Doctoral Dissertation Evaluation Review

Degree requested Doctor of Life Science Applicant's name Mutmainah

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Title of Doctoral Dissertation

Study on Malabaricone C Isolated From the Mace of *Myristica fragrans* as Natural Antiviral Against SARS-CoV-2 and Discovery of Its Mechanism of Actions
（香辛料由来マラバリコーン C による SARS-CoV-2 感染阻害とその機構解明）

Results of Evaluation of the Doctoral Dissertation (Report)

Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), is an enveloped beta coronavirus responsible for a global health crisis. The disease it causes, COVID-19, manifests respiratory symptoms such as cough, fever, and severe pneumonia, which can result in death. Similar to its predecessor, SARS-CoV-1, which occurred two decades earlier, this novel coronavirus invades human cells by attaching its spike protein to Angiotensin-Converting Enzyme-2 (ACE2) receptors. However, while SARS-CoV-1 uses endocytosis to enter host cells, SARS-CoV-2 favors a route through transmembrane serine protease 2 (TMPRSS2)-mediated membrane fusion. Although new vaccines and antiviral drugs have been created to combat SARS-CoV-2, the need for additional COVID-19 treatments remains due to limited options. From a safety perspective, bioactive compounds from foods and edible plants present potential and challenges as new therapeutic agents against SARS-CoV-2.

In this study the author highlights the efficacy of malabaricone C, a naturally occurring compound, along with its synthetic derivatives, in inhibiting SARS-CoV-2 infection. The first chapter introduces SARS-CoV-2 and recent treatment advancements, including a review of natural antiviral agents and their mechanisms for inhibiting infections. The second chapter centers on malabaricone C, a compound derived from nutmeg, identifying it as a novel natural inhibitor of SARS-CoV-2. Malabaricone C and its derivatives demonstrated an EC_{50} range of 1–1.5 μM against SARS-CoV-2 and its variants in mammalian cells, indicating its potential as a safe therapeutic candidate for treating and preventing COVID-19. Additionally, the chapter will present a novel method for visualizing virus-assisted cell fusion. The third chapter explores various possible mechanisms for malabaricone C inhibiting SARS-CoV-2 infection in vitro. Potential targets include disrupting the interaction between Spike RBD and ACE2, inhibiting TMPRSS2, and interfering with lipid raft assembly on the cell membrane. This study suggests that malabaricone C inhibits SARS-CoV-2 entry primarily by affecting lipid raft assembly in the host cell membrane.

In conclusion, malabaricone C and its derivatives were identified as natural inhibitors of anti-SARS-CoV-2 (WK-521) agents, similar to remdesivir, in VeroE6/TMPRSS2 cells. In particular, malabaricone C and its derivatives had a large selective index of over five values between EC_{50} and CC_{50}. Interestingly, those compounds exhibited potent antiviral effect against SARS-CoV-2 variants with an EC_{50} of 1.5–2.0 μM, and their antiviral activities were equal to or even more potent than those of chloroquine and hydroxychloroquine, the anti-SARS-CoV-2 drugs that have been FDA-approved for COVID-19. We also investigated the anti-SARS-CoV-2 variants (B.1.1.7, P.1, and B.1.617.2) activity of malabaricone C and its derivatives in human embryonic kidney cells HEK293TA cell lines. These results emphasize the potential implications of

malabaricone C and its derivatives as novel anti-SARS-CoV-2 candidates for the treatment of COVID-19. In addition, we established a novel visualized cell fusion assay for SARS-CoV-2 with SARS-CoV-2 persistently infected HEK293TA-G cells (green fluorescence) and HEK293TA-M cells (red fluorescence). This simple cell-cell fusion assay provides a versatile tool for study SARS-CoV-2 mediated fusion. Currently, there are no reports on the molecular mechanisms of infection between SARS-CoV-2 and host cells. However, the molecular and cellular mechanisms of malabaricone C antiviral activity have not been completely elucidated. Here, we first demonstrated the partial mechanism of the anti-SARS-CoV-2 activity of malabaricone via interference with lipid raft formation on the plasma membrane in Vero E6 cells. Further detailed elucidation of the mechanism is required. Finally, because malabaricone C is present in various edible plants for the prevention of COVID-19, it can be safely and easily administered to the elderly and small children. This finding could be a valuable approach for new drug development based on the malabaricone C structure, which has developed rapidly in recent years. Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Life Science from Hokkaido University.