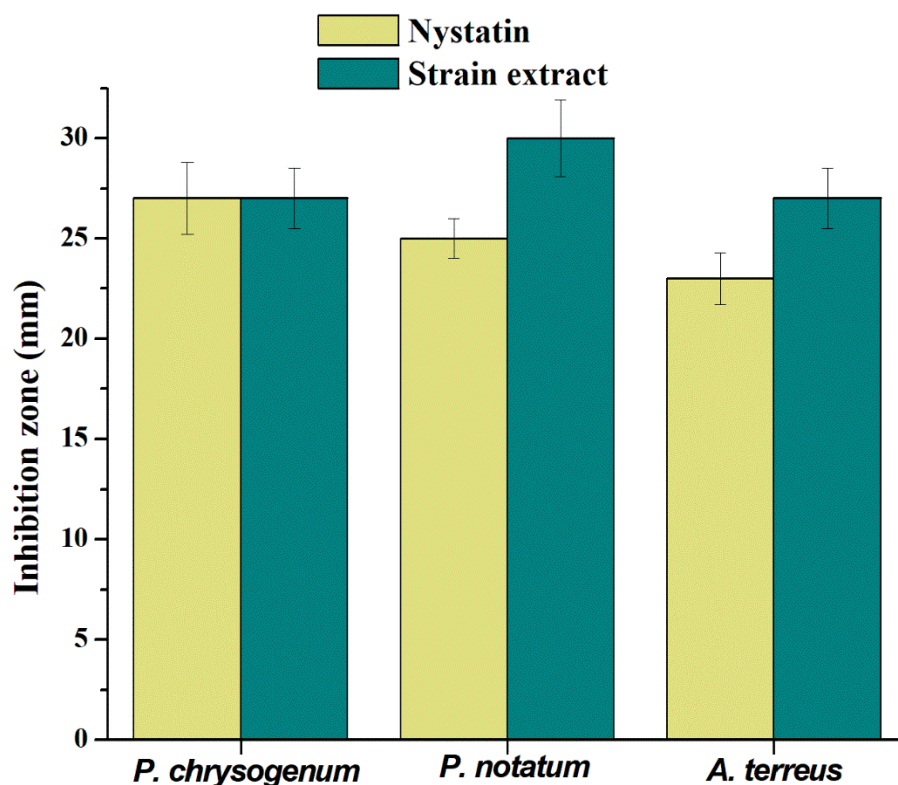
107 **Antimicrobial screening**

108 Antimicrobial effects of the extract and isolated compounds were examined by agar
109 diffusion and minimum inhibitory concentration (MIC) was calculated by the aid of the broth
110 microdilution method as in literature¹⁵⁻¹⁷.

111 **Statistical Analysis**

112 Student's t-test was used to evaluate the significant difference and compare results of the
113 antimicrobial activities of the different tested samples. A statistically significant difference
114 was considered when the p value was smaller than 0.05.

115



116

117 **Figure 1: Antifungal activity of *A. alternata* extract measured in terms of the**
118 **diameter of the inhibition zone in millimeters using nystatin as a positive control**

119 **Cytotoxic assay**

120 The cancer cell lines K-562, HUVEC and HeLa were cultured in Roswell Park Memorial
121 Institute (RPMI) 1640, Dulbecco's Modified Eagle's Medium (DMEM), and RPMI 1640,
122 respectively. 10 mL l⁻¹ ultraglutamine 1, 500 µl l⁻¹ gentamicin sulfate, and 10 % heat
123 inactivated fetal bovine serum were added at 37°C in high density polyethylene flasks for
124 supplementation of the cell culture medium and the cytotoxic assay was conducted as
125 previously published ^{11, 18}.

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127 **RESULTS AND DISCUSSION**

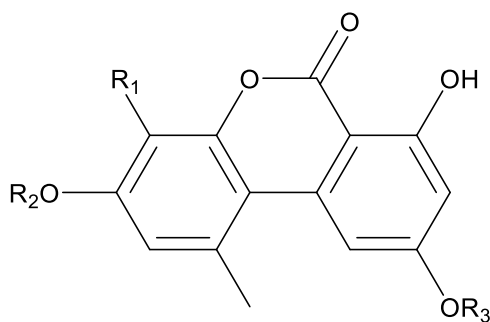
128 **Isolation of secondary metabolites**

129 The endophytic extract revealed significant cytotoxicity (CC₅₀= 3.71 µg mL⁻¹) against human
130 immortal cervical cancer (HeLa), human immortalized myelogenous leukemia (K-562) and
131 human umbilical vein endothelial (HUVEC) cell lines (CC₅₀ = 3.65 µg mL⁻¹ and 3.86 µg
132 mL⁻¹, respectively). Moreover, the extract exerted antifungal activity against several fungal
133 strains (Fig. 1) in agar diffusion assay. Accordingly, bio-guided fractionation using different
134 chromatographic approaches was conducted on the bioactive fractions to explore the active
135 metabolites which resulted in the isolation of four fungal secondary metabolites (Fig. 2).

136 **Structure elucidation of bioactive metabolites**

137 The molecular weight of 258 g/mol was deduced for the first metabolite by the obtained
138 negative and positive ESI-MS at *m/z* 257.4 [M-H]⁻ and *m/z* 259.2 [M+H]⁺. The NMR data
139 of the compound led to the deduction of a molecular formula of C₁₅H₁₂O₅. The ¹³C and ¹H
140 NMR spectra revealed four aromatic protons and an aromatic methyl group for the
141 compound. All spectral data obtained for this metabolite were identical to previously
142 published data for alternariol ¹⁹ (Fig. 2). The second metabolite was obtained with similar
143 UV absorbances to alternariol derivatives. Its HRESI-MS indicated a molecular formula of
144 C₁₄H₁₀O₈S which was corroborated with the equimolecular ion peak at *m/z* 339.0170 [M+H]
145 ⁺. ¹H NMR indicated the presence of an aromatic methyl group in addition to two pairs of
146 meta-coupled aromatic protons. The ¹³C NMR data of alternariol were comparable to those
147 obtained for this metabolite except for the up-field shift of C-5 and downfield shifts of C-4
148 and C-6 suggesting the presence of substitution by a sulphate group at C-5 ²⁰, which was

149 confirmed by literature ²¹ and resulted in the identification of this metabolite as alternariol-
 150 5-O-sulphate (alternariol-Sul). Further, alternariol-5-O-methyl ether (alternariol-ME) (Fig.
 151 2) showed typical UV absorbances for alternariol derivatives. A molecular weight of 272
 152 g/mol and a molecular formula of C₁₅H₁₂O₅ were deduced through negative and positive
 153 ESI-MS which showed molecular ion peaks at *m/z* 271.3 [M-H]⁻ and *m/z* 273.2 [M+H]⁺.
 154 From the ¹H and ¹³C NMR spectra, it was concluded that the compound contained a methoxy
 155 group, an aromatic methyl group and four aromatic protons. Comparison of the obtained
 156 spectral data for this compound with previously published data confirmed its identity as
 157 alternariol-ME ²². The molecular formula C₁₅H₁₂O₈S of the fourth endophytic metabolite
 158 was revealed for its HRESIMS with the equimolecular ion peak at *m/z* 353.0320 [M+H]⁺
 159 which showed 14 mass units higher compared to alternariol-Sul. A close resemblance of the
 160 structure of this secondary metabolite with alternariol-Sul and alternariol-ME was concluded
 161 from the ¹H and ¹³C NMR spectra. The main difference observed in this compound was the
 162 up-field shift of C-4' and downfield shifts of C-3' and C-5', indicating the attachment of a
 163 sulphate group to C-4' ²⁰. The obtained spectral data were identical with literature data and
 164 led to its identification as alternariol-5-O-methyl ether-4'-O-sulphate (alternariol-MESA) ²¹.



Compound	R ₁	R ₂	R ₃
Alternariol	H	H	H
Alternariol-5-O-sulphate	H	H	SO ₃ H
Alternariol-5-O-methyl ether	H	H	CH ₃
Alternariol-5-O-methyl ether-4'-O-sulphate	H	SO ₃ H	CH ₃

165

166 **Figure 2: Chemical structures of alternariol, alternariol-Sul, alternariol-ME,**
 167 **alternariol-MESA**

168

169 **Bioactivity of isolated metabolites**

170 The isolated fungal metabolites were tested for their antifungal activity in agar diffusion
 171 assay against several fungal strains (*Aspergillus terreus* ATCC 74135, *Penicillium notatum*
 172 ATCC 9478, *Penicillium chrysogenum* ATCC 10106) using nystatin (1 µg mL⁻¹) and as a
 173 positive control. Highest antifungal activity was observed for all metabolites against *A.*
 174 *terreus* with a MIC of 2.64 µg mL⁻¹ for alternariol, 3.67 µg mL⁻¹ for alternariol-Sul, 7.73 µg
 175 mL⁻¹ for alternariol-ME and 8.52 µg mL⁻¹ for alternariol-MESA (Table 1). Alternariol and
 176 alternariol-Sul also exhibited antifungal effect against the plant pathogen *Fusarium*
 177 *oxysporum* with MIC values of 36 and 44 µg mL⁻¹, respectively compared to the positive
 178 standard amphotericin B (MIC= 2.9 µg mL⁻¹). Alternariol exerted higher antifungal activity
 179 against *P. notatum* and *P. chrysogenum* followed by alternariol-Sul (Table 1).

180

Compounds Fungal strain	alternariol	alternariol-5-O-sulphate	alternariol-5-O-methyl ether	alternariol-5-O-methyl ether-4'-O-sulphate
MIC against <i>A. terreus</i>	2.64 µg mL ⁻¹	3.67 µg mL ⁻¹	7.73 µg mL ⁻¹	8.52 µg mL ⁻¹
MIC against <i>F. oxysporum</i>	36 µg mL ⁻¹	44 µg mL ⁻¹	-----	-----
MIC against <i>P. notatum</i>	3.54 µg mL ⁻¹	4.45 µg mL ⁻¹	9.05 µg mL ⁻¹	10.67 µg mL ⁻¹
MIC against <i>P. chrysogenum</i>	4.26 µg mL ⁻¹	5.62 µg mL ⁻¹	10.31 µg mL ⁻¹	11.98 µg mL ⁻¹

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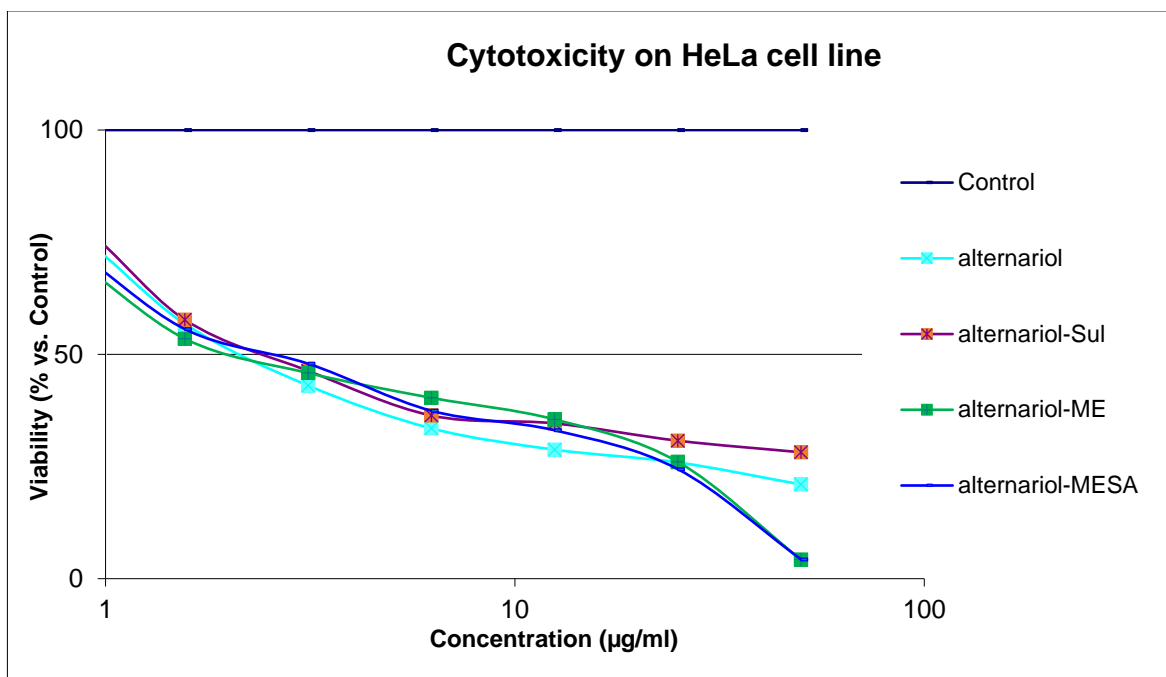
182 **Table 1: Antifungal activities of the isolated compounds against *A. terreus* and *F.***
 183 ***oxysporum***

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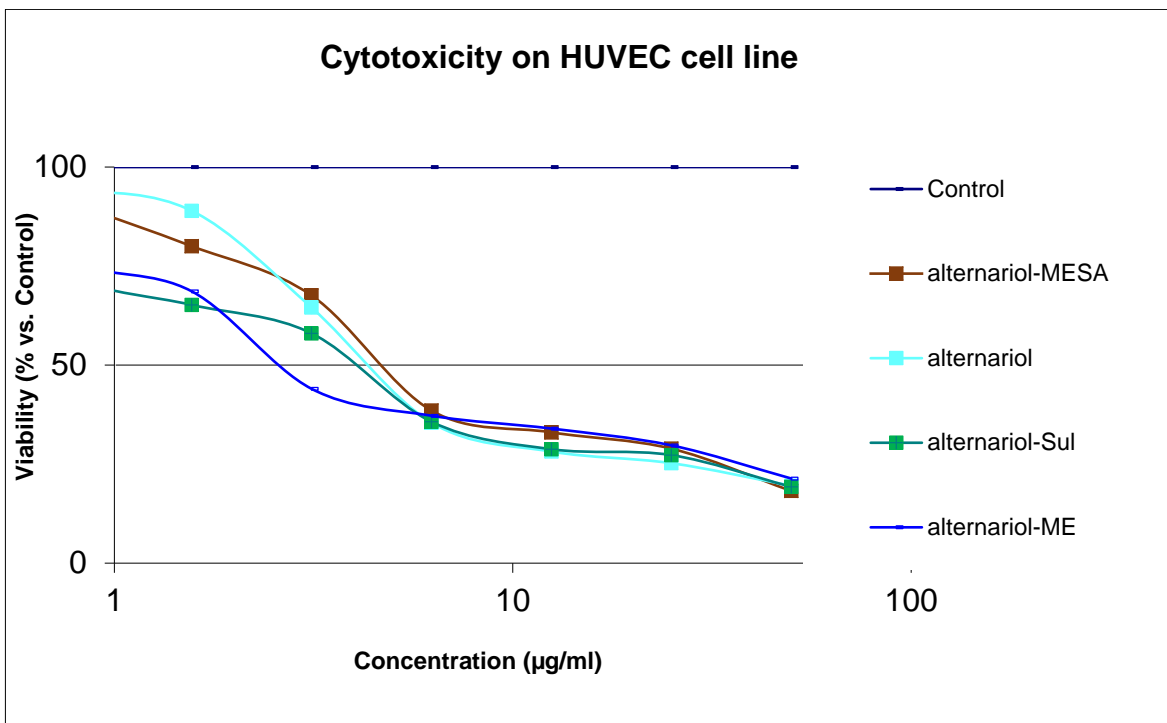
186 Furthermore, the isolated molecules were subjected to a cytotoxic assay against the cancer
187 cell lines K-562, HUVEC and HeLa. All metabolites exerted significant cytotoxic activities
188 against HeLa cell line (Fig. 3-5) with highest cytotoxicity observed for alternariol-ME (CC_{50}
189 = $2.06 \mu\text{g mL}^{-1}$) followed by alternariol-MESA ($CC_{50} = 2.16 \mu\text{g mL}^{-1}$). Strong cytotoxic
190 activity was observed for all compounds against HUVEC cell line with the highest activity
191 detected for alternariol-ME ($CC_{50}=3.72 \mu\text{g mL}^{-1}$). All isolated metabolites exerted similar
192 cytotoxicity against K-562 cells with CC_{50} values ranging from 4.31 to $4.75 \mu\text{g mL}^{-1}$
193 mL^{-1} (Fig 3-5). These results highlight the importance of *Alternaria alternata* as a rich source
194 of bioactive metabolites which has been supported by the detected cytotoxicity of a recently
195 discovered natural product, alternate C against the cancer cell lines MDA-MB-231 and
196 MCF-7 ²³.

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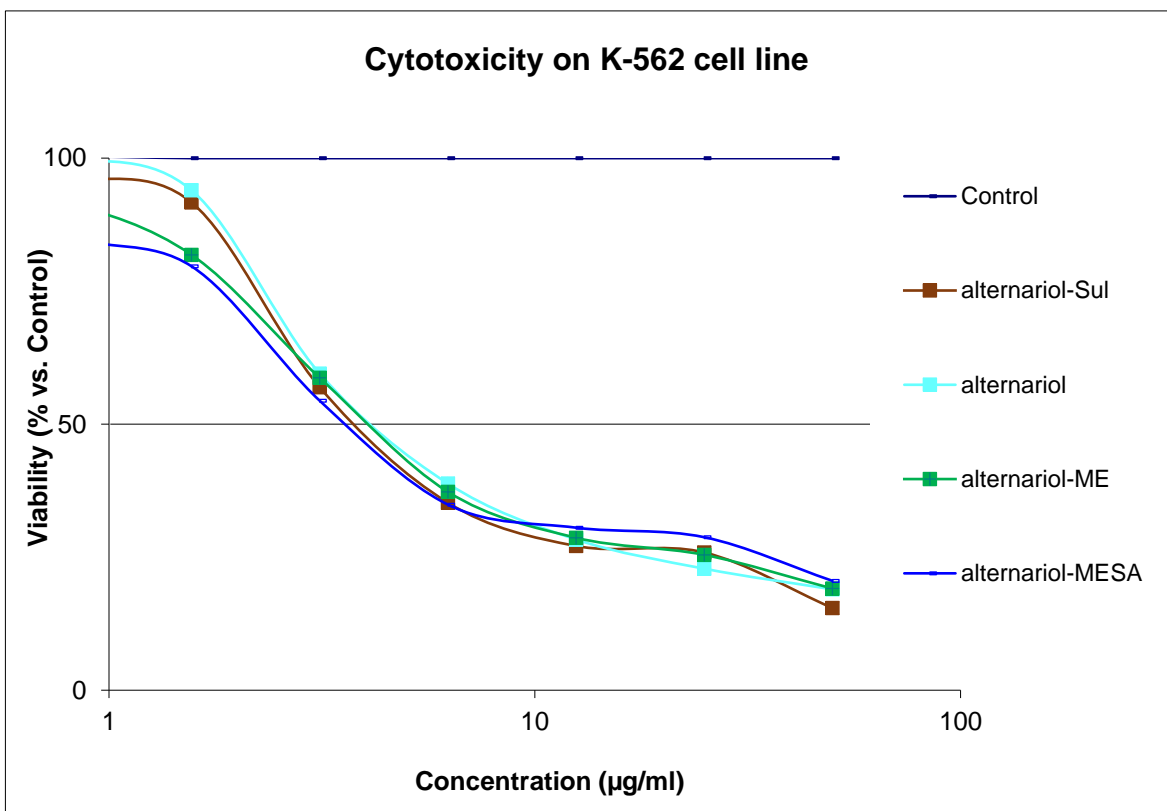
Figure 3: Cytotoxic (CC_{50}) activities of the isolated metabolites on HeLa



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Figure 4: Cytotoxic (CC_{50}) activities of the isolated metabolites on HUVEC



205

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Figure 5: Cytotoxic (CC_{50}) activities of the isolated metabolites on K-562

207 **CONCLUSION:**

208 In conclusion, from the medicinal plant *Ficus carica* L. fam *Moraceae* growing in the
209 tropical weather of Makkah, KSA the endophyte *Alternaria alternata* was isolated and
210 studied for its bioactive metabolites. Bioguided fractionation led to the isolation of four
211 alternariol derivatives from their bioactive fraction and identified by different spectroscopic
212 analyses. The highest cytotoxicity against HeLa and HUVEC cell lines was observed for
213 alternariol-5-O-methyl ether. Interestingly, all alternariol derivatives showed potent
214 cytotoxic and antifungal activities suggesting contribution of this endophyte at least in part
215 in the antimicrobial and anticancer activities reported for the host plant *F. carica*.
216 Furthermore, the detected antifungal effects of these compounds suggest a possible
217 protection of the host plant by this endophyte which supports previous assumptions on the
218 protective relationship between endophytes and host plants.

219

220 **ACKNOWLEDGEMENT**

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222 University for supporting this work by Grant Code: (22UQU4350488DSR02).

223

224 **CONFLICT OF INTEREST**

225 The authors have no conflicts of interest to report.

226

227 **ABBREVIATIONS**

228 **CC₅₀**: cytotoxic concentration 50; **MIC**: minimum inhibitory concentration; **GI₅₀**: growth
229 inhibition 50%; **NMR**: nuclear magnetic resonance; **HRESIMS**: high-resolution
230 electrospray ionization mass spectrometry; **HMBC**: heteronuclear multiple bond
231 correlations; **HUVEC**: human umbilical vein endothelial cell; **K-562**: human immortalized
232 myelogenous leukemia; **HeLa**: human immortal cervical cancer.

233

234 **SUMMARY**

235 Endophytes are a rich source of bioactive natural products and suggested to contribute to the
236 biological or defense activities of their host plants. Following our research on the discovery

237 of bioactive metabolites from endophytes, the fungal strain *Alternaria alternata* was isolated
238 from the leaves of *Ficus carica* L. fam *Moraceae*. In preliminary screening, this endophytic
239 extract exerted promising antifungal and cytotoxic activities. Bio-guided fractionation
240 resulted in the isolation and identification of four alternariol derivatives. The isolated
241 compounds were tested for their cytotoxicity against HeLa, K-562 and HUVEC cancer cells.
242 Alternariol-5-O-methyl ether exhibited the highest cytotoxicity against HeLa and HUVEC
243 cell lines. All alternariol derivatives showed potent cytotoxicity and antifungal activity
244 suggesting contribution of this endophyte at least in part in the biological activities reported
245 for the host plant *F. carica*. Results revealed highest antifungal activity for alternariol against
246 *A. terreus* (MIC= 2.64 $\mu\text{g mL}^{-1}$) and *F. oxysporum* (MIC= 36 $\mu\text{g mL}^{-1}$). The detected
247 antifungal effects of these compounds suggest a possible protection of the host plant by this
248 endophyte which supports previous assumptions on the protective relationship between
249 endophytes and host plants ²⁴.

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252 REFERENCES

- 253 1.Newman D J, Cragg G M. Natural Products as Sources of New Drugs over the Nearly Four Decades
254 from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803.
- 255 2.Strobel G. Harnessing endophytes for industrial microbiology. Curr Opin Microbiol.
256 2006;9(3):240-4.
- 257 3.Vieira L M, Kijjoa A. Naturally-occurring xanthenes: recent developments. Curr Med Chem.
258 2005;12(21):2413-46.
- 259 4.Herre E A, Jander, C., Machado, C. A., . Evolutionary Ecology of Figs and Their Associates: Recent
260 Progress and Outstanding Puzzles Annu Rev Ecol Evol Syst , . 2008;39:p. 439-58.
- 261 5.Joseph B, Raj S J. Pharmacognostic and phytochemical properties of *Ficus carica* Linn- An
262 overview. Int J Pharmtech Res. 2011;3(1):08-12.

- 263 6.Joeng M R, Kim H Y, Cha J D. Antimicrobial activity of methanol extract from *Ficus carica* leaves
264 against oral bacteria. Journal of Bacteriology and Virology 2009;39(2):97-102.
- 265 7.Aref H L, Salah K B H, Chaumont J P, Fekih A, Aouni M, Said K. In vitro antimicrobial activity of four
266 *Ficus carica* latex fractions against resistant human pathogens (antimicrobial activity of *Ficus carica*
267 latex). Pakistan Journal of Pharmaceutical Sciences. 2010;23(1):53-8.
- 268 8.Purnamasari R, Winarni D, Permanasari A A, Agustina E, Hayaza S, Darmanto W. Anticancer
269 activity of methanol extract of *Ficus carica* leaves and fruits against proliferation, apoptosis and
270 necrosis in Huh7it cells. Cancer Informatics. 2019;18:1-7.
- 271 9.Abdou R, Mojally M, Attia G H. Investigation of bioactivities of endophytes of *Ficus carica* L. fam
272 Moraceae. Bulletin of the National Research Centre. 2021;45(49).
- 273 10.Kour D, Rana K L, Kaur T, Devi R, Negi C, Yadav A N, et al. Endophytic fungi from medicinal plants:
274 biodiversity and biotechnological applications,. Microbial Endophytes. 2020:273-305.
- 275 11.Abdou R, Scherlach K, Dahse H M, Sattler I, Hertweck C. Botryorhodines A-D, antifungal and
276 cytotoxic depsidones from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens*
277 *pilosa*. Phytochemistry. 2010;71(1):110-6.
- 278 12.Jariwala B D B. Isolation and identification of endophytic fungi from various medicinal plants. .
279 BMR Microbiology 2018 Apr 7. Edition 1. .
- 280 13.Abdou R, Mojally, M. & Attia, G.H. . Investigation of bioactivities of endophytes of *Ficus carica* L.
281 Fam Moraceae. . Bull Natl Res Cent 2021;45(49).
- 282 14.Barnett HL, BB H. Illustrated Genera of Imperfect Fungi. APS press St Paul Minnesota USA. 1998.
- 283 15.Bowling J E, Owens A E, McElmeel M L, Fulcher L C, Herrera M L, Wickes B L, et al. Detection of
284 inducible clindamycin resistance in beta-hemolytic streptococci by using the CLSI broth

285 microdilution test and erythromycin-clindamycin combinations. J Clin Microbiol. 2010;48(6):2275-
286 7.

287 16.Jorgensen J H, Ferraro M J. Antimicrobial susceptibility testing: a review of general principles
288 and contemporary practices. Clin Infect Dis. 2009;49(11):1749-55.

289 17.Jorgensen J H, McElmeel M L, Fulcher L C, McGee L, Richter S S, Heilmann K P, et al. Collaborative
290 evaluation of an erythromycin-clindamycin combination well for detection of inducible clindamycin
291 resistance in beta-hemolytic streptococci by use of the CLSI broth microdilution method. J Clin
292 Microbiol. 2011;49(8):2884-6.

293 18.Macabeo A. P. G., Lopez A. D. A., Schmidt S., Heilmann J., Dahse H. M., Alejandro G. J. D., et al.
294 Antitubercular and cytotoxic constituents from *Goniothalamus gitingensis* Rec Nat Prod.
295 2013;8(1):41-5.

296 19.Stinson E E, Wise W B, Moreau R A, Jurewicz A J, Pfeffer P E. Alternariol: Evidence for biosynthesis
297 via norlichexanthone. . Can J Chem. 1986;64(8):1590B4.

298 20.Ragan M A. Phenol sulfate esters: ultraviolet, infrared, 1H and 13C nuclear magnetic resonance
299 spectroscopic investigation. . Can J Chem. 1978;56(20):2681B5.

300 21.Plasencia J, Mirocha C J. Isolation and characterization of zearalenone sulfate produced by
301 *Fusarium* spp. Phytochemistry. 1991;57(1):146-50.

302 22.Onocha P A, Okorie D A, Connolly J D, Roycroft D S. Monoterpene diol, iridoid glucoside and
303 dibenzo- α -pyrone from *Anthocleista djalonenensis*. Phytochemistry. 1995;40(4):1183-9.

304 23.Wang J T, Ma Z H, Wang G K, Xu F Q, Yu Y, Wang G, et al. Chemical constituents from plant
305 endophytic fungus *Alternaria alternata*. Nat Prod Res. 2021;35(7):1199-206.

306 24.Tan R X, Zou W X. Endophytes: a rich source of functional metabolites. Nat Prod Rep.
307 2001;18(4):448-59.