Two novel aliphatic unsaturated alcohols isolated from a pathogenic fungus

Fusarium proliferatum

Wanying Lu a,1, Guoliang Zhu a,1, Weize Yuan a, Zhaoxi Han a, Huanqi Dai b, Mostafa Basiony a, Lixin Zhang a, Xueting Liu a, Tom Hsiang c, Jingyu Zhang a,1.

a State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, 200237, China
b The State Key Laboratory of Mycology (SKLM), Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China
c School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada

ARTICLE INFO

Keywords:
Pathogenic fungus
Aliphatic unsaturated alcohols
Antibacterial
Natural products

ABSTRACT

Phytopathic fungi have attracted great attention as a promising source for new drug discovery. In the progress of our ongoing study for bioactive natural products from an in-house phytopathic fungus library, a pathogenic fungus, Fusarium proliferatum strain 13294 (FP13294), was selected for chemical investigation. Two novel aliphatic unsaturated alcohols named fusariumnols A and B (1 and 2), together with one previously characterized sesquiterpenoid lignoren (3) were identified. Structures of 1–3 were assigned by mass spectrometry and NMR spectroscopy. Their bioactivities were assessed against Staphylococcus epidermidis, S. aureus, and Methicillin-resistant S. aureus (MRSA). Compounds 1 and 2 exhibited weak antibacterial activity against S. epidermidis (MIC = 100 μM).

1. Introduction

Fungi inhabiting special environments are well-known producers of secondary metabolites with diverse biological activities. Phytopathogenic fungi have attracted increasing attention because of frequent discoveries of natural products with diverse structural properties and promising biological activities [1,2]. These are treasure troves for mining novel products especially in phytopathogenic fungi where genes have arisen during the long-term co-evolutionary process with host plants; these gene products have high potential in pharmaceutical and agricultural applications [3,4]. For example, phytoxins derived from phytopathogenic fungi have been used as ecofriendly tools in the development of safe bioherbicides [5,6]. In addition, fungal phytoxins exhibited a variety of biological activities, including promising antifungal, anticancear, and anti-inflammatory activities [7–10].

The genus Fusarium, often isolated from different plant tissues and from plant debris, can afford diverse mycotoxins, causing reductions of crop quality and harvest, as well as affecting human health [11]. Nevertheless, there is abundant evidence indicating Fusarium sp. have the capacity to afford different kinds of natural products, such as polyketides, terpenoids, alkaloids, and peptides, which show significant biological activities [12–15]. F. proliferatum is a widespread plant pathogenic fungi associated with diverse crops, including rice, wheat, maize, garlic, asparagus, date palm, and Chinese chive [16]. F. proliferatum has been reported to produce multiple mycotoxins, such as fumonisins, beauvericin, fusaproliferin, moniliformin, and fusaric acid [17].

Staphylococci, which are Gram-positive bacteria, can colonize humans and cause infections as the immune system weakens. Pathogens are introduced through wounds and also associated with the insertion of medical devices [18,19]. They are able to form biofilms in chronic wounds, resulting in doubling of time for recovery. Due to extremely difficult to treat, these infections lead to a serious burden for the public health system [20]. The Staphylococcus species usually contain S. aureus and S. epidermidis. Because of increased bacterial resistance to current antibiotics in clinical use, there is a need to explore natural products to identify novel compounds for drug discovery.

During an ongoing study of novel bioactive natural products in our in-house phytopathic fungi library, a strain of F. proliferatum (FP13294) was subjected to investigate secondary metabolites based on...
bioassay guided strategy. Its crude extract showed weak antibacterial activity against S. epidermidis (MIC = 200 μM). Fractionation of crude extracts obtained from a rice fermentation culture yielded two new secondary metabolites, which we named fusariumnols A and B (1 and 2), in addition to one known compound lignoren (3). We herein provide details of the structure identification and biological activities of compounds 1–3.

2. Materials and methods

2.1. General experimental procedures

1D and 2D NMR spectra were measured on a Bruker Avance DRX 600 MHz spectrometer with TCI cryoprobe. Chemical shifts are expressed as δ (ppm) referenced to the solvent peaks at δC 49.0 and δH 3.31 for Methanol-d4. HRESIMS spectra were measured on a Thermo Orbitrap Q Exactive mass spectrometer. Materials including Sephadex LH-20 (GE Healthcare) and ODS-A (YMC) were utilized for column chromatography. Reverse phase HPLC chromatography was carried out by an Agilent 1100 Series HPLC, and ChemStation Rev.B.02.01 software was used to analyse the data. Semi-preparative RP-HPLC was performed equipped with an ACE Excel 5C18-AR column (10 × 250 mm, 5 μm).

2.2. Microbial strain culture and identification of F. proliferatum 13,294

F. proliferatum 13,294 (FP13294) was prepared on potato dextrose agar (PDA) at 28 °C. Strain FP13294 was identified based on morphology and 18S rRNA gene sequence. The primer pair EF-1a-F (5′- AAGGCTGGTTCAAGACTGGG-3′) and EF-1a-R (5′- TGGTCGTCTCTTCTGGCTCT-3′) were used to amplify the translation elongation factor 1 alpha (EF-1a) gene region of FP13294. The PCR amplification (25 μl in total) contained 1 μl of DNA template, 0.4 μl of rTaq polymerase, 0.4 μl of each primer, 2.5 μl of 2.5 mM dNTP, and 2.5 μl of 10 × buffer. The PCR reaction was carried out on an ABI PCR Thermal Cycler with following condition: denaturation at 94 °C for 5 min, 25 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, elongation at 72 °C for 45 s, and a final elongation at 72 °C for 10 min. Both forward and reverse primers were used for PCR product sequencing, and the consensus sequence was compared to the GenBank database using BLASTn. Sequences with high similarity were acquired from GenBank. Phylogenetic neighbor-joining tree was constructed by MEGA 6.0 software [21], and rooted on Trichoderma harzianum.

2.3. Fermentation and isolation of compounds 1–3

FP13294 was prepared on PDA plate medium at 28 °C for 7 days, and agar plugs (0.5 × 0.5 × 0.5 cm³) were inoculated into six Erlenmeyer flasks (250 ml) each containing 100 ml potato dextrose broth (PDB). Then the Erlenmeyer flasks were incubated for five days at 28 °C on a rotary shaker at 200 rpm as the seed culture. Fermentation was then carried out in 200 aseptic-bags each containing 80 g of autoclaved rice and 120 ml sterilized distilled H2O for 30 days at 28 °C. The fermentation material was extracted with EtOAc three times, and then was evaporated under in vacuo to give an extract (18.64 g).

The crude extract was subjected to Sephadex LH-20 eluting with 100% MeOH to yield seven fractions (A–G). Fraction E (11.33 g) was fractionated by Sephadex LH-20 again eluting with 100% MeOH to afford eight sub-fractions (E3C1–E3C8). Then Fraction E3C4 (641 mg) was chromatographed over ODS-MPLC with an ACN-H2O gradient (5%–100%) elution for 100 min to get seven sub-fractions (E3A–E3J). Sub-fraction E3C (2.56 g) was further purified by Sephadex LH-20 eluting with 100% MeOH to afford seven fractions (E3C1–E3C6). Fraction E3C4 (641 mg) was chromatographed over ODS-MPLC with an ACN-H2O gradient (10%–100%) elution for 100 min to yield eight sub-fractions (E3C4A–E3C4H). Sub-fraction E3C4D (148.2 mg) was then purified by semi-preparative RP-HPLC equipped with an ACE Excel 5C18-AR (10 × 250 mm) eluting with 35% ACN-H2O for 60 min (flow rate: 4 ml/min) to obtain 1 (2.4 mg, tR = 30.9 min), 2 (0.5 mg, tR = 52.9 min), and 3 (17.4 mg, tR = 21.4 min).

2.4. Accession number

Fusarium proliferatum strain 13,294 has been deposited at the China General Microbiological Culture Collection Center with the accession number 21945. The sequence data of the EF-1a gene has been deposited under GenBank accession number M3282914.

3. Results

3.1. Characterization and identification of strain FP13294

Isolate 13294 was originally obtained from wheat tissue. It appeared as white villous colonies which produce light purple pigment and have a colony diameter of 7 mm after 7 d on potato dextrose agar (PDA) at 28 °C (Fig. 1). The EF-1a gene sequence and neighbor-joining tree (Fig. 1) confirmed it as Fusarium proliferatum.

3.2. Structure elucidation

The HRESIMS (Fig. S1a) analysis revealed the molecular formula of 1 as C13H23O2 (241.2162 [M + H]+, calc'd for 241.2162), implying two degrees of unsaturation. Based on combined analysis of the 1H, 13C, and HSQC spectra of 1 (Table 1 and Figs. S1b, S1c, S1e), 15 carbon resonances could be observed, composed of one olefinic quaternary carbon (δC 137.1), three conjugated sp² methines [δC/δH 133.0/5.57 (dt, J = 15.2, 6.9 Hz), 128.3/6.29 (dd, J = 15.2, 10.5 Hz), 126.3/5.83 (d, J = 10.5 Hz)], two oxymethine groups [δC/δH 73.7/3.44 (m), 73.4/3.47 (m)], six sp³ methines, two terminal methyl triplets [2 × δC/δH 10.5/0.95 (t, J = 7.4 Hz), and one methyl singlet [δC/δH 16.7/1.72 (s)], indicating that the structure of 1 comprises a bishydroxylated alkyl chain with two conjugated olefinic groups. The 1H-1H COSY (Fig. S1d) correlations of 1 revealed the existence of two sub-units C-1–C-5 and C-7–C-14, which were deduced to connect at C-6 based on the key HMBC (Fig. S1i) correlations of H-2 with C-5/C-6/C-7/C-9. Further HMBC analysis revealed that the two hydroxyl groups were substituted at C-3 and C-12, based on the crosspeaks between H-2/C-3 and H-3/C-12. The density functional theory (DFT) based 13C NMR calculation and DP4 analysis of two epimers 1α/1β was conducted, resulting in higher Bayes’s theorem probability of 1β (75%) compared with 1α (25%) (Table S1). Therefore, the structure of 1 was established as illustrated in Fig. 3 and named fusariumnon A.

The molecular formula of 2 could be deduced as C13H25O2 with three degrees of unsaturation from its HRESIMS (Fig. S2a) spectrum (239.2005 [M + H]+, calc'd for 239.2006). The 1H and 13C NMR data of 2 (Figs. S2b and S2c) showed high similarity with those of 1, except for an additional keto-carbonyl group (δC 214.0) replacing one of the original O-substituted methines in 1. Further HMBC (Fig. S2b) analysis revealed that compound 2 is the 3-oxo derivative of 1 based on crosspeaks of H-1 with C-2/C-3, and H-2 with C-3. The Δ⁶β and Δ⁸β double bonds were also determined to be E configuration based on the NOESY correlations (Fig. S2g) between H-3/H-8, H-5/H-7, and H-7/H-9 combined with 1H-1H NOE value (15.1 Hz). Compound 2 was proposed as a new analog of 1 and was named fusariumnon B (Fig. 2).

The structure of one known compound, lignoren (3), was determined based on the HRESIMS 1H and 13C NMR data (Figs. S3a–S3c) and by comparison with those of published work [22].
3.3. Bioactivity tests

The crude extract, purified subfractions, and isolated compounds 1–3 were assessed for their anti-bacterial activity against Gram-positive bacterium, including *S. aureus*, MRSA, and *S. epidermidis*. The crude extract and subfraction E3C4D showed anti-bacterial activity against *S. epidermidis* (MIC = 200 μM). Finally, we found that compounds 1 and 2 exhibited weak anti-bacterial activity against *S. epidermidis* (MIC = 100 μM).

4. Discussion and conclusion

The discovery of penicillin initiated an era wherein natural products derived from microorganisms revolutionized medicine. Afterwards, human life expectancy was extended by nearly 40 years [23]. Historically, the majority of approved drugs have been found from natural sources or derivatives of natural compounds. However, due to the repeat discovery of known compounds in other microorganisms, the discovery ratio of new natural compounds with bioactivity has declined in recent years. Additionally, many pharmaceutical companies have reduced their investment in natural product research. Thus, to further advance drug discovery, new strategies for the discovery of novel natural products are of great importance.

Recently, poorly tapped biological resources, including rare actinobacteria, endophytic fungi, and even phytopathogenic fungi, have been explored as innovative resources for discovering novel natural compounds with promising bioactivity [24–26]. Among them, phytopathogenic fungi play an important, but yet rarely explored role. They are classified as biotrophs, hemibiotrophs, and necrotrophs, acquiring nutrition during invading process of their host plants. Between the interactions of phytopathogenic fungi and plants, fungi usually metabolize and produce series of low-molecular weight secondary metabolites, which are not essential for life of phytopathogenic fungi while are known for their versatility. These secondary metabolites always play an important role in ecological defense and biological competition. In association with host plants, phytopathogenic fungi can become rich sources of cyclic peptides, terpenes, alkaloids, aliphatic hydrocarbons, aromatic polyketones, and heterocyclic compounds, showing special values to humans.

![Fig. 1. Morphology and phylogenetic tree of *F. proliferatum* 13294.](image)

*a* Morphology characteristic of FP13294 cultured on PDA at 28 °C for 7 days. *b* Phylogenetic neighbor-joining tree of FP13294 based on EF-1α gene sequences. NCBI accession numbers are given in parentheses. Numbers at nodes indicate levels of bootstrap support (percentage) based on 1000 resampled datasets; only values > 50% are presented. The bar indicates 0.5 amino acid substitutions per site. *Trichoderma aurantiifusum* was chosen as outgroup.

![Fig. 2. Structure of isolated compounds (1–3) from *F. proliferatum* 13294.](image)
Recently, there has been great progress in the research of the secondary metabolites from phytopathogenic fungi. Many natural products they produce e.g., sirodesmin PL [28,29], AF-toxin I/II [30], and cytochalasins B [31] show different promising bioactivities. *Fusarium* species are distributed worldwide, and mainly attack grain crops causing quality and yield reductions; they also produce a variety of mycotoxins, such as corn gibbereleneone [11], beauvericin [32], fusaproliferin [33], and fumonisins [34]. In addition, abundant active secondary metabolites have also been isolated from *Fusarium* sp., including antibacterial and anticancer naphthoquinone derivatives [35,36], anti-cancer and anti-inflammatory alkaloids [37,38], antibacterial terpenoids [39], antifungal and anti-malarial cyclodepsipeptide [40], etc. For example, *F. solani* has the ability to produce cyclooxygenase-2 (COX-2) inhibitor fusopolitide A [41] and anti-tumor naphthoquinone compound solaninarthoquinone [42]. Kakeya et al. reported the isolation and structure elucidation of lucilactaene with unique structure, a cell cycle inhibitor fusopoltide A [41] and anti-tumor naphthoquinone compound solaninarthoquinone [42].

Fig. 3. Key 2D NMR correlations of compounds 1 and 2.

| ![1H-1H COSY](image1) | ![HMBC](image2) | ![NOESY](image3) |

Wanying Lu: Visualization, Investigation, Writing – original draft. Guoliang Zhu: Visualization, Investigation, Writing – original draft. Weize Yuan: Investigation. Zhaoxi Han: Investigation. Huanqin Dai: Investigation, Activity screening. Mostafa Basiony: Writing – review & editing. Lixin Zhang: Conceptualization, Supervision. Xueling Liu: Conceptualization, Supervision. Tom Hsiang: Resources, Writing – review & editing. Jingyu Zhang: Conceptualization, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2020YFA0907200, 2019YFA0906200, and 2020YFA0907800), the National Natural Science Foundation of China (21877038, 21907031, 21977029, 31720103901, and 81903529), Shanghai Rising-Star Program (20QA1402800), the Open Project Funding of the State Key Laboratory of Bioreactor Engineering, and the 111 Project (B18022). Discovery and isolation of *F. proliferatum* strain 13294 was supported by the Natural Sciences and Engineering Research Council of Canada funding to T. Hsiang.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2021.10.001.

References

[1] Blackwell M. The Fungi: 1, 2, 3,. 5.1 million species? Am J Bot 2011;98:426–38. https://doi.org/10.3732/ajb.1000928.
[2] Stergiopoulos I, Collemare J, Mehrabi R, De Wit Pj. Phytotoxic secondary metabolites and pesticides produced by plant pathogenic Dothideomycete fungi. FEMS Microbiol Rev 2013;37:67–93. https://doi.org/10.1111/1574-6976.2013.00349.x.
[3] Kusari S, Hertweck C, Spiteller M. Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 2012;19:792–8. https://doi.org/10.1016/j.chembiol.2012.06.004.
[4] Zhang JY, Wang ZZ, Song ZJ, Karlthai L, Hou CJ, Zhu GL, et al. Brocasolid D, a novel compound isolated from a wheat pathogenic fungus, Microdochium majus 99049. Synth Syst Biotechnol 2019;4:173–6. https://doi.org/10.1016/j.synbio.2019.09.001.
[5] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[6] Sugawara F, Strobel G, Fisher L, Van Dyne G, Clardy J. Bipolarixin, a selective phytotoxic antibiotic produced by Bipolaris cynodontis. Proc Natl Acad Sci U S A 1985;82:8291–4. https://doi.org/10.1073/pnas.82.24.8291.
[7] Dixon RA. Natural products and plant disease resistance. Nature 2001;411:843–7. https://doi.org/10.1038/35081178.
[8] Hammerschmidt R. Phytotoxins: what have we learned after 60 years? Annu Rev Phytopathol 1999;37:285–302. https://doi.org/10.1146/annurev.phyto.37.1.285.
[9] Li JW-H, Vederas JC. Drug discovery and natural products: end of an era or an exponential growth phase? Curr Opin Chem Biol 2018;39:24–32. https://doi.org/10.1016/j.cbpa.2017.12.001.
[10] W. Lu et al. https://doi.org/10.1038/s41598-019-45645-8.
[11] Jayasinghe L, Abbas HK, Jacob MR, Herath WH, Nanayakkara ND. N-Methyl-4-hydroxy-2-pyridinone analogues from Fusarium oxysporum and isolation of a novel compound A protoporphyrin adduct as an antitubercular prodrug. Angew Chem Int Ed Engl 2013;52:114–5. https://doi.org/10.1002/anie.201208801.
[12] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[13] Nadeem M, Ram M, Alam P, Ahmad MM, Mohammad A, Al-Qurainy F, et al. Supraman U, Hiri N, Santra PK, Wasimul H, Malik A, Anan S, et al. New naphthoquinone derivatives from Fusarium napiforme of a mangrove plant. Nat Prod Res 2021;35:1406–12. https://doi.org/10.1080/14786419.2019.1650358.
[14] Tadpetch K, Chukong C, Jeannard C, Lhiraoporn A, Ruchsasichirakul V, Phongpaichit S, et al. Cytochalasins Z1, Z2 and Z3, a new mycotoxin from Fusarium sambucinum sp. J Antibiot (Tokyo) 2001;54:850–7. https://doi.org/10.7164/antibiotics.54.850.
[15] Jiang B, Chen K, Sun W, Bie Q, Liu X, Chen C, et al. Fusopoltide A and fusosteride A, a new polyketide with a pentaleno [1,2-c] pyran ring system and a degraded steride, from Fusarium sambucinum. J Nat Prod 2020;83:703–9. https://doi.org/10.1021/acs.jnatprod.9b00717.
[16] Down G, Cui L, Li XJ, Duan RT, Shu Y, Chen FY, et al. Production of a new tetracyclic triterpenoid sulfate metabolite sambacide by solid-state cultured Fusarium sambucinum B10.2 using potato as substrate. Bioreourc Technol 2016;218:1266–70. https://doi.org/10.1016/j.biortech.2016.07.014.
[17] Strukelj B. Endophytic fungi – the role of plant pathology in food safety and food security. Curr Top Med Mycol 2014;18:257–72. https://doi.org/10.5943/cream/6/1/3.
[18] Evidente A, Andolfi A, Vurro M, Zonno MC, Motta A. Cytochalasins Z1, Z2 and Z3, new cell cycle inhibitor in p53-transfected cancer cells, produced by a fungal plant pathogen. FEMS Microbiol Lett 2017;10:175–81. https://doi.org/10.1016/j.femsle.2016.07.014.
[19] Santini A, Ritieni A, Fogliano V, Randazzo G, Mannina L, Logrieco A, et al. Characterization of vinblastine and vincristine from endophytic fungus Panax pseudoginseng. J Asian Nat Prod Res 2018;20:75–9. https://doi.org/10.1080/14786419.2017.1407035.
[20] Palmieri N, De Laurentiis A, Di Paolo S, Amendola G, Izzo A, et al. M. C1000F, an endophyte from the root of Panax notoginseng. Nat Prod Res 2012;26:391–6. https://doi.org/10.1080/14786419.2010.531967.
[21] Tadpetch K, Chukong C, Jeanard C, Lhiraoporn A, Ruchsasichirakul V, Phongpaichit S, et al. Cytochalasins Z1, Z2 and Z3, a new mycotoxin from Fusarium sambucinum sp. J Antibiot (Tokyo) 2001;54:850–7. https://doi.org/10.7164/antibiotics.54.850.
[22] Kusari S, Hertweck C, Spiteller M. Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 2012;19:792–8. https://doi.org/10.1016/j.chembiol.2012.06.004.
[23] Abbas HK, Vederas JC. Drug discovery and natural products: end of an era or an exponential growth phase? Curr Opin Chem Biol 2018;39:24–32. https://doi.org/10.1016/j.cbpa.2017.12.001.
[24] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[25] Abbas HK, Vederas JC. Drug discovery and natural products: end of an era or an exponential growth phase? Curr Opin Chem Biol 2018;39:24–32. https://doi.org/10.1016/j.cbpa.2017.12.001.
[26] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[27] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[28] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[29] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[30] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[31] Abbas HK, Duke SO. Phytotoxins from plant pathogens as potential herbicides. J Toxicol Toxicol Rev 2015;14:523–43. https://doi.org/10.3109/1613890X.2015.1059640.
[50] Tsavkelova EA, Bionke C, Netrusov AI, Weiner J, Tudzynski B. Production of gibberellic acids by an orchid-associated Fusarium proliferatum strain. Fungal Genet Biol 2008;45:1393–403. https://doi.org/10.1016/j.fgb.2008.07.011.

[51] Lu YC, Chang HS, Peng CF, Lin CH, Chen IS. Secondary metabolites from the unripe pulp of Persea americana and their antimycobacterial activities. Food Chem 2012;135:2904–9. https://doi.org/10.1016/j.foodchem.2012.07.073.

[52] Loizzo M, Tundis R, Menichini F, Saab A, Statti G, Menichini F. Antiproliferative effects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells. Cell Prolif 2008;41:1002–12. https://doi.org/10.1111/j.1365-2184.2008.00561.x.

[53] Chiang LC, Chiang W, Chang MY, Ng LT, Lin CC. Antileukemic activity of selected natural products in Taiwan. Am J Chin Med 2003;31:37–46. https://doi.org/10.1142/S0192415X03000825.

[54] Brodmann J, Twele R, Francke W, Yi-Bo L, Xi-qiang S, Ayasse M. Orchid mimics honey bee alarm pheromone in order to attract hornets for pollination. Curr Biol 2009;19:1368–72.