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Strategic Researches for Combating Antibiotic-Resistant Infections

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ปัญหาแบคทีเรียดื้อยาปฏิชีวนะได้แพร่กระจายเป็นวงกว้างและเป็นปัญหาสำคัญทางสาธารณสุขทั่วโลก ทำให้ประสิทธิภาพของยาในการใช้รักษาโรคติดเชื้อแบคทีเรียเริ่มมีข้อจำกัด อีกทั้งการระบาดของเชื้อดื้อยาเกิดขึ้นอย่างรวดเร็วกว่าการพัฒนายาใหม่ ดังนั้นการพัฒนายาและ/หรือการค้นหาวิธีใหม่ในการรักษาโรคติดเชื้อดื้อยาจึงมีความสำคัญยิ่ง โครงการวิจัยนี้ได้มีการวางกลยุทธการจัดการควบคุมและรักษาโรคติดเชื้อแบคทีเรียโดยเน้นการใช้สารธรรมชาติและ green technology เพื่อรักษาสิ่งแวดล้อม งานวิจัยแบ่งเป็นสองส่วนที่ได้ดำเนินการแบบคู่ขนาน ได้แก่ งานวิจัยด้านวิทยาศาสตร์พื้นฐานเพื่อสร้างองค์ความรู้ใหม่ และงานวิจัยที่เน้นการใช้ประโยชน์อย่างเป็นรูปธรรมโดยมุ่งสู่การสร้างความร่วมมือกับภาคอุตสาหกรรม ในส่วนของการสร้างงานวิจัยที่เป็นองค์ความรู้ใหม่จากการพัฒนางานวิจัยที่ต่อเนื่อง ได้ค้นพบกลไกใหม่ในการออกฤทธิ์ต้านแบคทีเรียของสารโรโดไมรโทนที่มีศักยภาพสูง (PLoS Pathogens 14: e1006876) ซึ่งอาจนำไปสู่การใช้เป็นยาใหม่สำหรับรองรับการใช้รักษาโรคติดเชื้อที่ดื้อยา นอกจากนี้ได้มีการค้นพบสารธรรมชาติอื่น ๆ ที่มีฤทธิ์ต่อเชื้อดื้อยา งานวิจัยการใช้นาโนเทคโนโลยีในการเพิ่มประสิทธิภาพของสาร รวมถึงค้นหาสารที่มีคุณสมบัติในการยับยั้ง quorum sensing molecule ของแบคทีเรีย และสารที่มีคุณสมบัติในการเสริมฤทธิ์ยาปฏิชีวนะที่มีอยู่ทำให้นำกลับมาใช้ได้อย่างมีประสิทธิภาพ และการศึกษาตำรับยาแผนไทยเชิงบูรณาการและการประยุกต์ใช้ ได้ผลิตผลงานวิจัยที่เกิดจากการทำงานร่วมกันของนักวิจัยต่างศาสตร์ ในระยะเวลา 3 ปี มีผลงานวิจัยที่ได้รับการตีพิมพ์ในฐาน ISI จำนวน 36 รายการ (4 articles ใน quartile 1, 20 articles ใน quartile 2, 10 articles ใน quartile 3, 2 articles ใน quartile 4) และ หนังสือ 1 เล่ม รวมทั้งผลงานประดิษฐ์ที่สามารถนำไปใช้ประโยชน์เชิงพาณิชย์ โดยมี อนุสิทธิบัตร 7 รายการ ที่สำคัญยิ่งคือการสร้าง ‘นักวิจัยมืออาชีพ’ และ ประชาคมวิจัยที่เข้มแข็ง ผลิตนักวิจัยรุ่นใหม่-รุ่นกลางคน ระดับหลัง 16 คน 4 ปริญญาเอกบัณฑิตระดับปริญญาเอก คน 9 และ ปริญญาโท คน 8 โดยคณาจารย์ได้มีตำแหน่งทางวิชาการที่สูงขึ้น ได้แก่ ตำแหน่งผู้ช่วยศาสตราจารย์ 3 คน และรองศาสตราจารย์ 6 คน ได้มีการวางแผนงานให้เกิดการมีส่วนร่วมและเชื่อมโยงระหว่างองค์ความรู้และภาคการผลิต ภาคการบริการ ภาคสังคม ซึ่งสามารถตอบสนองนโยบายการวิจัย

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Abstract

The emergence of antibiotic-resistant pathogenic bacteria is a healthcare problem worldwide. The efficacy of most current antibiotics for treatment of bacterial infections has become quite limited due to the occurrence of multiple antibiotic-resistant organisms. Moreover, antibiotic resistance is spreading faster than the introduction of new compounds into clinical uses, leading to increasing public health crisis. The discovery of new compounds or alternative ways to overcome this serious problem could lead to considerable global benefits. In hopes of vanquishing the growing drug-resistant pathogens, we have been searching for new compounds as well as studying alternative means to overcome the problems encountered by antibiotic-resistant bacteria. Paralled studies including basic researches and applied researches have been integratingly designed with the hope to bring our findings to application levels. We discovered a new mechanism of rhodomyrtone, a compound that possesses equivalent potency as vancomycin, which enable us to move forwards, challenging it as a possible new antibiotic (PLoS Pathogens 14: e1006876). In addition to searching for new biologically active compounds, this target-based research on natural products covered the following areas including green nanomaterial synthesis to enhance antibacterial activity of plant extracts and their purified compounds, studies on quorum sensing inhibitors and resistance modifying agents with specific focus on Gram-negative bacteria, studies on scientific information to support the use of Thai herbal formulations for bacterial infections, and applications of natural products for control and treatment of infectious diseases. By the

end of the granting period, we could produce 36 scientific articles in ISI database (4 articles in quartile 1, 20 articles in quartile 2, 10 articles in quartile 3, 2 articles in quartile 4) and 7 petty patents. More importantly, with a strong passion to promote collaboration and support networking among researchers from different disciplines, a long-term development of human resources in multidisciplinary fields both graduate students (9 Ph.D., 8 M.Sc.) and academic career path scientitists (6 Associate Professor, 3 Assistant Professors, 4 postdoctoral fellows) was established.

Executive Summary

The efficacy of most current antibiotics for treatment of bacterial infections has become quite limited due to the occurrence of multiple drug-resistant bacterial strains. In hopes of combating the growing antibiotic-resistant organisms, we have been searching for new compounds. Rhodomyrtone, an acylphloroglucinol derivative isolated from the leaf of *Rhodomyrtus tomentosa* (Aiton) Hassk., (Myrtaceae) presented extremely potent antibacterial activity against Gram-positive bacteria including multidrug-resistant organisms, comparable to that of vancomycin. Proteomic and transcriptomic mapping of both intracellular and extracellular proteins in methicillin-resistant *S. aureus* (MRSA) after rhodomytone treatment were undertaken to provide insight into possible antibacterial mechanisms. In this study, both the computational aided approach for drug discovery and experimental methods were integratingly analyzed to reveal a possible candidate target for rhodomyrtone.

The global emergence of multi-drug-resistant clones of Gram-negative bacterial nosocomial pathogens has narrowed treatment options for life-threatening infections. In addition to continued search for new active compounds against pathogenic bacteria of medical and economic importance, the project has been designed to study alternative approaches to control and treat these antibiotic-resistant pathogens. Candidate resistance modifying agents from plants which can restore extensive drug resistance in extensively drug-resistant bacteria, to be used in combination with current recommended antibiotics for the treatment of infections were investigated.

Since series of works from our laboratory have clearly demonstrated a strong antibacterial potency of a member of Myrtaceae family, it would make a valuable scientific

contribution to this area by extending our studies to cover other species within this plant family.

The search for quorum sensing inhibitors from medicinal plants would be a useful option in controlling quorum sensing dependent bacterial pathogenesis in host. In other context, as many phytochemicals present in certain plants can act as strong reducing agents, extended antibacterial spectrum using nanotechnology for the treatment of especially Gram-negative bacteria was studied. Green synthesis of nanoparticles using plant extracts or their purified compounds was

demonstrated to be an appropriate mean to enhance their antibacterial properties. Furthermore, to evaluate possible modes of action, computer-aided drug discovery approach was adopted to restructure phytochemicals with quorum sensing inhibitor potential from plant extracts.

It is recognized that herbal remedies prepared from medicinal plants may have several traditional applications, especially in developing nations like Thailand. In recent years, the demand for herbal remedies in Thailand has been on the rise. The increasing commercial promotion of herbals necessitate the need for claims, assessment of safety, and validity of medicinal claims. However, there have been very little or no scientific data to support medicinal claims of Thai herbal formulations. We have set up multidisciplinary researches on herbal formulations used in southern Thailand including chemical studies on herbal formulations, study on biological activities, studies on antimicrobial activity of herbal formulations. Lupinifolin isolated from a formulation used for oral infection was further studied.

Previous results demonstrated the potential of rhodomyrtone for further drug development for the treatment of biofilm-forming staphylococcal infections. Liposomal encapsulated rhodomyrtone was developed as an effective therapeutic anti-acne agent. An

investigation on the effects of rhodomyrtone on *Propionibacterium acnes* as well as appropriate drug delivery system confirmed its application as a novel anti-acnes drug. Other applications included uses of *Rhodomyrtus tomentosa* extract as preventive methods in medical gloves, bovine mastitis, as well as streptococcosis in fish of economic importance. Plant extracts with promising antibacterial activity were studied as mouth wash and food additives.

Tuberculosis, a severe infectious disease caused by *Mycobacterium tuberculosis*, remains a major global health problem. The role of macrophage migration inhibitory factor as an inflammatory mediator has been implicated in several diseases including an infectious disease such as tuberculosis. Several reports have demonstrated that macrophage migration inhibitory factor plays either a protective or deleterious role in the immune response to different pathogens. In order to gain a better understanding on the role of macrophage migration inhibitory factor, we looked at a correlation between the gene promoter polymorphism and susceptibility of individuals to active pulmonary tuberculosis.

By the end of the granting period, we could produce 36 fruitful scientific articles with relatively high impact factor in ISI database. More importantly, with a strong passion to promote collaboration and support networking among researchers from different disciplines, a long-term development of human resources in multidisciplinary fields, both graduate students and academic career path scientitists was well-established.

Our findings under objectives stated in proposal were outlined as follows:

Detailed studies on *Rhodomyrtus tomentosa* and rhodomyrtone, a novel antibiotic candidate

Rhodomyrtone, an acylphloroglucinol derivative isolated from the leaf of *Rhodomyrtus tomentosa* (Aiton) Hassk. (Myrtaceae) is a promising new antibiotic against Gram-positive bacteria including multidrug-resistant organisms, comparable to last-resort antibiotics including vancomycin and daptomycin. While many studies have demonstrated its antibacterial potential in a variety of clinical applications, very little is known about the mechanism of action of rhodomyrtone. During the past years, proteomic and transcriptomic mapping of both intracellular and extracellular proteins in methicillin-resistant *Staphylococcus aureus* (MRSA) after rhodomytone treatment were undertaken by our research group to provide insight into possible antibacterial mechanisms. Further information to elucidate antibacterial mechanisms is inevitably required for further drug development for the treatment of bacterial infections. During this RTA granting period, both the computational aided approach for drug discovery and experimental designs following criteria from proteomic and transcriptomic data have been integratingly analyzed to reveal a possible candidate target for rhodomyrtone.

We applied computer-aided technique to identify a rhodomyrtone protein target from vital MRSA protein structure with the hope that an investigation of rhodomyrtone action at the molecular level could be more precisely focused. Fifty MRSA proteins, playing roles in vital processes, was screened for rhodomyrtone molecular targets. The molecular docking study was operated using AutoDock4. To confirm two possible targets, checkerboard assay and cell visualization were further carried out. Rhodomyrtone exhibited an interesting efficacy towards

one-fifth of the given proteins. Moreover, metal dependent phosphate binding proteins were excluded from possible targets because of electrostatic forces. Amongst chosen proteins, rhodomyrtone, both enantiomers, displayed significant potency to dihydrofolate reductase (DHFR) and filamenting temperature-sensitive Z (FtsZ) proteins, compared with their natural substrates/inhibitors. In contrast, protein cofactors such as nicotinamide adenine dinucleotide phosphate (NADP) or guanosine diphosphate (GDP) decreased rhodomyrtone binding affinity. This information suggested a cofactor free DHFR and a ligand-unbound FtsZ are likely to be rhodomyrtone targets for MRSA inhibition. In addition, checkerboard assay and cell visualization gave a hint on target confirmation. We have proposed potential rhodomyrtone targets, and DHFR and FtsZ caught our interest. (*Year 1: Publication No. 6*).

Our previous molecular docking study suggested that the compound could competitively bind to the main bacterial cell division protein FtsZ. We further applied a computational approach (*in silico*), *in vitro*, and *in vivo* experiments to investigate the effects of rhodomyrtone on Gram-positive bacterial tubulin homologue FtsZ. Using molecular simulation, FtsZ conformational changes were observed in both (S)- and (R)-rhodomyrtone binding states, compared with three natural states of FtsZ (ligand-free, GDP-, and GTP (guanosine triphosphate)-binding states). Calculations of free binding energy showed a higher affinity of FtsZ to (S)-rhodomyrtone (-35.92±0.36 kcal/mol) than GDP substrate (-23.47±0.25 kcal/mol), while less affinity was observed in the case of (R)-rhodomyrtone (-18.11±0.11 kcal/mol). In *vitro* experiments further revealed that rhodomyrtone reduced FtsZ polymerization by 36% and inhibited GTPase activity up to 45%. However, the compound had no effect on FtsZ localization in *Bacillus subtilis* at inhibitory concentrations and cells also did not elongate after treatment. Higher concentrations of rhodomyrtone did affect localization of FtsZ and also affected localization of its membrane anchor proteins FtsA and SepF, showing

that the compound did not specifically inhibit FtsZ but rather impaired multiple divisome proteins. Furthermore, a number of cells adopted a bean-like shape suggesting that rhodomyrtone possibly possesses further targets involved in cell envelope synthesis and/or maintenance (*Year 2: Publication No. 9*).

Preceding studies have been focused on intracellular targets, but no specific intracellular protein could be confirmed as main target. Using live cell, high-resolution, and electron microscopy we demonstrate that rhodomyrtone causes large membrane invaginations with a dramatic increase in fluidity, which attract a broad range of membrane proteins. Invaginations then form intracellular vesicles, thereby trapping these proteins. Aberrant protein localization
impairs several cellular functions, including the respiratory chain and adenosine triphosphate (ATP) synthase complex. Being uncharged and devoid of a particular amphipathic structure,

rhodomyrtone did not seem to be a typical membrane-inserting molecule. In fact, molecular dynamics simulations showed that instead of inserting into the bilayer, rhodomyrtone transiently
binds to phospholipid head groups and causes distortion of lipid packing, providing explanations for membrane fluidization and induction of membrane curvature. Both its transient binding mode and its ability to form protein-trapping membrane vesicles are unique, making it an attractive new antibiotic candidate with a novel mechanism of action (*Year 3: Publication No. 8*).

Early effects of rhodomyrtone on the bacterial membrane integrity were detected in a time-course study. Flow cytometry revealed a reduction in green fluorescent emission and increase in uptake of propidium iodide in rhodomyrtone-treated bacterial cells in a concentration- and time-dependent manner. Disruption of cytoplasmic membrane was further

monitored by measuring cellular ATP and potassium ion (K^+). Leakage of both ATP and K^+ and significant decrease in intracellular ATP in MRSA were observed following treatment. Pronounced changes in the bacterial ultrastructure and morphology were confirmed by transmission electron microscopy and scanning electron microscopy. Bacterial cell disruption, holes in cell surface, and bulge formations were noted in rhodomyrtone-treated cells. In this study, we provided relevant data to clarify that rhodomyrtone is a bacterial cell membrane-damaging agent. A possible early effect of this novel compound involves bacterial membrane disruption (*Year 2: Publication No. 11*).

Rhodomyrtone exhibited pronounced anti-pneumococcal activity against a broad collection of clinical isolates. We studied the effects at the molecular level by integrated proteomic and metabolomic analysis. The results revealed alterations in enzymes and metabolites involved in several metabolic pathways including amino acid biosynthesis, nucleic acid biosynthesis, glucid, and lipid metabolism. Notably, the levels of two enzymes (glycosyltransferase and UTP-glucose-1-phosphate uridylyl transferase (UDP) and three metabolites (UDP-glucose, UDP-glucuronic acid and UDP-N-acetyl-D-galactosamine) participating in the synthesis of the pneumococcal capsule clearly diminished in the bacterial

cells exposed to rhodomyrtone. Rhodomyrtone-treated pneumococci significantly possessed less amount of capsule, as measured by a colorimetric assay and visualized by electron microscopy.

The findings revealed the utility of combining proteomic and metabolomic analyses to provide insight into phenotypic features of *Streptococcus pneumoniae* treated with this potential novel

antibiotic. This can lead to an alternative antibiotic for the treatment of *Streptococcus pneumoniae* infections (*Year 2: Publication No. 5*).

Rhodomyrtus tomentosa, a medicinal plant possessing several phytochemical constituents, has been considered as a potential source of antimicrobial and immunomodulatory agents. Enterohaemorrhagic *Escherichia coli* O157:H7 is one of the most virulent causative agents of foodborne disease. Use of antibiotics for the treatment against *Escherichia coli* O157:H7 infection leads to haemolytic uraemic syndrome. We evaluated the potential of *Rhodomyrtus tomentosa* ethanolic leaf extract in enhancing the killing activity of human neutrophils against *Escherichia coli* O157:H7. In addition, the effects of the extract on membrane permeability of the organisms were studied. In the killing assay, percentage survival of the bacterial cells after being exposed to human neutrophils in the presence of various concentrations of the extract were determined. At 45 min, percentage survival of *Escherichia coli* O157:H7 and *Escherichia coli* ATCC 25922 after treated with neutrophils in the presence of the extract at 125-250 μg/mL was 58.48%-50.28% and 69.13%-35.35%, respectively. Furthermore, upon treatment with *Rhodomyrtus tomentosa* at 250 μg/mL uptake of crystal violet by *Escherichia coli* O157:H7 and *Escherichia coli* ATCC 25922 was increased to 40.07% and 36.16%, respectively. Therefore, it is suggested that the extract exhibited dual effects as immunostimulant and membrane permeabilizing agent perhaps resulted in enhancing the killing activity of neutrophils against the organisms (*Year 1: Publication No. 2*).

Methicillin-resistant *Staphylococcus aureus* has an ability to invade nonprofessional phagocytic cells, resulting in persistent infections and most likely host cell death. This study was to further investigate potency of the extract in intracellular killing of human HaCaT keratinocytes. Pretreatment of MRSA with the extract resulted in a remarkable reduction in the bacterial adhesion to HaCaT keratinocytes, compared with untreated control ($P<0.001$). In addition, at least 60% inhibition of the bacterial invasion into HaCaT cells was observed.

Intracellular killing assay demonstrated that the extract exhibited strong antibacterial activity against intracellular MRSA at non-toxic concentrations (128 mg/L), which may have resulted from the increase in bactericidal activity under phagolysosomal pH. Transmission electron microscopy displayed the effects of the extract on alterations in the bacterial cell morphology with cell lysis. Fluorescence microscopy revealed that the extract decreased MRSA-induced apoptosis in HaCaT cells. In addition, cytotoxicity of HaCaT cells caused by MRSA supernatant was reduced at least 50% by the extract at concentration 8 and 16 mg/L ($P<0.001$). The potential activities of *Rhodomyrtus tomentosa* extract may be useful in an alternative treatment of MRSA infections in slight acidic compartments, particularly skin infections (*Year 2: Publication No. 13*).

Candida albicans has become a major problem of oral candidiasis due to increase in antibiotic resistance. The aim of this study was to investigate antivirulence and immunostimulatory activity of *Rhodomyrtus tomentosa* ethanolic leaf extract against *Candida albicans*. The effects of the extract on *Candida albicans* were assessed on germ tube production, adherence of the organisms to surfaces, and biofilm. In addition, the effects of the extract on phagocytosis and killing activity of neutrophils against the pathogen were investigated. Suppression of germ tube production following 30 min exposure to the extract at 256 μg/mL was significantly increased in comparison with that of the unexposed cells ($P<0.05$). The pathogens demonstrated a significant reduction in adherence ability to surfaces in a dose dependent manner, compared with the control ($P<0.05$). At 48 h, the extract at 512-1024 μg/mL significantly reduced biofilm forming ability of the organisms up to 42.31-64.58% ($P<0.05$). Compared with the control group, a significant inhibition of mature biofilm after treated with the extract at 256 μg/mL was observed ($P<0.05$). The extract at 50 μg/mL significantly enhanced phagocytosis and killing activity of neutrophils against the organism, compared with the control ($P<0.05$). The findings suggest that *Rhodomyrtus tomentosa* extract

displayed a dual mode of action, inhibiting virulence factors of *Candida albicans* and enhancing neutrophil functions. Further pharmaceutical development of the extract might be useful as an alternative therapeutic agent against oral candidiasis (*Year 3: Publication No. 3*).

Green nanomaterial synthesis techniques to enhance antibacterial activity of plant extracts and their purified compounds

Since series of works from our laboratory have clearly demonstrated a strong antibacterial potency of *Rhodomyrtus tomentosa* which is a member of Myrtaceae family, it may

make a valuable scientific contribution to this area by extending our studies to cover other species within this plant family. In other context, as many phytochemicals present in certain plants can act as strong reducing agents, extended antibacterial spectrum using nanotechnology for the treatment of especially Gram-negative bacteria was set up. Green synthesis of nanoparticles using plant extracts or their purified compounds from Myrtaceae family could be an appropriate mean to enhance their antibacterial properties.

Silver nanoparticles (AgNPs) were synthesized using ethanolic leaf extracts from Myrtaceae family including *Callistemon lanceolatus*, *Decaspermum parviflorum*, *Eucalyptus citriodor*a, *Melaleuca cajuputi*, *Rhodomyrtus tomentosa*, *Syzygiupam campanulatum*, and *Xanthostemon chrysanthus*. Silver nanoparticles were verified by UV-visible spectroscopy, transmission electron microscopy, energy dispersive x-ray spectroscopy, zeta potential, and Fourier-transform infrared spectroscopy. Surface plasmon resonance bands of AgNPs occurred in the wavelength range of 417-462 nm. AgNPs morphology was spherical and ranged in size

from 5-55 nm. Zeta potential of AgNPs were 36.49-22.25 mV. Fourier-transform infrared spectroscopy results showed the binding properties of constituents responsible for capping and stabilizing the nanoparticles. Minimum inhibitory concentration (MIC) and minimum inhibitory concentration (MBC) of AgNPs against *Enterococcus faecalis*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* ranged between 7.8-62.5 and 62.5-125 µg/mL, respectively (*Year 2: Publication No. 8*). Formulation of silver nanoparticle as antimicrobial agent using green process synthesis from Myrtaceae family (*Thai Petty Patent 1601005192*) was registered.

Metallic nanoparticles augmented the antibacterial potency of *Rhodomyrtus tomentosa* acetone extract against *Escherichia coli*. The study was focused on the preparation of gold (Au),

silver (Ag), and gold-silver-alloy NPs using *Rhodomyrtus tomentosa* acetone extract. The synthesized NPs showed the surface plasmon resonance absorption peak corresponding to AuNPs and AgNPs. However, Au-Ag-Alloy nanoparticles showed the single peak between the peaks of AuNPs and AgNPs. Transmission electron microscopy observation ascertained the shape and size of nanoparticles. Fourier-transform infrared spectroscopy results indicated the involvement of the extract for the synthesis and capping of nanoparticles. Study on antibacterial activity demonstrated the enhanced activity of *Rhodomyrtus tomentosa* acetone extract capped on silver and Au-Ag-Alloy NPs against *Escherichia coli* (*Year 2: Publication No. 10*).

Search for quorum sensing inhibitors as innovative therapeutic approaches for Gram-negative antibiotic-resistant bacteria and investigate possible modes of action

Considering the role of virulence factors in bacterial pathogenicity, interfering with the virulence factor production could afford a novel way for the treatment of infections caused by pathogenic bacteria. Virulence factors regulated by quorum sensing play a critical role in the pathogenesis of pathogenic bacteria in causing infections to the host. The search for quorum sensing inhibitors from medicinal plants in controlling quorum sensing dependent bacterial pathogenesis in host was performed. Furthermore, to evaluate possible modes of action, computer aided drug discovery approach were adopted to restructure phytochemicals with quorum sensing inhibitor potential from plant extracts.

In the present work, the anti-virulence potential of the medicinal plant extracts and their derived phytochemicals from Myrtaceae family was evaluated against *Pseudomonas aeruginosa*, an opportunistic human pathogen. In the preliminary screening of the tested medicinal plant extracts, *Syzygium jambos* and *Syzygium antisepticum* demonstrated a maximum inhibition in quorum sensing-dependent violacein pigment production by *Chromobacterium violaceum* DMST 21761. These extracts demonstrated an inhibitory activity over a virulence

factor, pyoverdin, production by *Pseudomonas aeruginosa* ATCC 27853. Gas chromatography-mass spectrometric analysis revealed the presence of 23 and 12 phytochemicals from the extracts

of *Syzygium jambos* and *Syzygium antisepticum*, respectively. Three top-ranking phytochemicals, including phytol, ethyl linoleate, and methyl linolenate, selected on the basis of docking score in molecular docking studies lowered virulence factors such as pyoverdin

production, protease and haemolytic activities of *Pseudomonas aeruginosa* to a significant level. In addition, the phytochemicals reduced rhamnolipid production by the organism. The work demonstrated an importance of plant-derived compounds as anti-virulence drugs to conquer *Pseudomonas aeruginosa* virulence towards the host (*Year 2: Publication No. 6*).

Eugenyl acetate, a well-known aromatic component of *Syzygium aromaticum* (clove bud), was assessed for its anti-virulence potential against both Gram-negative and Gram-positive pathogens. Eugenyl acetate at 150 µg/mL, significantly inhibited virulence factor production such as pyocyanin and pyoverdin by *Pseudomonas aeruginosa* ATCC 27853 up to 9.4 ($P<0.01$) and 3.7-fold ($P<0.01$), respectively. In addition, protease activity of *Pseudomonas aeruginosa* was significantly reduced upon treatment with eugenyl acetate ($P<0.05$). The test compound lowered haemolytic activity of *Staphylococcus aureus* ATCC 29213 up to ten-fold ($P<0.01$). Furthermore, a decrease in staphyloxanthin pigment production was observed when the bacteria were treated with increasing concentrations of eugenyl acetate (37.5-150 µg/mL). The test compound at 75 µg/mL exhibited quorum sensing inhibitory potential in inhibiting violacein production by *Chromobacterium violaceum* DMST 21761 up to 27.7-fold ($P<0.01$). Thus, results of the present work reveal the potential of eugenyl acetate as an alternative candidate to control pathogenicity of both Gram-negative and Gram-positive organisms (*Year 1: Publication No. 10*).

We further evaluated the prospective of eugenyl acetate as an antibacterial and anti-virulence agent against drug-resistant *Acinetobacter baumannii* clinical isolates. The minimum inhibitory concentration of the compound against the clinical isolates (n=20) was found to be in the range between 0.05% and 0.4% v/v. In time-kill assay, eugenyl acetate at MIC retarded the growth of the isolates and inhibited further proliferation. Moreover, a significant inhibition in the growth dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide by *Acinetobacter baumannii* was observed upon treatment with eugenyl acetate, compared with no effects observed with ampicillin ($P<0.01$). Scanning electron microscopic studies demonstrated cell rupture, wrinkled cell morphology, and an irregular shape of the bacterial cells upon treatment with the compound. Eugenyl acetate at sub-MIC (0.025% v/v) exhibited anti-virulence effects by reducing motility and protease production by *Acinetobacter baumannii*. The percentage motility inhibitory effects of the compound ranged from 75.63-87.20%, significantly different from the control ($P<0.01$). Furthermore, the compound reduced proteolytic activity of the isolates in the range of 50.81-92.66%. The results established the prospective of eugenyl acetate against *Acinetobacter baumannii* (*Year 1: Publication No. 3*).

Antifungal potential of eugenyl acetate against clinical isolates of *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata* was evaluated. Minimum inhibitory concentrations of eugenyl acetate against candida isolates were in the range between 0.1%-0.4% (v/v). Spot assay further confirmed the susceptibility of candida isolates to the compound upon treatment with respective MIC. Growth profile measured in time kill study evidence that the compound at MIC and 1/2×MIC retarded the growth of the yeast cells, divulging the fungicidal activity. Light microscopic observation demonstrated that upon treated with eugenyl acetate, rough cell morphology, cell damage, and fragmented patterns were observed in all *Candida* spp. Furthermore, unusual morphological changes of the organism were observed in scanning electron microscopic study. Therefore, it is validated that the compound could cause cell damage resulting in the cell death of candida clinical isolates. Eventually, the compound at sub-MIC (0.0125% v/v) significantly inhibited serum-induced germ tube formation by *Candida albicans*. Eugenyl acetate inhibited biofilm forming ability of the organisms as well as reduced the adherence of yeast cells to HaCaT keratinocytes cells. In addition, upon treatment with eugenyl acetate, the phagocytic activity of macrophages was increased significantly against *Candida albicans* ($P<0.05$). The results demonstrated the

potential of eugenyl acetate as a valuable phytochemical to fight against emerging candida infections (*Year 1: Publication No. 4*).

Search for resistance modifying agents with specific focus on Gram-negative bacteria and investigate possible modes of action

The global emergence of multi-drug-resistant clones of Gram-negative bacterial nosocomial pathogens has narrowed treatment options for life-threatening infections. In addition to continued search for new active compounds against pathogenic bacteria of medical and economic importance, our strategic research project has been designed to study alternative approaches to control and treat antibiotic-resistant pathogens. The bacteria of primary concern include recent isolates of carbapenem-resistant strains of the four main Gram-negative ESKAPE pathogens, namely, *Enterococcus faecium, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.

Holarrhena antidysenterica has been employed as an ethnobotanical plant for the treatment of dysentery, diarrhoea, fever, and bacterial infections. Biological activities of the principle compound, conessine including anti-diarrhoea and anti-plasmodial effects were documented. *Holarrhena antidysenterica* was previously demonstrated to be an interesting candidate resistance modifying agent to be used in combination with current recommended antibiotics for the treatment of infections for restoring drug resistance in extensively drug-resistant *Acinetobacter baumannii*. The efficacy of the plant extract including its major steroidal alkaloid conessine as resistance-modifying agents on the susceptibility of the

organisms to novobiocin and rifampicin was investigated. A significant synergistic activity of both the extract and conessine in combination with either novobiocin or rifampicin with fractional inhibitory concentration index ≤0.5 was demonstrated. Fluorescent dyes and different efflux pump inhibitors were used to further investigate the synergism. Increase in the uptake of 1-N-phenylnaphthylamine in the bacterial cells treated with the extract and conessine was not observed indicating that both substances did not act as permeabilizers. With regard to efflux pump inhibition, no accumulation in ethidium bromide was noticed suggesting that the AdeABC pump was not involved. In contrast, accumulation in pyronin Y was significantly increased ($P<0.05$) demonstrating that the synergism was due to interference with the AdeIJK pump. Study on frequencies of the spontaneous mutational resistance to the extract in combination with

antibiotics demonstrated attenuation in drug-resistant organisms. Thus, *Holarrhena antidysenterica* extract and conessine as RMAs may offer a combinatory therapy to restore antibiotic susceptibility in the extensively drug-resistant *Acinetobacter baumannii* (*Year 1: Publication No. 7*).

Our previous study reported potency of *Holarrhena antidysenterica* extract and conessine as resistance modifying agents against extensively drug-resistant *Acinetobacter baumannii*. This study aimed to investigate (i) whether conessine, a steroidal alkaloid compound, could act as a resistance modifying agent against multidrug-resistant *Pseudomonas aeruginosa*, and (ii) whether MexAB-OprM efflux pump involved in the mechanism. Conessine combined with various antibiotics were determined for synergistic activity against *Pseudomonas aeruginosa* PAO1 strain K767 (wild-type), K1455 (MexAB-OprM overexpressed), and K1523 (MexB deletion). H33342 accumulation assay was used to evaluate efflux pump inhibition while

NPN uptake assay was assessed membrane permeabilization. Conessine significantly reduced

MICs of all antibiotics by at least 8-fold in MexAB-OprM overexpressed strain. The levels were comparable to those obtained in wild-type strain for cefotaxime, levofloxacin, and tetracycline. With erythromycin, novobiocin, and rifampicin, MICs were 4- to 8-fold less than MICs of the wild-type strain. Loss of MexAB-OprM due to deletion of mexB affected susceptibility to almost all antibiotics, except novobiocin. Synergistic activities between other antibiotics (except novobiocin) and conessine observed in MexB deletion strain suggested that conessine might inhibit other efflux systems present in *Pseudomonas aeruginosa*. Inhibition of H33342 efflux in the tested strains clearly demonstrated that conessine inhibited MexAB-OprM pump. In contrast, the mode of action as a membrane permeabilizer was not observed after treatment with conessine as evidenced by no accumulation of 1-N-phenylnaphthylamine. The results suggested that conessine could be applied as a novel efflux pump inhibitor to restore antibiotic activity by inhibiting efflux pump systems in *Pseudomonas aeruginosa*. The findings speculated that conessine may also have a potential to be active against homologous resistance-nodulation-division (RND) family in other Gram-negative pathogens (*Year 2: Publication No. 12*).

Search for new biologically active compounds

In our continued searching for new biologically active compounds, the green branches extract of *Garcinia dulcis* was studied in the search for biflavonoids. Two new prenylated biflavonoids, named dulcisbiflavonoid and dulcisbiflavonoid, along with 6 previously reported biflavonoids and flavonoids were isolated from the acetone extract of the green branches of *Garcinia dulcis*. The structures of the isolates were determined on the basis of spectroscopic

analysis and the data was compared with those previously reported. Antimicrobial activities of some isolated compounds were evaluated (*Year 3: Publication No. 1*). Three new prenylated xanthones, named dulcisxanthone, dulcisxanthone, and dulcisxanthone, along with 38 known compounds have been isolated from the stem bark of *Garcinia dulcis*. Their structures were elucidated mainly by analysis of 1D and 2D spectroscopic data. 12b-hydroxy-des-D-garcigerrin A and dulcisxanthone displayed moderate antibacterial activity against both penicillin-susceptible *Staphylococcus aureus* and MRSA with MIC values of 4 and 16 μg/mL, respectively (*Year 2: Publication No. 14*).

The chemical investigation of the crude CH_2Cl_2 extract from the stems of *Zanthoxylum nitidum* was performed. A new alkylamide, named (*2E,6E,8E*)-*N*-(2-methylpropyl)-10-oxo-2,6,8-decatrienamide (**1**), together with 22 known compounds (**2**-**23**), were isolated from the stems of *Zanthoxylum nitidum*. Their structures were elucidated by spectroscopic methods, including 1D and 2D NMR spectroscopy. The isolated compounds exhibited slightly antioxidant activities through DPPH and ABTS radical scavenging assays but showed no antibacterial activity against *Streptococcus mutans* ATCC2517, a dental caries causing bacteria (*Year 3: Publication No. 2*).

Albizia myriophylla Benth. wood (Fabaceae) is a medicinal herb which is used as a traditional remedy for various ailments in Thailand. The ethanol extract, fractions and the isolated compounds from the wood of *Albizia myriophylla* were evaluated for *in vitro* α-glucosidase inhibition using spectrophotometric method. The plant ethanol extract and its different fractions possessed α-glucosidase inhibitory activity in a concentration-dependent manner. Dichloromethane fraction of the wood ethanol extract exhibited the highest percent

inhibition against α-glucosidase (69.30%) among all fractions. Subsequent α-glucosidase inhibition assay proved that indenoic acid, 8-methoxy-7,3′,4′-trihydroxyflavone, and 3,4,7,3′-tetrahydroxyflavan were partially rational for antidiabetic effect of this plant species. Among

these compounds, 3,4,7,3′-tetrahydroxyflavan (IC_{50}=98.59 μg/ mL) exhibited potent inhibition of α-glucosidase, compared with a positive control acarbose (IC_{50}=125 μg/mL). The inhibitory effect towards α-glucosidase of these compounds was reported herein for the first time (*Year 2: Publication No. 1*).

Studies on scientific information to support the use of Thai herbal medicine for bacterial infections

It is recognized that herbal remedies prepared from medicinal plants may have several traditional applications, especially in developing nations like Thailand. In recent years, the demand for herbal remedies in Thailand has been on the rise. The increasing commercial promotion of herbals necessitate the need for claims, assessment of safety, and validity of medicinal claims. However, there have been very little or no scientific data to support medicinal claims of Thai herbal remedies. We set up multidisciplinary researches on traditional medicine used in southern Thailand including chemical studies on important herbs and herbal formulations, study on biological activities, studies on antimicrobial activity of herbal formulations used for skin, oral and upper respiratory tract, and gastrointestinal tract infections.

Nutgall of *Quercus infectoria* (*Qi*) G. Olivier (Fagaceae) has been used for centuries in traditional medicine in several Asian countries for the treatment of infectious diseases and inflammatory disorders. Previous works have identified a number of pharmacological activities of *Qi* extract including antimicrobial, anti-inflammatory, and anti-oxidant activity. *Qi* extract contains high concentrations of tannins such as gallic acid, ellagic acid, along with flavonoids that are known to have anti-inflammatory properties. Potential wound healing activity of *Qi* formulation was carried out in diabetic rats. Twenty *Qi* formulations were pharmaceutically

formulated and antibacterial activity of all formulations was performed. The best formulation based on an antibacterial activity was selected for evaluation of wound healing property. Total phenolics, total flavonoids, and an anti-oxidant activity of the selected formulation were also investigated. Wound healing activity was assessed in streptozotocin-induced diabetic rats and control rats. Streptozotocin injection (50 mg/kg) was found to induce marked hyperglycaemia, compared with citrate-injected controls. Two wounds were created on the upper back of each animal. *Qi*F was topically applied three days after wounding to one of the duplicate wounds on each animal and physiological saline (control) was applied to the other. All wounds were cleaned once a day until wound closure. *Qi*F10, which exhibited antibacterial and anti-oxidant activities, had the ability to enhance the wound healing process in diabetic rats with abundant cellular infiltration, collagen deposition, and re-epithelialization when compared with the control. This study suggested that *Qi*F10 could be a novel alternative treatment for diabetic wounds (*Year 2: Publication No. 2*).

Chronic inflammation plays a key role in the pathogenesis of myriad complications associated with diabetes and thus anti-inflammatory therapies may ameliorate these complications. *Qi* extract has been shown to downregulate inflammatory processes; however, the molecular mechanisms of this anti-inflammatory activity remain unclear. The hypothesis of our study was that *Qi* extract exerts its anti-inflammatory effect by downregulating the Set7/NF-κB pathway. Bone marrow-derived macrophages were treated with high glucose plus palmitate medium to simulate the diabetic environment. Compared with control conditions, HG/Pa elevated expression Set7, expression and activity of NF-κB along with expression of several inflammatory cytokines. These changes were associated with increased levels of intracellular reactive oxygen species. Moreover, similar alterations were demonstrated in bone marrow-derived macrophages derived from mice fed a high fat diet compared to those from lean mice, suggesting that a high fat diet-induced changes in bone marrow progenitors persist

throughout differentiation and culture. Importantly, *Qi* extract dose-dependently reduced Set7, p65 and inflammatory cytokine expression relative to vehicle controls in both high glucose plus palmitate medium and a high fat diet-treated bone marrow-derived macrophages. Finally, macrophages/monocytes isolated from wounds of diabetic mice that were treated with *Qi* solution exhibited lower expression of the inflammatory cytokines, IL-1β and TNF-α, compared with vehicle treated wounds, demonstrating translation to the *in vivo* diabetic environment.

Taken together, data from this study suggests that *Qi* downregulates diabetes-induced activity of the Set7/NF-κB pathway (*Year 2: Publication No. 3*).

Antibacterial mechanisms of action of lupinifolin from *Albizia myriophylla* against cariogenic *Streptococcus mutans* was assessed to provide scientific evidence to support the traditional use of the plant against dental caries. Minimum inhibitory concentration was evaluated using the broth microdilution method. The effects of lupinifolin on bactericidal activity, bacterial cell walls, and membranes were investigated by time-kill, lysis, and leakage assays, respectively. Electron microscopy was utilized to observe any cell morphological changes caused by the compound. Localization of lupinifolin in *Streptococcus mutans* was detected using the thin layer chromatography technique. The MIC range of lupinifolin against *Streptococcus mutans* (n=6) was 2-4 μg/mL. This compound displayed bactericidal effects on *Streptococcus mutans* ATCC 25175 by 90-99.9% killing at 4xMIC-16xMIC after 8-24 h. Lupinifolin-treated cells demonstrated no lysis. However, significant cytoplasmic leakage through the bacterial membrane was observed after treatment with lupinifolin at 4xMIC-16xMIC. As revealed by ultrastructural analysis, lupinifolin produced some changes in bacterial cell walls and membranes. Moreover, the compound was observed in the cytoplasmic fraction of the lupinifolin-treated cells. These results suggest that lupinifolin can enter the cell

of bacteria but does not accumulate in the cell envelope and subsequently disrupts the integrity of the cytoplasmic membrane, leading to cell death. The scientific evidence from this study offers valuable insights into the potential role of lupinifolin in pharmaceutical and antibiotic applications and supports the therapeutic effects of *Albizia myriophylla*, which has traditionally been used as an alternative treatment for dental caries (*Year 3: Publication No. 4*).

Anti-infective properties of a Thai traditional polyherbal formula, namely, Ya-Sa-Marn-Phlae (YSMP), its herbal components (*Curcuma longa*, *Areca catechu*, *Oryza sativa*, and *Garcinia mangostana*), and representative chemical constituents (catechin, α-mangostin, and curcumins) were investigated. Ethanol extracts of YSMP and *Garcinia mangostana*, and α-mangostin exhibited potent antibacterial effects against *Staphylococcus* spp. isolated from mastitis cows with MIC values of 1-32 μg/mL. These tested agents inhibited biofilm formation of the isolates on both polypropylene (hydrophobic) and glass (hydrophilic) surfaces. The

current study indicated that YSMP had strong antibacterial activity and anti-biofilm abilities against the tested isolates similar to that of α-mangostin and *Garcinia mangostana*. The anti-staphylococcal effects were confirmed with both scanning and transmission electron

microscopes. The main abnormalities in the microstructure of the treated cells were the severe alterations of the cell wall with the formation of holes and morphological disorganization. Therefore, it might be proposed that *Garcinia mangostana* is the major active component of YSMP and α-mangostin may be used as an active marker compound for YSMP to indicate its activity against bovine mastitis-isolated staphylococci (*Year 2: Publication No. 4*).

Applications of natural products to control and treat diseases

Industrial applications from *Rhodomyrtus tomentosa* leaf, both the ethanolic extract and its purified compound-rhodomyrtone, have been performed. Previous results demonstrated the potential of rhodomyrtone for further drug development for the treatment of biofilm-forming staphylococcal infections. Liposomal encapsulated rhodomyrtone was developed as an effective therapeutic anti-acne agent. An investigation on anti-acne effects of rhodomyrtone on *Propionibacterium acnes* was performed to confirm its application as a novel anti-acnes drug. The effects of rhodomyrtone against enzyme production and biofilm formation production by clinical isolates and a reference strain of *Propionibacterium acnes* were evaluated. The degree of hydrolysis by both lipase and protease enzymes significantly decreased upon treatment with the compound at 0.125-0.25 mg/mL ($P<0.05$). Lipolytic zones significantly reduced in all isolates while decrease in proteolytic activities was found only in 50% of the isolates. Rhodomyrtone at 1/16xMIC and 1/8xMIC caused significant reduction in biofilm formation of the clinical isolates ($P<0.05$). Percentage viability of *Propionibacterium acnes* within mature biofilm upon treated with the compound at 4xMIC and 8xMIC ranged between 40% and 85%. As rhodomyrtone was clearly demonstrate to cause inhibition of lipase production, biofilm formation, and disorganize established biofilm in *Propionibacterium acnes*, the findings suggest a path towards developing a novel anti-acne agent (*Year 2: Publication No. 15*). Rhodomyrtone formulation as anti-inflammatory agent: Biological Property and Process (*Thai Petty Patent* 1603002560) has been registered.

Previous results demonstrated the potential of rhodomyrtone for further drug development for the treatment of bacterial infections. The information on other biological activities of rhodomyrtone will support its use. The effects of rhodomyrtone on the proliferation, growth arrest, and apoptosis of HaCaT keratinocytes were investigated. Percentage anti-proliferative activity of rhodomyrtone on HaCaT cells at concentrations of 2-32 mg/mL after 24, 48, and 72 h ranged from 13.62-61.61%, 50.59-80.16%, and 61.82-85.34%, respectively. In scratch assay, rhodomyrtone at 2 and 4 mg/mL significantly delayed

closure of a wound by up to 61.78% and 71.65%, respectively, after 24 h incubation. HaCaT keratinocytes treated with rhodomyrtone showed chromatin condensation and fragmentation of nuclei when stained with Hoechst 33342. The results indicated that rhodomyrtone could induce apoptosis in the keratinocytes. In addition, flow cytometric analysis demonstrated an increase in the percentage of apoptosis of keratinocytes after treatment with rhodomyrtone at 2-32 µg/mL from 1.2-10%, 8.2-35.4%, and 21.0-77.8% after 24, 48, and 72 h, respectively, compared with the control. To further develop the compound as a potential anti-psoriasis agent, rhodomyrtone formulation was prepared and subjected to skin irritation tests in rabbits. The formulation caused no skin irritation such as erythema and edema. The results suggested that rhodomyrtone had the potential as a promising candidate for further development as a natural anti-psoriasis agent (*Year 1: Publication No. 1*).

The potential of the extract for further development as a bio-food supplement to prevent the incidence of *Listeria monocytogenes* contamination in food have been studied. Twenty-two *Listeria monocytogenes* isolates were checked with 16 commercial antibiotics and isolates displayed resistance to ten antibiotics. All the tested isolates were sensitive to the extract with inhibition zones ranging from 14-16 mm. Minimum inhibition concentration and minimum bactericidal concentration values ranged from 16-32 µg/mL and 128-512 µg/mL, respectively. Time-kill assay showed that the extract had remarkable bactericidal effects on *Listeria monocytogenes*. The extract at a concentration of 16 µg/mL reduced tolerance to 10% NaCl in *Listeria monocytogenes* in 4 h. Stationary phase of the bacterial cells were rapidly inactivated by greater than 3-log units within 30 min of contact time with *Rhodomyrtus tomentosa* extract at 128 µg/mL. Electron microscopy revealed fragmentary bacteria with changes in the physical and morphological properties (*Year 1: Publication No. 11*). For industrial applications, the use

of *Rhodomyrtus tomentosa* ethanolic leaf extract as a bio-control agent against *Listeria monocytogenes* in cooked chicken meat model system was carried out. The extract was observed as a potent bio-additive agent to control contaminations from *Listeria monocytogenes* and ensure safety in ready-to-eat meat. The antilisterial activity of the plant extract was better under microwave condition and enhanced as storage temperature increased from 4 to 37°C. The extract could reduce the bacterial numbers at low (10^4 CFU/g) and high (10^6 CFU/g) inoculum levels in cooked chicken by both rinse and injection application methods. A 5 min rinse in 8% w/v *Rhodomyrtus tomentosa* extract reduced the bacterial number by 2-log before storage and 3-log after storage at 4°C for 5 d. Injection with 0.4% w/w *Rhodomyrtus tomentosa* extract resulted in approximately 2-log reduction in the cell numbers both before and after storage at 4°C for 5 d. Five minute rinse in the extract bath demonstrated better sensory preferences which were not significantly different from the control. Addition of black pepper powder to the extract rinsed samples improved odour but not appearance, colour, and texture preferences (*Year 1: Publication No. 5*). '*Rhodomyrtus tomentosa* leaf extract: Formulation and Process' (*Thai Petty Patent 1503002121*) has been registered.

Other applications included use of *Rhodomyrtus tomentosa* extract as preventive methods in bovine mastitis and streptococcosis in fish of economic importance. Bovine mastitis is one of the most important infectious diseases in dairy herds. Antibiotics and chemical agents used in livestock for prevention and cure of the disease can accumulate in milk and give rise to food safety concerns. *Rhodomyrtus tomentosa* leaf extract was studied as an alternative approach for the treatment of staphylococcal bovine mastitis. Staphylococcal isolates (n=44) were isolated from milk samples freshly squeezed from individual cows. All staphylococcal isolates were resistant to ampicillin, ciprofloxacin, erythromycin, gentamicin, penicillin, except vancomycin. *Rhodomyrtus tomentosa* leaf ethanolic extract was assessed for its antibacterial activity and anti-inflammatory potential. The extract exhibited profound antibacterial activity against all of staphylococcal isolates with MIC and MBC ranged from 16-

64 μg/mL and 64-128 μg/mL, respectively. Moreover, the extract also exerted anti-protein denaturation and human red blood

cell membrane stabilizing activity. The results support the use of *Rhodomyrtus tomentosa* extract that could be applied to cure bovine mastitis and to reduce inflammatory injury caused by the bacterial infections (*Year 1: Publication No. 8*). To further extend the antibacterial efficacy,

silver nanoparticles synthesized with the extract, a pure rhodomyrtone, and liposomal encapsulated rhodomyrtone were applied and their inhibitory effects on bacterial adhesion and invasion were determined by *ex vivo* study in a bovine udder epidermal tissue model. These agents exerted remarkable antibacterial activity against staphylococci and decreased the adhesion of the bacterial cells to the tissues. These results supported that *Rhodomyrtus tomentosa* ethanolic extract could be applied as an alternative agent for bovine udder care in dairy farms (*Year 1: Publication No. 9*). Moreover, a petty patent on '*Rhodomyrtus tomentosa* leaf extract cream for bovine mastitis prevention' (*Thai Petty Patent No. 1603000119*) and 'Dairy cow teat

dipping formulation from *Rhodomyrtus tomentosa* leaf extract' (*Thai Petty Patent 1603001360*) have been registered.

In vitro and *in vivo* assessments of *Rhodomyrtus tomentosa* leaf extract as an alternative anti-streptococcal agent in Nile tilapia (*Oreochromis niloticus* L.) have been performed. The purpose was to evaluate the antibacterial properties of *Rhodomyrtus tomentosa* leaf extract against *Streptococcus agalactiae* and *Streptococcus iniae* isolated from infected tilapia. The anti-streptococcal activity of *Rhodomyrtus tomentosa* was determined using broth microdilution

assays. The extract demonstrated strong antibacterial activity against the fish pathogens, with MICs ranging from 7.8-62.5 μg/mL. It was found to possess a dose-dependent bacteriostatic

effect on this organism. Scanning electron microscopy revealed and long chains of swollen cells as well as corkscrew shapes and incomplete separation of cell division of *Streptococcus agalactiae* cells following the treatment at sub-MIC. Moreover, *Streptococcus agalactiae* cells pre-treated with the extract became more sensitive to oxidative stress induced by H_2O_2 than the untreated cells. Based on the mortality of Nile tilapia after intraperitoneal infection of *Streptococcus agalactiae* at median lethal dose, the pre-treated cells caused a significant ($P<0.01$) reduction in mortality of *Streptococcus agalactiae*-infected Nile tilapia. The results suggested that *Rhodomyrtus tomentosa* could be further developed as a simple and effective agent for the treatment of streptococcosis in Nile tilapia (*Year 2: Publication No. 7*).

Rhodomyrtus tomentosa is a medicinal plant that shows biological effects including immunomodulatory activity on human and other mammals. In this study, we evaluated *in vitro* effects of *Rhodomyrtus tomentosa* leaf extract and its active compound, rhodomyrtone, on the

immune responses, using rainbow trout (*Oncorhynchus mykiss*) head kidney macrophages as a model. The tested immune functions included the expression of genes involved in innate immune and inflammatory responses and the production of reactive oxygen species. Gene expression was evaluated after exposure to 10 μg/mL of *Rhodomyrtus tomentosa* and 1 μg/mL of rhodomyrtone for 4 and 24 h. *Rhodomyrtus tomentosa* and rhodomyrtone induced changes in the expression of pro-inflammatory cytokines (il1β, il8, and tnfα), anti-inflammatory cytokines (il10 and tgfβ), inducible en- zymes (inos, cox2, and arginase), and an antioxidant enzyme (gpx1). Co-exposure of *Rhodomyrtus tomentosa* with lipopolysaccharide (LPS) resulted in a prominent reduction in the expression of genes related to an inflammatory process (il1β, il8, tnfα, inos, saa, hepcidin, and gpx1), suggesting anti- inflammatory effects. Similarly, co-exposure of rhodomyrtone with LPS led to a downregulation of inflammation-related genes (il1β, inos, saa, and hepcidin). In addition, exposure to both natural plant products caused a reduction in cellular reactive oxygen species levels by head kidney macrophages. The present

results indicate that *Rhodomyrtus tomentosa* and rhodomyrtone exerted immunostimulatory and anti-inflammatory effects on fish macrophages, thus opening up the possibility of using these natural products to further develop immunostimulants for health management in aquaculture (*Year 3: Publication No. 6*).

The immunostimulatory effects of *Rhodomyrtus tomentosa* leaf extract were evaluated in rainbow trout through changes in expression profile of genes involved in innate immune and antioxidant response, haematology and stress indicators. The concentrations of *Rhodomyrtus tomentosa* at 10 and 100 µg per fish were administrated by intraperitoneal injection, alone or in combination with LPS. After 6 h of administration, the gene expression was measured in head kidney, spleen, and intestine. Results indicated that *Rhodomyrtus tomentosa* caused an increase in the expression of *il10*, *saa*, *hepcidin*, and *sod* in head kidney and the expression of *il10*, *tgfβ*, and *inos* in intestine. In combination with LPS, the plant suppressed the expression of pro-inflammtory cytokine *il1β*, *il8* and other consisting of *saa* and *gpx1* in head kidney and *il1β* in spleen, pointing out its anti-inflammatory activities. Furthermore, the plant did not exert any impact on hematological parameters, but it was able to reduce cortisol levels when co-administered with LPS, indicating that *Rhodomyrtus tomentosa* could attenuate stress response in rainbow trout. Our observations suggest that *Rhodomyrtus tomentosa* induced the expression of genes involved in cytokine and innate immune response and modulated the physiological stress response as indicated by the suppressed cortisol in rainbow trout (*Year 3: Publication No. 6*.

Eleuterine americana, another plant extract with promising antibacterial activity was applied as a food additive. Microencapsulation using extrusion and emulsion techniques was prepared for *Bifidobacterium longum* protection against sequential exposure to simulated

gastric and intestinal juices, refrigeration storage and heat treatment. *Eleutherine americana* was used as the co-encapsulating agent. Hydrolysis of *Eleuterine americana* by gastric and intestinal juices was also determined. *Eleuterine americana* and its oligosaccharide extract demonstrated their resistance to low pH and partial tolerance to human α-amylase. Microencapsulated *Bifidobacterium longum* with *Eleuterine americana* and oligosaccharide extract prepared by the extrusion technique survived better than that by the emulsion technique under adverse conditions. Survival of microencapsulated cells after exposure to the juices and refrigeration storage was higher than free cells at week 2 and 4. In addition, the viability of microencapsulated cells was better than free cells at 65°C for 15 min. This work suggested that microencapsulated *Bifidobacterium longum* with *Eleuterine americana* offers the effective delivery of probiotics to colon and maintains their survival in food products (*Year 1: Publication No. 12*).

To further study an application of natural extract as food additive, *Bifidobacterium longum* was microencapsulated by extrusion technique and added in fresh milk tofu and pineapple juice. Microencapsulation of *Bifidobacterium longum* with *Eleuterine americana* extract, oligosaccharides extract, and commercial fructo-oligosaccharides was assessed for the bacterial survival after sequential exposure to simulated gastric and intestinal juices, and refrigeration storage. Microencapsulated *Bifidobacterium longum* with the extract and oligosaccharides extract in the food products showed better survival than free cells under adverse

conditions. Sensory analysis demonstrated that the products containing co-encapsulated bacterial cells were more acceptable by consumers than free cells. Pineapple juice prepared with

co-encapsulated cells had lower values for over acidification, compared with the juice with free cells added. This work suggested that microencapsulated *Bifidobacterium longum* with

Eleuterine americana could enhance functional properties of fresh milk tofu (*Year 1: Publication No. 13*).

Study on the role of macrophage migration inhibitory factor in pathogenesis of tuberculosis

Tuberculosis, a severe infectious disease caused by *Mycobacterium tuberculosis*, remains a major global health problem. The role of macrophage migration inhibitory factor as an inflammatory mediator has been implicated in several diseases including an infectious disease such as tuberculosis. Several reports have demonstrated that macrophage migration inhibitory factor plays either a protective or deleterious role in the immune response to different pathogens. In order to gain a better understanding on the role of macrophage migration inhibitory factor, project on the role of macrophage migration inhibitory factor (MIF) as an upstream immunoregulatory and pro-inflammatory cytokine related to the progression of tuberculosis was conducted in collaboration with Kunming Medical University, China. We looked at a correlation

between the gene promoter polymorphism and susceptibility of individuals to active pulmonary tuberculosis. CATT short tandem repeat polymorphism at position -794 in the MIF gene promoter is associated with susceptibility to TB. The objective of this study was to study the relationship of *mif*-794 CATT polymorphism with new patients and retreatment cases of tuberculosis. Genotyping of *mif*-794 CATT polymorphism and quantifying of serum MIF were performed to associate *mif*-794 CATT polymorphism with new patients and retreatment cases. Significant increases in *mif* -794 CATT genotypes 7/8 and allele CATT 8 were observed in TB patients. Significant differences in the genotypic frequencies of *mif*-794 CATT (5/X + 6/X vs. 7/7 + 7/8) were demonstrated upon comparing the total cases and the new cases of TB with the controls. Significant differences in the allelic frequencies of *mif* -794 CATT (5 + 6 vs. 7 +

8) were observed in the total cases and new cases of TB. No differences in the genotypic frequencies of the *mif*-794 CATT (5/X + 6/X vs. 7/7 + 7/8) were observed between the retreatment cases and the controls or between the new cases and retreatment cases. The *mif* -794 CATT genotypes 7/8 and allele CATT 8 were highly associated with TB, no differences in the genotypic frequencies of the *mif* -794 CATT (5/X + 6/X vs. 7/7 + 7/8) were observed between the new cases and retreatment cases (*Year 3: Publication No. 5*).

Outputs

Published works in ISI จำนวน 36 *รายการ* (ภาคผนวก 1)

Year 3 จำนวน 8 *รายการ*

(1) Abdullah I, Phongpaichit S, **Voravuthikunchai SP**, Mahabusarakam W. 2018. Prenylated biflavonoids from the green branches of *Garcinia dulcis*. PHYTOCHEMISTRY LETTERS 23: 176-179. **JIF =1.148**, **Q 2**.

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(3)* Hmoteh J, Musthafa KS, **Voravuthikunchai SP**. 2018. Effects of *Rhodomyrtus tomentosa* extract on virulence factors of *Candida albicans* and human neutrophil function. ARCHIVES OF ORAL BIOLOGY 87: 35-42. **JIF = 1.748**, **Q 2**.

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Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). FISH PHYSIOLOGY AND BIOCHEMISTRY 44: 543-555. **JIF = 1.647**, **Q 2**.

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(8) Saeloh D, Tipmanee V, Dekker M, **Voravuthikunchai SP,** Wenzel M, Hamoen LW. 2018. The novel antibiotic rhodomyrtone traps membrane proteins in vesicles with increased fluidity. PLOS PATHOGENS 14: Art. No. e1006876 (1-35) **JIF = 6.608**, **Q 1**.

Year 2 จำนวน 15 *รายการ*

(1) Joycharat N, Issarachote P, Sontimuang C, **Voravuthikunchai SP**. 2018. Alpha-glucosidase inhibitory activity of ethanol extract, fractions, and purified compounds from the wood of *Albizia myriophylla*. NATURAL PRODUCT RESEARCH 32: 1291-1294. **JIF = 1.418**, **Q 2**.

(2)* Chokpaisarn J, Chusri, S, Amnuaikit T, Udomuksorn W, **Voravuthikunchai SP**. 2017. Potential wound healing activity of *Quercus infectoria* formulation in diabetic rats. PEERJ 5: e3608. **JIF = 2.177**, **Q 2**.

(3) Chokpaisarn J, Urao N, **Voravuthikunchai SP**, Koh TJ. 2017. *Quercus infectoria* inhibits set7/NF-ΚB inflammatory pathway in macrophages exposed to a diabetic environment. CYTOKINE 94: 29-36. **JIF = 3.488**, **Q 2**.

(4) Chusri S, Tongrod S, Saising J, Mordmuang A, Limsuwan S, Sanpinit S, **Voravuthikunchai SP**. 2017 .Antibacterial and anti-biofilm effects of a polyherbal formula and its constituents against coagulase-negative and -positive staphylococci isolated from bovine mastitis. JOURNAL OF APPLIED ANIMAL RESEARCH 45: 364-372. **JIF = 0.426**, **Q 4**.

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(15)* Wunnoo S, Saising J, **Voravuthikunchai SP**. 2017. Rhodomyrtone inhibits lipase production, biofilm formation, and disorganizes established biofilms in *Propionibacterium acnes*. ANAEROBE 43: 61-68. **JIF = 2.278**, **Q 3**.

Year 1 จำนวน 13 *รายการ*

(1)* Chorachoo J, Saeloh D, Srichana T, Amnuaikit T, Musthafa KS, Sretrirutchai S, **Voravuthikunchai SP**. 2016. Rhodomyrtone as a potential anti-proliferative and apoptosis inducing agent in HaCaT keratinocyte cells. EUROPEAN JOURNAL OF PHARMACOLOGY 772: 144-151. **JIF = 2.896**, **Q 2**.

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(4)* Musthafa KS, Mohtae J, Thamjarungwong B, **Voravuthikunchai SP**. 2016. Antifungal potential of eugenyl acetate against clinical isolates of *Candida albicans*. MICROBIAL PATHOGENESIS 99: 19-29. **JIF = 2.009**, **Q 3**.

(5)* Odedina GF, Vongkamjan K, **Voravuthikunchai SP**. 2016. Use of *Rhodomyrtus tomentosa* ethanolic leaf extract for the bio-control of *Listeria monocytogenes* post-cooking contamination in cooked chicken meat. JOURNAL OF FOOD SCIENCE AND TECHNOLOGY 53: 4234-4243. **JIF = 1.262**, **Q 3**.

(6) Saeloh D, Tipmanee V, **Voravuthikunchai SP**. 2016. Rhodomyrtone target exporation: Computer aided search on *Staphylococcus aureus* key proteins as a potential therapeutic target. CURRENT COMPUTER-AIDED DRUG DESIGN 12: 119-134. **JIF = 0.732**, **Q 4**.

(7)* Siriyong T, Chusri S, Srimanote P, Tipmanee V, **Voravuthikunchai SP**. 2016. *Holarrhena antidysenterica* and its steroidal alkaloid, conessine, as resistance modifying agents against extensively drug-resistant *Acinetobacter baumannii*. MICROBIAL DRUG RESISTANCE 22: 273-282. **JIF = 2.306**, **Q 3**.

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(10)* Musthafa KS, **Voravuthikunchai SP**. 2015. Anti-virulence potential of eugenyl acetate against pathogenic bacteria of medical importance. ANTONIE VAN LEEWENHOEK INTERNATIONAL JOURNAL OF GENERAL AND MOLECULAR MICROBIOLOGY 107: 703-710. **JIF = 1.795**, **Q 3**.

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(12)* Phoem AN, Chanthachum S, **Voravuthikunchai SP**. 2015. Preparation of *Eleuterine americana*-alginate complex microcapsules and application in *Bifidobacterium longum*. NUTRIENTS 7: 831-848. **JIF = 3.550**, **Q 2**.

(13)* Phoem AN, Chanthachum S, **Voravuthikunchai SP**. 2015 .Applications of microencapsulated *Bifidobacterium longum* with *Eleutherine americana* in fresh milk tofu and pineapple juice. NUTRIENTS 7: 2469-2484. **JIF = 3.550**, **Q 2**.

*** Voravuthikunchai SP** as corresponding author.

อนุสิทธิบัตร จำนวน7 รายการ (ภาคผนวก 2)

Year 3 จำนวน 1 รายการ

(1) ฉลองรัฐ แดงงาม สุภากิจ เภาเสน ศักรินทร์ เหล่าทองคำ **ศุภยางค์ วรวุฒิคุณชัย** กรรมวิธีการสังเคราะห์ชั้นเคลือบอนุภาคซิลเวอร์นาโนบนพื้นผิวท่อช่วยหายใจ
อนุสิทธิบัตรไทย เลขที่คำขอ 1803000673 วันยื่นคำขอ 20 มีนาคม 2561

Year 2 จำนวน 5 รายการ

(1) **ศุภยางค์ วรวุฒิคุณชัย** *สุทธิรัตน์ ศรีสุวรรณ*
สารเจือปนอาหารที่มีฤทธิ์ต้านแบคทีเรียและสปอร์จากกระทุ (โทะ)
อนุสิทธิบัตรไทย เลขที่คำขอ 1703000724 วันยื่นคำขอ 28 เมษายน 2560

(2)**ศุภยางค์ วรวุฒิคุณชัย** จุฬาลักษณ์ ช่อระชู ธนภร อำนวยกิจ สูตรตำรับโรโดไมรโทนที่มีฤทธิ์ต้านการอักเสบและกรรมวิธีการผลิต
อนุสิทธิบัตรไทย เลขที่คำขอ 1603002560 วันยื่นคำขอ 16 ธันวาคม 2559

(3) **ศุภยางค์ วรวุฒิคุณชัย** สุภากิจ เภาเสน สูตรองค์ประกอบของซิลเวอร์นาโนที่สังเคราะห์โดยใช้กรรมวิธีการสกัด
สารจากพืชวงศ์ชมพู่เป็นสารต้านจุลินทรีย์
อนุสิทธิบัตรไทย เลขที่คำขอ 1601005192 วันยื่นคำขอ 8 กันยายน 2559

(4) **ศุภยางค์ วรวุฒิคุณชัย** ธนภร อำนวยกิจ เอื้อมพร หมวดเมือง
สูตรองค์ประกอบน้ำยาจุ่มเต้านมโคที่มีสารสกัดใบกระทุเป็นองค์ประกอบ
อนุสิทธิบัตรไทย เลขที่คำขอ 1603001360 วันยื่นคำขอ 2 สิงหาคม 2559

(5) **ศุภยางค์ วรวุฒิคุณชัย** ธนภร อำนวยกิจ
สูตรผสมที่มีส่วนผสมของสารสกัดใบกระทุและกรรมวิธีการผลิต
อนุสิทธิบัตรไทย เลขที่ 12588)9 ธันวาคม 2558-8 ธันวาคม 2564)

Year 1 จำนวน 1 รายการ

(1) **ศุภยางค์ วรวุฒิคุณชัย** เอื้อมพร หมวดเมือง
สูตรองค์ประกอบครีมป้ายเต้านมโคที่มีสารสกัดใบกระทุเป็นองค์ประกอบ
อนุสิทธิบัตรไทย เลขที่คำขอ 1603000119 วันยื่นคำขอ 22 มกราคม 2559

หนังสือ จำนวน 1 รายการ

ศุภยางค์ วรวุฒิคุณชัย สุกัลญา หลีแจ้
สมุนไพรไทยต้านจุลินทรีย์ 2559
สำนักพิมพ์แห่งจุฬาลงกรณ์มหาวิทยาลัย 562 หน้า ISBN 978-974-03-3612-9.

บทความ จำนวน 5 รายการ (ภาคผนวก 3)

(1) **ศุภยางค์ วรวุฒิคุณชัย** เสาวรัตน์ เอื้อเพิ่มเกียรติ ธนภร อำนวยกิจ จงกล สายสิงห์ จุฬาลักษณ์ ช่อระชูและ สุทธิวรรณ วุ่นหนู 2559 ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน หนังสือลองแล…งานวิจัยใน ม.อ. หน้า 47-48

(2) **ศุภยางค์ วรวุฒิคุณชัย** และ ปิ่นอนงค์ ณ พัทลุง 2559
การศึกษาประสิทธิภาพของสารสกัดใบกระทุในสัตว์น้ำโดยใช้เซลล์มาโครเฟจที่สกัดจากไตส่วนต้นของปลาเทราต์สายรุ้ง
หนังสือลองแล…งานวิจัยใน ม.อ. หน้า 67-69

(3) **ศุภยางค์ วรวุฒิคุณชัย** จุฬาลักษณ์ โชคไพศาล ศศิธร ชูศรี และ ธนภร อำนวยกิจ 2559
ปูดเบญกานีสำหรับรักษาแผลเรื้อรังในผู้ป่วยเบาหวาน
หนังสือลองแล …งานวิจัย ในม.อ. หน้า 70-71

(4) **ศุภยางค์ วรวุฒิคุณชัย** เอื้อมพร หมวดเมือง อุษา เชษฐานนท์ และ ธนภร อำนวยกิจ 2559
สารสกัดใบกระทุ: ทางเลือกใหม่เพื่อการควบคุมโรคเต้านมอักเสบในฟาร์มโคนมอินทรีย์
หนังสือลองแล …งานวิจัย ในม.อ. หน้า 106-109

(5) สูตรตำรับยารักษาแผลติดเชื้อเรื้อรังจากสมุนไพรปูดเบญกานี 2559

Industry Focus April หน้า 25

การไปนำเสนอผลงานในที่ประชุมวิชาการโดยกลุ่มวิจัย จำนวน 37รายการ

Year 3 จำนวน 12 รายการ

(1) Saeloh D, Leeha M, Visutthi M, **Voravuthikunchai SP**. Efficacy of meropenam in combination with Myrtaceae extracts against extensively drug-resistant *Acinetobacter baumannii*. International Conference on Biomedical Sciences 2018, 22-23 March, 2018 at Walailak University, Nakhon Si Thammarat, Thailand.

(2) Saeloh D, Varomyalin T, **Voravuthikunchai SP**. Antibacterial mechanisms of rhodomyrtone against Gram-positive bacteria. The first National Conference on Health Sciences Research and Innovation, 7-8 December, 2017 at Mae Fah Luang, Phayao, Thailand.

(3) **Chorachoo J**, Paosen S, Voravuthikunchai SP. 2017. Green synthesis of silver nanoparticles as an antimicrobial agent using *Cassia alata* Linn. bark extract. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia.

(4) Daus M, **Chakthong S**. Acylphoroglucinols from the leaves of *Eucalyptus camaldulensis*. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia.

(5) Lethongkan S, Paosen S, **Voravuthikunchai SP**. 2017. Silver Nanoparticles synthesized from *Eucalyptus citriodora* Leaf extract inhibit biofilm of extended spectrum β-lactamase producing *Escherichia coli*. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia.

(6) Mitsuwan W, Wintachai P, **Voravuthikunchai SP**. 2017. Effects of *Rhodomyrtus tomentosa* leaf extract on *Streptococcus pneumoniae* adhesion and invasion to human

alveolar epithelial cell line A549. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at Selangor, Malaysia.

(7) Paosen S, **Voravuthikunchai SP**. 2017. Enhanced antibacterial activity of silver nanoparticles synthesized by *Eucalyptus citriodora* on *Escherichia coli* O157: H7. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia.

(8) Phetkul U, Hayiawae N, Khunthong S, Geok CS, **Chakthong S**. 2017. Lignans and phenylpropanoids from the roots of *Zanthoxylum nitidum*. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia.

(9) **Sianglum W**, Muangngam K, Joycharat N. 2017. Antibacterial activity and mechanisms of lupinifolin from *Albizia myriophylla* Benth. against enterococci and vancomycin-resistant enterococci. UKM-UR-UII-PSU Joint Seminar, 20-22 November, 2017 at UKM Selangor, Malaysia.

(10) Soontarach, Paosen S, **Voravuthikunchai SP**. 2017. Biosynthesis of silver nanoparticles using *Eucalyptus citriodora* Hook. leaf extract and its antibacterial activity against clinical isolates of *Enterococcus* spp. and *Klebsiella pneumoniae*. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia.

(11) **Wintachai P**, Voravuthikunchai SP. 2017. Antibacterial activity and cytotoxicity of phenolic compounds against hospital-acquired infections. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at Selangor, Malaysia.

(12) Wunnoo S, Amnuaikit T, Auepemkiate S, **Voravuthikunchai SP**. 2017. A randomized, double-blind clinical trial for topical treatment of acne vulgaris: the comparison among three groups of 1% liposomal encapsulated rhodomyrtone serum, marketing product of 1% clindamycin gel, and placebo. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at Selangor, Malaysia.

Year 2 จำนวน 8 รายการ

(1) **Joycharat N**, Issarachote P, Sianglum W, Voravuthikunchai SP. 2017. Bioactive prenylated flavanones from *Derris reticulate* Craib. 18th Tetrahedron Symposium Asia Edition, Melbourne, Australia, 24-26 July 2017.

(2) Mordmuang A, Brouillette E, **Voravuthikunchai SP,** Malouin F. 2017. Potential of *Rhodomyrtus tomentosa* and rhodomyrtone against *Staphylococcus aureus* infection in mastitis model. FEMS Microbiology Congress, Valencia, Spain, 3-7 July 2017.

(3) Srisuwan S, MacKin KE, Hocking D, Lyras D, **Voravuthikunchai SP,** Robins-Browne R. 2017. Effects of rhodomyrtone on hypervirulent *Clostridium difficile* strain. International Consortium Prevention & Infection Control, Geneva, Switzerland, 20-23 June 2017.

(4) Siriyong T, Coote PJ, **Voravuthikunchai SP**, 2017. Interaction of conessine with resistance-nodulation-division efflux pumps in multidrug-resistant *Pseudomonas aeruginosa* assessed by Hoechst 33342 accumulation. 15th International Pharmaceutical Microbiology and Biotechnology Conference, London, United Kingdom, 21-23 June 2017.

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(6) Chorachoo J, Lambert S, Wolterink L, Reingold L, .**SP Voravuthikunchai**Johnston A. 2017 .Rhodomyrtone suppresses TNF-α and IL-17A-induced keratinocyte inflammatory

responses: A potential therapeutic for psoriasis. Society for Investigative Dermatology Annual Meeting, Portland, Oregon, United States, 26-29 May 2017.

(7) Wenzel M, Saeloh D, Tipmanee V, **Voravuthikunchai SP,** Hamoen LW. 2017. The new antibiotic rhodomyrtone interacts with phospholipid head groups to generate protein-trapping membrane invaginations. 69th Annual Meeting on the DGHM, Wurzburg, Germany, 7 March 2017.

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Year 1 จำนวน 17 รายการ

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(2) **Tipmanee V**. Computer-aided apparatus in biomedical research. The 3rd Joint Symposium BMS-BME-EU ณ คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ระหว่างวันที่ 30-31 พฤษภาคม 2559

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(8) **Sianglum W.** Antibacterial activity of lupinifolin against enterococci from clinical isolates and characterization of virulence factors. The 6th International Conference on Natural Products for Health and Beauty at Pullman Raja Orchid Hotel, Khon Kaen, Thailand, 21-23 January, 2016.

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(10) **Limsuwan S**. Anti-biofilm and anti-quorum sensing activity of Thai herbal formula on *Streptococcus mutans*. The 1st National Conference in Traditional Thai Medicine at Hansa JB Hotel, Songkhla, Thailand, 23-25 December 2015.

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(12) **Chakthong S**, Madmanang S, Siseng H. Chemical constituents from the twigs of *Feronia limonia*. International Symposium of the Korean Society for Applied Biological Chemistry, 17-19, August 2015 at Pyeongchang, Korea.

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(15) **Musthafa KS**, Saising J, Voravuthikunchai SP. 2015. Anti-quorum sensing potential of certain phytochemicals against *Pseudomonas aeruginosa*. International Symposium of the Korean Society for Applied Biological Chemistry at Seoul National University, Pyeongchang, Korea, 17-19 August 2015.

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การได้รับเชิญไปเป็นวิทยากร

Year 3

(1) **ศุภยางค์ วรวุฒิคุณชัย** นักวิจัยพี่เลี้ยง: เข็มทิศชีวิตนักวิจัย โครงการฝ่ายวิชาการ สกว. สัญจร ครั้งที่ 2 ณ โรงแรมสยาม ออเรียนทัล สงขลา วันที่ 8-9 พฤษภาคม 2561

(2) **Voravuthikunchai SP.** 2018. Intellectual Properties: Top quality natural products from *Rhodomyrtus tomentosa* extract and rhodomyrtone. International Conference on Biomedical Sciences, 22-23 March, 2018 at Walailak University, Nakon Si Thammarat, Thailand.

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Year 2

(1) **ศุภยางค์ วรวุฒิคุณชัย** การอบรมเชิงปฏิบัติการในการเขียนผลงานตีพิมพ์ ในวารสาร

วิชาการระดับนานาชาติ คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏสงขลา ณ บ้านเคียงเขา รีสอร์ท หมู่บ้านคีรีวง นครศรีธรรมราช ระหว่างวันที่ 30 กรกฎาคม-1 สิงหาคม 2560

(2) **ศุภยางค์ วรวุฒิคุณชัย** จรรยาบรรณการเขียนผลงานและเทคนิคการเขียนเอกสารประกอบการสอน/เอกสารคำสอน วันที่ 25 พฤษภาคม พ.ศ. 2560 ณ มหาวิทยาลัยสงขลานครินทร์ จังหวัดสงขลา

(3) **Voravuthikunchai SP**. Natural compound: from basic research to pharmaceutical applications. 2nd International Conference on Herbal and Traditional Medicine Asia Hotel, Bangkok 24-27 January2017.

(4) **ศุภยางค์ วรวุฒิคุณชัย** เทคนิคการตีพิมพ์ในวารสารวิชาการระดับนานาชาติ ครั้งที่ 1 ณ คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏสงขลา วันที่ 4 สิงหาคม 2559

(5) **ศุภยางค์ วรวุฒิคุณชัย** การอบรมเชิงปฏิบัติการในการเขียนผลงานตีพิมพ์ในวารสารวิชาการระดับนานาชาติ ณ สถาบันวิจัยและพัฒนา มหาวิทยาลัยทักษิณ ระหว่างวันที่ 26-27 กรกฎาคม 2559

(6) **ศุภยางค์ วรวุฒิคุณชัย** การอบรมเชิงปฏิบัติการในการเขียนข้อเสนอโครงการและบทความทางวิชาการ สาขาวิทยาศาสตร์และเทคโนโลยี ฝ่ายวิชาการ สกว. สัญจร ครั้งที่ 4 ณ มหาวิทยาลัยสงขลานครินทร์ วันที่ 13 กรกฎาคม 2559

(7) **Voravuthikunchai SP**. Rhodomyrtone: From basic research to pharmaceutical applications. The 2nd CRS International Congress: From Medicinal Chemistry-Natural Products to Pharmaceutics & Pharmaceutics & Pharmacokinetics/Pharmacodynamics at Aegli Zappeio, Athens, Greece, 22-24 June 2016.

(8) **Voravuthikunchai SP**. Application of *Quercus infectoria* gall extract to cure chronic infected wound in diabetic patients. International Symposium of the Korean Society for Applied Biological Chemistry at ICC JEJU, Jeju Island, Korea, 16-17 June 2016.

(9) **Voravuthikunchai SP**. *Rhodomyrtone*: from basic research to applications. The 6th International Conference on Natural Products for Health and Beauty at Pullman Raja Orchid Hotel, Khon Kaen, 21-23 January 2016.

(10) **Voravuthikunchai SP**. The discovery of rhodomyrtone, a new antibiotic ในการประชุมนักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 15 ณ โรงแรมเดอะรีเจนท์ ชะอำบีช รีสอร์ท หัวหิน ชะอำ จังหวัดเพชรบุรี ระหว่างวันที่ 6-8 มกราคม 2559

(11) **ศุภยางค์ วรวุฒิคุณชัย** เทคนิคการตีพิมพ์ในวารสารวิชาการระดับนานาชาติ ณ สถาบันวิจัยและพัฒนา มหาวิทยาลัยทักษิณ ระหว่างวันที่ 16-17 กันยายน 2558

Year 1

(1) **Voravuthikunchai SP**. *Rhodomyrtus tomentosa* (Aiton) Hassk: from basic research to applications 2015 International Symposium of the Korean Society for Applied Biological Chemistry at Seoul National University, Pyeongchang, Korea, 17-19 August 2015.

(2) **ศุภยางค์ วรวุฒิคุณชัย** การประยุกต์ใช้ประโยชน์จากสารธรรมชาติทางการแพทย์ ณ คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ วันที่ 11 สิงหาคม 2558

การจัดการประชุมและอบรม

(1) การประชุมวิชาการ โครงการทุนเมธีวิจัยอาวุโส สกว .ประจำปี พ.ศ. 2561 เรื่อง ‘แผนงานวิจัยแบบมุ่งเป้าเพื่อแก้ปัญหาการติดเชื้อดื้อยาปฏิชีวนะ’ ณ ห้องประชุมคณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

ในวันที่ 13 กรกฎาคม 256180 ผู้เข้าร่วมประชุมประมาณ) คน(

(2) 'เครื่องมือออนไลน์สำหรับการเขียนวิชาการ' บรรยายพิเศษโดยวิทยากรรับเชิญ ดร. วรมย์ญลิน ทิพย์มณี ภาควิชาชีวเวชศาสตร์ คณะแพทยศาสตร์ ระหว่างวันที่ 9-10 สิงหาคม 2560 ณ อาคารวิทยาศาสตร์และเทคโนโลยี .วท)504) คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

(3) การประชุมวิชาการ โครงการทุนเมธีวิจัยอาวุโส สกว .ประจำปี พ.ศ. 2560 เรื่อง 'แผนงานวิจัยแบบมุ่งเป้าเพื่อแก้ปัญหาการติดเชื้อดื้อยาปฏิชีวนะ' ณ ห้องประชุมคณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ในวันที่ 16 พฤษภาคม 2560 50 ผู้เข้าร่วมประชุมประมาณ) คน(

(4) 'Stress and immunostimulation in farmed fish: A cellular approach' บรรยายพิเศษโดยวิทยากรชาวต่างประเทศ Dr. Camino Fierro-Castro, Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Spain ในวันที่ 15 พฤษภาคม 2560 ณ อาคารวิทยาศาสตร์และเทคโนโลยี .วท)504) คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

(5) ' Lessons learnt from the23Annual ASM Conference for Undergraduate Educators rd, value and build up Happy class experiences to motivate learning21 century skills st บรรยายพิเศษโดยวิทยากรรับเชิญ ผู้ช่วยศาสตราจารย์ ดร.กนกวรรณ กิตตินิยม ภาควิชาจุลชีววิทยา มหาวิทยาลัยมหิดล ณ ณ อาคารวิทยาศาสตร์และเทคโนโลยี .วท) 504) คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ในวันที่1 7 มกราคม 2560

(6) 'Flavonoid biotransformation by human intestinal bacteria' บรรยายพิเศษโดย

วิทยากรชาวต่างประเทศProfessor Dr . Jaehong Han, Department of Integrative Plant Science, Chung- Ang Universityณ อาคารวิทยาศาสตร์และเทคโนโลยี .วท)504) คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ในวันที่1 7 มกราคม 2560

(7) 'Manipulating macrophages to improve wound healing in diabetes' บรรยายพิเศษโดยวิทยากรชาวต่างประเทศProfessor Dr . Timothy Koh, Department of Kinesiology and Nutrition, iversity of Illinois at ChicagoUn ณ อาคารวิทยาศาสตร์และเทคโนโลยี .วท)504) คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ในวันที่ 21 ธันวาคม 2559

(8) 'The Microbiome and Psoriasis' บรรยายพิเศษโดยวิทยากรชาวต่างประเทศ Professor Dr. drew JohnstonnA, Department of Dermatology, University of Michigan ณ อาคารวิทยาศาสตร์และเทคโนโลยี .วท)504) คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ในวันที่ 7 ธันวาคม 2559

(9) การประชุมวิชาการ โครงการทุนเมธีวิจัยอาวุโส สกว .ประจำปี พ.ศ. 2559 เรื่อง 'แผนงานวิจัยแบบมุ่งเป้าเพื่อแก้ปัญหาการติดเชื้อดื้อยาปฏิชีวนะ' ณ อาคารศูนย์ทรัพยากรการเรียนรู้ มหาวิทยาลัยสงขลานครินทร์ ในวันที่ 19 กันยายน 2559) ผู้เข้าร่วมประชุมประมาณ 100 คน(

(10) การจัดกิจกรรมวิชาการ ค่ายวิทยาศาสตร์ภาคฤดูร้อน (ระดับอุดมศึกษา) ครั้งที่ 30 โครงการส่งเสริมผู้มีความรู้ความสามารถพิเศษทางวิทยาศาสตร์และเทคโนโลยี ประจำปี พ.ศ. 2559 เรื่อง 'ผลิตภัณฑ์ธรรมชาติ' ณ สถานวิจัยความเป็นเลิศด้านผลิตภัณฑ์ธรรมชาติ มหาวิทยาลัยสงขลานครินทร์ วันที่ 4 กรกฎาคม 2559) ผู้เข้าประชุม 60 คน(

(11) 'Multifunctional nanomaterial ultra thin film : fabrication characterization and application' บรรยายพิเศษโดยวิทยากรรับเชิญ ดร.ฉลองรัฐ แดงงาม ภาควิชาฟิสิกส์ คณะวิทยาศาสตร์ ในวันที่ 11 มีนาคม 2559 ห้องสมุดภาควิชาจุลชีววิทยา .วท)504) อาคารวิทยาศาสตร์และเทคโนโลยี คณะวิทยาศาสตร์

(12) TRF Seminar Series in Basic Research ณ มหาวิทยาลัยสงขลานครินทร์ ในวันที่ 25 กันยายน 2558) ผู้เข้าร่วมประชุม 200 คน(

กิจกรรมที่เกี่ยวข้องกับการนำผลงานจากโครงการไปใช้ประโยชน์

(1) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน: ซิลเวอร์นาโนโกลฟ์สำหรับใช้ทางการแพทย์ งานมหกรรมแสดงสินค้าและประชุมทางวิชาการด้าน**ยางพารา** (Natural Rubber Innovation Expo 2018) มหาวิทยาลัยสงขลานครินทร์ และอุทยานวิทยาศาสตร์ภาคใต้ และสถาบันวิจัยและ**นวัตกรรมยางพารา** ณ โรงแรมสยามธานี จังหวัด**สุราษฎร์ธานี** ระหว่างวันที่ 3-5 พฤษภาคม 2561

(2) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน: ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน Spark STSP innovation Fair 2018 เปิดบ้านนวัตกรรมนำธุรกิจไทยสู่ยุค 4.0 อุทธยานวิทยาศาสตร์ภาคใต้และกระทรวงวิทยาศาสตร์และเทคโนโลยี ณ ศูนย์การค้าเซ็นทรัลเฟสติวัล หาดใหญ่ ระหว่างวันที่ 27-29 เมษายน 2561

(3) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-ผลิตภัณฑ์จุ่มเต้านมโคนมจากสารสกัดใบกระทุสำหรับป้องกันโรคเต้านมโคอักเสบ
-อาหารเสริมสุขภาพปลาเพาะเลี้ยงที่มีส่วนผสมของสารสกัดใบกระทุเพื่อใช้เป็นอาหารเสริมภูมิคุ้มกันต่อการติดเชื้อ
-ถุงมือซิลเวอร์นาโน
-ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน
มหกรรมงานวิจัยส่วนภูมิภาค ประจำปี 2561: ภาคใต้ (Regional Research Expo 2018) สำนักงานคณะกรรมการวิจัยแห่งชาติ ณ มหาวิทยาลัยเทคโนโลยีราชมงคล**ศรีวิชัย** จังหวัด**สงขลา** ระหว่างวันที่ 22-24 มีนาคม 2561

)4(**ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-ผลิตภัณฑ์จุ่มเต้านมโคนมจากสารสกัดใบกระทุสำหรับป้องกันโรคเต้านมโคนมอักเสบ
-อาหารเสริมสุขภาพปลาเพาะเลี้ยงที่มีส่วนผสมของสารสกัดใบกระทุเพื่อใช้เป็นอาหารเสริมภูมิคุ้มกันต่อการติดเชื้อ
-ถุงมือซิลเวอร์นาโน
-ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน
งานวันนักประดิษฐ์ สำนักงานคณะกรรมการวิจัยแห่งชาติ ณ ไบเทค บางนา ระหว่างวันที่ 2-6 กุมภาพันธ์ 2561

)5(**ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-ผลิตภัณฑ์จุ่มเต้านมจากสารสกัดธรรมชาติ
-อาหารเสริมสุขภาพปลาเพาะเลี้ยงจากสารสกัด
-ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน
บูธผลงานที่ได้จดอนุสิทธิบัตรและพร้อมนำไปใช้ประโยชน์เชิงพาณิชย์ Idea Market การประชุม TRG- OHEC Annual Congress2018 ณ โรงแรมเดอะรีเจนท์ชะอำบีชรีสอร์ท เพชรบุรี ระหว่างวันที่1 0-12 มกราคม 2561

(6) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-ผลิตภัณฑ์จุ่มเต้านมโคนมจากสารสกัดใบกระทุสำหรับป้องกันโรคเต้านมโคนมอักเสบ
-อาหารเสริมสุขภาพปลาเพาะเลี้ยงที่มีส่วนผสมของสารสกัดใบกระทุเพื่อใช้เป็นอาหารเสริมภูมิคุ้มกันต่อการติดเชื้อ
- ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน
บูธผลงานที่ได้จดอนุสิทธิบัตรและพร้อมนำไปใช้ประโยชน์เชิงพาณิชย์
งาน 25 ปี **สกว**.: สร้างคน สร้างความรู้ สร้างอนาคต ณ รอยัล พารากอน ฮอลล์ 2 **สยามพารากอน** ระหว่างวันที่ 25-26 สิงหาคม 2560

(7) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-ผลิตภัณฑ์จุ่มเต้านมจากสารสกัดธรรมชาติ
-สูตรตำรับปูดเบญกานีสำหรับรักษาแผลในผู้ป่วยเบาหวาน
-อาหารเสริมสุขภาพปลาเพาะเลี้ยงจากสารสกัด
-ครีมรักษารอยโรคสะเก็ดเงิน
-ถุงมือซิลเวอร์นาโน
-ซีรัมแต้มสิวไลโปโซมโรโดไมรโทน
บูธผลงานที่ได้จดอนุสิทธิบัตรและพร้อมนำไปใช้ประโยชน์เชิงพาณิชย์ Idea Market

การประชุม TRG- OHEC Annual Congress2017 ณ โรงแรมเดอะรีเจนท์ชะอำบีชรีสอร์ท เพชรบุรี ระหว่างวันที่1 1-13 มกราคม 2560

(1) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-ผลิตภัณฑ์ AcneClear
-ผลิตภัณฑ์จากปูดเบญกานีสำหรับแผลเบาหวาน
งาน ม.อ. วิชาการ ณ ศูนย์ประชุมนานาชาติ ม.อ. ระหว่างวันที่ 17-18 สิงหาคม 2559

)9(**Voravuthikunchai SP**. et al.
-'*Chronicare*' for chronic diabetic wounds
-Potential use of *Rhodomyrtus tomentosa* leave extract for prevention and control of bovine mastitis
1st Joint Seminar in Science and Technology for ASEAN Community' PSU Basic Science Building, 1-2 August 2016.

(10) **ศุภยางค์ วรวุฒิคุณชัย** สูตรตำรับยารักษาแผลติดเชื้อเรื้อรังจากสมุนไพรปูดเบญกานี 2559 Industry Focus April หน้า 25

(11) **ศุภยางค์ วรวุฒิคุณชัย** สมุนไพรปูดเบญกานี สำหรับรักษาแผลติดเชื้อเรื้อรังในผู้ป่วยเบาหวาน รายการเส้นทางทำกิน ออกอากาศทางสถานีวิทยุศึกษา FM 92 และ AM 1161 วันที่ 9 มีนาคม 2559

(12) **Voravuthikunchai SP**. et al. Rhodomyrtone AcneClear-Best Quality Anti-Acnes Herbal Product' Brussels Innova 2015 at Brussels Exhibition Center, Brussels, Belgium, 19-21 November 2015.

(13) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน:
-Rhodomyrtone AcneClear-Best Quality Anti-Acnes Herbal Product
-อาหารเสริมสุขภาพปลาเพาะเลี้ยงที่มีส่วนผสมของสารสกัดใบกระทุเพื่อใช้เป็นอาหารเสริมภูมิคุ้มกันต่อการติดเชื้อ

มหกรรมนวัตกรรมไทยภาคใต้ (Southern Innovation Expo 2015)' กระทรวงวิทยาศาสตร์และเทคโนโลยี ณ หาดใหญ่ฮอลล์ ศูนย์การค้าเซ็นทรัลเฟสติวัล หาดใหญ่ ระหว่างวันที่ 30-31 กันยายน 2558

(14) **ศุภยางค์ วรวุฒิคุณชัย** และคณะ ผลงาน: Rhodomyrtone AcneClear-Best Quality Anti-Acnes Herbal Product งาน Technology Show' สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ ณ โรงแรมเดอะ สุโกศล กรุงเทพมหานคร วันที่ 15 กันยายน 2558

การเชื่อมโยงกับต่างประเทศ

(1) Professor Dr. Freidrich Gotz, Tubingen University, Germany.

(2) Professor Dr. Manuel J. Rodríguez-Ortega, Universidad de Córdoba, Spain.

(3) Professor Dr. Royle Robins-Browne, The University of Melbourne, Australia.

(4) Professor Dr. François Malouin, The University of Sherbrooke, Canada.

(5) Professor Dr. Prof. Dr. Leendert Hamoen, Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands.

(6) Professor Dr. Lluis Tort, Dr. Maria Camino Fierro-Castro, Universitat Autonoma de Barcelona, Spain

(collaboration (1)-(6) in order to fulfill objective: To study rhodomyrtone as a novel antibiotic candidate).

(7) Professor Dr. Timothy Koh, The University of Illinois, USA (in order to fulfill objective: To apply natural products for control and treatment of diseases).

(8) Dr. Peter Coote, University of St. Andrews, Scotland (in order to fulfill objective: To search for resistance modifying agents with specific focus on Gram-negative bacteria and investigate possible modes of action).

(9) Professor Dr. Fukai Bao (in order to fulfill objective: To study the role of macrophage migration inhibitory factor in pathogenesis of tuberculosis).

รางวัลที่ได้รับ

(1) Leaders in Innovation Fellowship Royal Academy of Engineering, UK ประจำปี2561

(2) บุคลากรดีเด่น คณะวิทยาศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ประจำปี2560

(3) สตรีตัวอย่างแห่งปี สาขาวิจัยและพัฒนา คณะกรรมการมูลนิธิเพื่อสังคมไทย 2559

(4) อาจารย์ดีเด่นแห่งชาติ พ 2559 .ศ.สาขาวิทยาศาสตร์เทคโนโลยี ที่ประชุมประธานสภาอาจารย์มหาวิทยาลัยแห่งประเทศไทย

(5) **สุภากิจ เภาเสน** ซิลเวอร์นาโนโกลฟ์สำหรับใช้ทางการแพทย์ ‘งานมหกรรมแสดงสินค้าและประชุมทางวิชาการด้าน**ยางพารา** (Natural Rubber Innovation Expo 2018)’ มหาวิทยาลัยสงขลานครินทร์ อุทยานวิทยาศาสตร์ภาคใต้ และสถาบันวิจัยและ**นวัตกรรมยางพารา** ณ โรงแรมสยามธานี จังหวัด**สุราษฎร์ธานี**
รางวัลชมเชย (อาจารย์ที่ปรึกษาวิทยานิพนธ์ระดับปริญญาเอก)

(6) **Paosen S**, Voravuthikunchai SP. 2017. Enhanced antibacterial activity of silver nanoparticles synthesized by *Eucalyptus citriodora* on *Escherichia coli* O157: H7. UKM-UR-UII-PSU Joint Seminar 2017, 20-22 November, 2017 at UKM Selangor, Malaysia. Best Poster Award (อาจารย์ที่ปรึกษาวิทยานิพนธ์ระดับปริญญาเอก)

(7) **Saeloh D**, Varomyalin T, Voravuthikunchai SP. 2017. Antibacterial mechanisms of rhodomyrtone against Gram-positive bacteria. The first National Conference on Health Sciences Research and Innovation, 7-8 December, 2017 at Mae Fah Luang, Phayao, Thailand. รางวัลการนำเสนอผลงานวิจัยด้วยวาจาระดับดีมาก (อาจารย์ที่ปรึกษาวิทยานิพนธ์ระดับปริญญาเอก)

(8) **Sutthirat S**, Voravuthikunchai SP. 2017. Effects of rhodomyrtone on hypervirulent *Clostridium difficile* strain. International Consortium Prevention & Infection Control, Geneva, Switzerland, 2017. International Award (อาจารย์ที่ปรึกษาวิทยานิพนธ์ระดับปริญญาเอก)

(9) **จุพาลักษณ์ ช่อระชู** ศักยภาพของสารโรโดไมรโทนจากใบกระทุในการรักษารอยโรคสะเก็ดเงิน รางวัลวิทยานิพนธ์ชมเชย ระดับปริญญาเอก ประจำปี 2559 (อาจารย์ที่ปรึกษาวิทยานิพนธ์)

(10) **เอื้อมพร หมวดเมือง** ประสิทธิภาพของสารสกัดจากใบกระทุและการประยุกต์ใช้ในการป้องกันและควบคุมโรคเต้านมอักเสบในโคนม รางวัลวิทยานิพนธ์ชมเชย ระดับปริญญาเอก ประจำปี 2559 (อาจารย์ที่ปรึกษาวิทยานิพนธ์)

(11) **สุทธิวรรณ วุ่นหนู** การศึกษาศักยภาพและประยุกต์ใช้ทางคลินิกของสารบริสุทธิ์ rhodomyrtone สำหรับรักษาสิว รางวัลวิทยานิพนธ์เกียรติคุณ ระดับปริญญาโท ประจำปี 2559 (อาจารย์ที่ปรึกษาวิทยานิพนธ์)