News

Student Research:

"Innovative research on shampoo and dishwashing detergent made from Kapok leaves"

A team of six students was recently awarded first place in the *"Our Business"* competition by His Excellency Dr Hang Chuon Naron, the Minister for Education, Youth and Sport, which included a prize of 1,000 USD plus a gold medal and certificate for each member. The team comprised four senior female students: KEM Tha, KAN Superjaria, KHENG Lada, and KHEUN Chenda from the Department of Bio-Engineering at the Royal University of Phnom Penh (RUPP); as well as two other senior female students: JHON Kaly from the National University of Management (majoring in Accounting), and RETH Panha from Institute of Foreign Language at the RUPP (majoring in Thai literature), both with a scholarship from the Harpswell Foundation. Eighteen teams entered, which was narrowed down to fifteen in the first round of the competition. Of the five finalists, only three teams were selected.

The team designed a factory for producing dishwashing detergent from kapok leaves. They decided to enter the *"Our Business"* because they believed their product was innovative, environmentally friendly, and had the potential to obtain a large market share both in Cambodia and overseas. Each team member invested 100 USD of their own funds in the project, using techniques learned at the Department of BioEngineering. The experiment was conducted solely by the six students. While the four students from the Department of BioEngineering worked on the production of the detergent; the two other students worked on budgeting, planning and market research.

The team demonstrated their commitment to each other by writing a proposal to enter the competition. From the outset, win or lose, they were determined to take advantage of the high demand for dishwashing detergent and successfully realise their dream of running their own business.

The project is innovative in its use of kapok leaves as an active ingredient in dishwashing detergent to improve its function as a detergent, while also being gentle on the skin of users. Their target market includes street food vendors, restaurants, and retailers in both rural and urban markets. The team were surprised that they were awarded first place, as it was the first time they had entered a competition of this nature. To enable to team to continue with their innovative research, His Excellency Dr Chet Chealy, the Rector of RUPP granted 1867.5 USD to the four students from the Department of BioEngineering. This grant will be managed by lecturers Dr TIENG Siteng and Dr LONG Solida to investigate how the properties of dishwashing detergents made from Kapok leaves may be improved, as well as how shampoo and liquid body soap products may be formulated.

The four students will work with the grant managers to further develop the concept and introduce these products to the market. The development team will run four simultaneous activities including product improvement, assessing the physical and chemical properties of kapok leaves, the development of a liquid body soap product, and the development of a shampoo product. These activities are expected to be completed by August 2021. The research will be published by the Department of Bioengineering in *Insight: Cambodia Journal of Basic and Applied Research*.

Faculty Research:
"The Approval of Higher Education Improvement Project (HEIP) Research Project Round II"

The 90.0 million USD Higher Education Improvement Project (HEIP) funded by IDA Credit, including a 2.5 million USD contribution from the Royal Government of Cambodia is being implemented over 6 years between July 2018 and June 2024. The objective is to improve the quality and relevance of higher education and research in the STEM and agricultural fields at target Higher Education Institutions (HEIs) in Cambodia, as well as to improve governance of the sector as a whole. As part of Component 2 of the project, *"Improving Research in STEM and Agriculture"*, the Royal University of Phnom Penh has been granted 14 projects with a total amount of 3.3 million USD to assist local academics to conduct and publish research in international peer-reviewed journals.

These publication are part of realizing the vision of RUPP to become Cambodia's leading university in applied and academic research, as stated in the Policy on Research Development and Innovation. Research proposals must fall into one of three windows: (i) research for industry; (ii) research for policy-making; or (iii) frontier research. In early 2021, six research projects across different disciplines, with a total budget of 2,455,810.31 USD on top of the eight research projects approved in early 2020.

Research Project 1

Research Topic: Synthesis, characterization, device fabrication, and the application of nanomaterials and 2-Dimensional materials

Duration of the Sub-project: 3 years

Total Cost of the Sub-project: 1,050,365.75 USD

Research Team: Dr SRIV Tharith (Principal investigator), Dr SOUM Veasna, Dr PECH Ouksaphea, Dr KHUN Kimleang, Dr CHEY Chan Oeurn, Dr SOU Kalyan, Mr YANN Rem, Miss. CHUONG Mary, Dr KHAN Sovann, Prof. Dr CHEON Hyeonsik, and Prof. Dr SHIN Kwanwoo

Nano- and 2D materials have attracted interest among the scientific community because of the suitability of their properties for technological applications in health care, thin-film solar cells, analytical sensors, and optoelectronics. An enhanced understanding of the properties of these materials is expected to also lead to other useful applications. In this project, nanomaterials will be synthesized using hydrothermal methods and thermal treatment to investigate the properties of AgNP, CNT, and ZnO nanoparticles, transition metal oxides (ZO_x) (Z = Mn, Fe, Ni, Mo, etc.), electrode materials, as well as 2D materials such as tin chalcogenides, transition metal di-chalcogenides and alloys (MoS_2, WSSe, MoSSe), and As_2Te_3, including phosphorous-arsenic alloy (P-As).

The methods used to analyze the nano- and 2D-materials include X-ray diffraction for structural analysis; UV-visible spectroscopy for bandgap analysis; AutoLab for electrochemical analysis, energy-dispersive x-ray spectroscopy (EDS/EDX) for chemical microanalysis; x-ray photoelectron spectroscopy (XPS) for elemental composition analysis; scanning electron microscopy (SEM) for micro-surface topology; atomic force microscopy (AFM) for micro and nanostructural analysis and determining the thickness

of the 2D materials, as well as Raman and photoluminescence (PL) spectroscopy. The UV-visible spectra, XRD patterns, Raman, PL and electrochemical analyses will be conducted locally using the existing facilities of the Laboratory of Applied Nanotechnology (LAN), and the some to-be-established Advanced Spectroscopy and Electronic Printing Technology Lab. The remaining experiments will be performed in the laboratories of foreign collaborators in Korea, Japan and Thailand.

OriginPro and CrystalMaker software will be used to support advanced scientific analyses to draw crystal structures for publication. The second part of the research will focus on printing techniques using well-understood materials to improve the fabrication of solar cells and analytical sensors. Specifically, the fabrication of glucose and iodide detection sensors and two corresponding sample sensor products will be registered and developed. The synthesis, characterization, and prototyping of these devices as part of this research are likely to result in higher quality, better performing and lower-cost devices that may contribute to the development of local industries similar to Thaung Enterprises. Scaling up production will be considered to improve the sustainability of the project.

Research Project 2

Research Topic: Analyzing bioactive compounds of marine algae to develop moisturizing, hyperpigmentation and anti-aging cosmeceutical creams or lotions

Duration of the Sub-project: 3 years

Total Cost of the Sub-project: 395,292.00 USD

Research Team: Dr LON Solida (Principal investigator), Dr HUY Hangsak, Dr CHENG Khley, Dr TIENG Siteng, Ms UNG Sivlin, and Associate Professor SOUSA Emilia

Specific objectives: To extract and characterize the bioactive compounds of brown, red and green algae to formulate cream products with the potential to be commercialized in Cambodia.

Hypothesis: Selected algae possess several saccharides and phenolic compounds that have anti-stress oxidation and photo-protective properties. Known compounds of interest may be enhanced by new molecular structures. Two types of emulsions will be formulated possessing a wide range of antioxidant, anti-melanogenesis, and moisturizing effects using *in vitro* and *in vivo* tests, which may be patented.

Methodology: Solvents with different polarities will be used to extract and further purify the compounds. Structure elucidation will use nuclear magnetic resonance (NMR) spectroscopy and high-performance liquid chromatography (HPLC). Antioxidant properties will be assessed using DPPH, metal, hydroxy radicals, and reducing power assays. UVA-UVB protection, anti-aging, anti-melanogenesis properties will be tested using HaCat and high expressing melanin cell lines. Cells viabilities will be examined using an MTT assay. Type-1 pro-collagen and melanin contents, and an expression of MMP-1 will be evaluated and confirmed using a western blot assay. Tyrosinase, elastase assays will also be included. Several types of day creams will be formulated by adding to the base formula of the algae extracts and/or isolated compounds. The most active compound will be selected for

development. Cream characteristics such as the rheology, chemical and physical stability, oxidation stability, and phase separation stability, pH, colour, smoothness, spreadability, and conductivity will be also determined. Volunteers will be selected to test the final products for 60 days. Skin characteristics such as epidermal water loss, capacitance, firmness, sebum, and wrinkles will be evaluated using a skin testing machine. Statistical analysis will be performed using ANOVA analysis.

Expected results: We expect that the extraction and purification process will render a significant number of bioactive compounds -carbohydrates and phenolic compounds with significant anti-aging, anti-pigmentation, antioxidant, and anti-UV radiation properties with potential applications in skincare products. We expect that the creams/lotions will demonstrate excellent potential as patentable and commercially viable products that may support new alga farming and related cosmetic industries in Cambodia. Three master's theses and six undergraduate theses are expected to be realized as part of the project. This will act as a basis for a research group focused on cosmeceutical products to become established within RUPP and Cambodia more broadly.

Research Project 3

Research Topic: Development of probiotic fermented vegetables in Cambodia and their effects on the immune system *in vitro*

Duration of the Sub-project: 3 years

Total Cost of the Sub-project: US$ 232,920.00

Research Team: Mrs HUOY Laingshun (Principal investigator), Ms UNG Sivlin, Dr HUY Hangsak, Dr TIENG Siteng, Associated Professor BOQVIST Sofia, and Asst. Prof. Dr KONSUE Nattaya

Lactic Acid Bacteria (LAB) are among the most advantageous microorganisms in fermented food because of their ability to enhance taste and texture (Bintsis, 2018). Moreover, LAB has been found to have potential antimicrobial (Turner et al., 2017), preservation, and immune system improvement properties both *in vitro* (Jeong et al., 2015) and *in vivo* studies (Karamese et al., 2016). This research project has four specific objectives related to the identification and characterization of LAB, followed by an investigation of optimal culture growth conditions. After obtaining the LAB samples, several compounds will be tested for their inhibitory and stimulating effects. Standard formula optimized for shelf life and quality control will be developed in collaboration with a private sector partner. In our research, we hypothesize that:

- Local fermented vegetable contains many useful LAB strains that have the potential to be used for future commercial food processing ventures.
- Isolating LAB strains may result in antimicrobial compounds, such as bacitracin and hydrogen peroxide that can be used to stimulate RAW 264.7 cell lines to produce TNF-α, IL-10, and nitrogen oxide (NO).
- Traditional fermented formula may be applied to a study of LABs in fermented food, whereby they may be optimized for shelf life and the improved quality of commercial fermented food products.

Methodology:

- The isolation and characterization of different LAB strains from various fermented vegetables will be conducted using the culture method on Man, Rogosa and Sharp (MRS) and M17 agar, before confirmation using the gram stain method and a biochemical test kit (API 50 CHL).
- An agar diffusion bioassay will be used for screening the bacteriocin production and *Lactobacillus sakei* and *Listeria innocua* will be used as indicator organisms. Then the production of H_2O_2 from the LAB will be determined using colourimetric methods.
- Total isolated LAB strains will be screened for activation of the macrophage cell line for TNF-α, IL-10, and nitrogen oxide (NO) production by the ELISA technique and Griess reactions; respectively. In brief, macrophage RAW 264.7, one of the model cell line for the study of the immune system *in vitro*, will be cultured and stimulated by the LAB characterized in Step 1 to determine whether lipoteichoic acid (LTA), an immune stimulatory protein found on the cell wall LAB, may induce the production of TNF-α, IL-10, and nitrogen oxide (NO). To determine the presence of TNF-α and IL-10, supernatant from the culture will be collected and tested using the specific antibodies for these two substances using an ELISA reader. To determine the amount of NO production, the supernatant of the stimulation culture will be tested by using a Griess reagent kit. The absorbance of the mixture will be measured at 540 nm on an automated EL800 plate reader

- LAB with antimicrobial and immunological stimulation criteria will be tested for optimum growth conditions for pH, salt bile, and temperature to clarify which LABs may be suitable for scaled-up production. The isolated LAB will be inoculated on a medium culture with a pH range of 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0, for the determination of the pH tolerance. Similarly, 0.1% - 0.5% bile salts (w/v) will be supplemented with MRS broth to test optimal bile salt concentrations. The strains will be incubated at temperatures of 20, 37 and 44 °C to investigate the function of LAB when progression through the digestive tract of humans.
- A standard formula for industrial production will be developed based on consumer preferences. Two target groups comprising 30 individuals who like fermented foods and another 30 individuals who do not like fermented foods will be recruited to participate in a sensory test of the products developed in the study to determine consumer acceptance. Microbial tests will also be conducted to determine product safety including culturable microorganisms at 22 ^{0}C and 37^{0}C; total coliforms, Escherichia coli; intestinal enterococci; spore of sulphite reducing anaerobes. Tests for physiochemical properties such as turbidity, pH, chlorides, ammonia, nitrites, nitrates, hardness, and iron will be conducted to determine the shelf life and quality of the final products, before launch. SPSS software will be used for the analysis.

Expected outcomes: It is expected that LAB strains from local vegetables and fermented foods will be isolated and characterized to identify those with the potential to be used as a starter for commercially produced traditional

Cambodian fermented foods. Moreover, it is anticipated that an acceptable formula will be established to transform the production of tradition fermented vegetables from the household scale to properly packaged items that meet food safety standards. This research will also serve as an opportunity for emerging researchers to develop collaborative work with the private sector to develop both local and export markets for these products. The work will be published in a peer-reviewed journal.

Research Project 4

Research Topic: Improvement of watermelon varieties through conventional breeding and tissue culture

Duration of the Sub-project: 2 years and 9 months

Total Cost of the Sub-project: US$ 327,037.00

Research Team: Ms Sivlin UNG (Principal investigator), Dr Phanna PHAT, Dr Siteng TIENG, Dr Solida LONG, and Professor Ho-Jong JU

Specific objective: To collect and characterize Indigenous and introduced varieties of watermelon for the selection of incorporation of traits into breeding programs for the development and production of potentially commercialized seeds of new diploid and tetraploid varieties.

Hypothesis: The quality and yield of introduced watermelon varieties and the adaptability of wild/indigenous varieties will be assessed for new varietal development. Five new open-pollinated varieties with traits of interests such as excellent flesh and rind colour, long shelf life, high soluble solids and high yield will be selectively bred using ten lines of tetraploids produced on plant

tissue culture with and without application of colchicine, a chemical agent-induced chromosome doubling.

Methodology: The seed will be collected from eleven provinces including Kandal, Kampong Cham, Kratie, Stoeng Treng, Mondulkiri, Ratanakiri, Preah Vihear, Kampong Thom, Siem Reap, Battambang, and Banteay Meanchey for bot Indigenous and introduced watermelon varieties. Each will be examined for the distinctness, uniformity and stability, and production of harmonized variety descriptions according to UPOV guidelines. Varieties with potential agronomic traits such as, high yield, long shelf life, high soluble solids with yellow, orange, or red flesh colour, that are adaptable to the Cambodian climate will be used in pedigree selection breeding. Two parents will be crossed to produce an F1 which will later be repeated using a self-pollinating or sib mate for the selection of the best line from generation to generation using advanced inbreeding. The best lines (F6/F7) will be tested for potential release as new open-pollinated varieties or new parents for hybrids. Measurement of fruit yield and quality will include fruit weight, length, and diameter, hollow heart, rind pattern, flesh colour and soluble solids.

Uniform and homogenous varieties with high yield and quality according to a first screening, and F4-F6 varieties derived from pedigree breeding will be germinated in vitro for excising of cotyledon for culturing on MS medium supplemented with 1mg/l BAP and 0.1mg/l IAA. These samples will be used to initiate adventitious calli, treated without and with aqueous colchicine at different levels (0, 0.1%, 0.2%, and 0.5%) to induce somaclonal variants showing superior or tetraploid traits. Morphological characteristics and the

number of chloroplasts in each stoma will be used as indicators for discrimination between diploids and tetraploids. Diploid somaclonal variants, F5 and F6 generation derived from pedigree breeding which becomes uniform and homogeneous and show superior traits will be further evaluated in trials. Successful samples may be registered as a New National Variety Release by the MAFF.

Field demonstrations will be conducted with forest communities to introduce and facilitate the evaluation of newly developed open-pollinated seeds based on consumer preferences. Knowledge will be transferred to forest communities on watermelon cultivating techniques utilizing these seeds. Tetraploids will be proposed for producing triploid hybrids for the production of seedless watermelon. Statistical analysis will be performed using ANOVA.

Expected results: It is expected that at least five open-pollinated seed varieties with high yield, long shelf life, high soluble solids with yellow, orange, or red flesh colour and adaptable to Cambodian climate will be produced. These will have potentially for registration as a National New Variety Release, which may be shared with forest communities and industrial partners for commercial production. Further, the industrial partner will be used to act as a seed distributor. Tetraploids and diploids from this project will be used to produce diploid and triploid hybrids of premium quality with an industrial partner to develop and issue patents for commercialization. Three graduate theses, four undergraduate theses, an article in an international peer-reviewed journal; and two articles in a national peer-

reviewed journal, and one watermelon breeding/cultivating book will also be produced as part of the project

Research Project 5

Research Topic: Growth optimization of *Haematococcus Pluvialis* for astaxanthin and algal powder supplements

Duration of the Sub-project: 3 years

Total Cost of the Sub-project: US$ 380,927.56

Research Team: Dr HUY Hangsak (Principal investigator), Dr LONG Solida, Dr TIENG Siteng, Prof. Dr AHN Chi-Yong, Ms UNG Sivlin, and Mrs HUOY Laingshun

Haematococcus Pluvialis shows variables in astaxanthin synthesis that range from 2 to 5% in an aplanospore cell state depending on the environmental factors, nutritional stress, derivatives and plant hormones. Research is required to increase this concentration. It is hypothesized that the growth of microalgae and enhancement of astaxanthin production can be achieved through cultural optimization and genetic manipulation. This research has several specific objectives: (i) To isolate, identify and characterize novel microalgae, closely-related to strains to or sub-strains of *H. Pluvialis* and compare their cellular contents with the commercial strain, *H. Pluvialis* UTEX 2505; (ii) To optimize growth conditions including cultural media, micronutrient supplements, carbon-to-nitrogen sources and ratios, light irradiation, temperature, pH, or nitrogen limitation; (iii) To analyze the impact on the algal growth and astaxanthin synthesis caused by the microalgal associated-bacteria and plant hormones; (iv) To chemically induce

mutation in some strains and select the best grown and stress-tolerant microalgae, barring higher astaxanthin content.

Multiple methodologies will be used. *First*, to collect microalgae from the freshwater sample, identify their morphologies and genetics microscopic and 18S rDNA sequencing will be used. The associated bacteria will be confirmed by 16S rRNA sequencing. The novel strains confirmed by sequencing will be further characterized. The growth and carotenoid accumulation in the isolates will be analyzed by cell counting and pigment reading. *Second*, to optimize the best growing medium, micronutrient supplements, C and N sources, C/N ratios, N limitation, and environmental conditions for the growth and carotenoid accumulation in the standard strain UTEX 2505. The growth and carotenoids production from the isolate, UTEX 2505, and induced-mutant microalgae will be compared. The effect of associated-bacteria and several plant hormones such as abscisic acid, jasmonic acid, methyl jasmonate or growth regulators like gibberellic acid, salicylic acid or brassinosteroid-2,4-epibrassinolide on astaxanthin accumulation will also be tested using real-time (RT) qPCR amplification, cell counting methods, and pigment analysis. *Third*, to induction of the mutation in *H. Pluvialis* will be facilitated using the chemical mutagen, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) to identify the best growth resistance under unfavourable growth conditions. *Fourth*, to extract and purify astaxanthin from the crude extract using the solvent base-extraction, the structure of astaxanthin will be analyzed by using NMR and HPLC. *Fifth*, to compare the ant oxidative activity of astaxanthin extracted from this study, it will be compared with a

commercial counterpart using mammalian cell lines and an anti-oxidative test kit. The expression of several antioxidant genes will also be confirmed by RT-qPCR amplification.

It is expected that various microalgae species will be isolated that have the potential to produce astaxanthin and other valuable sources of nutrition and that novel species of bacteria will be identified that may be grown with associated microalgae to explore their influence on microalgal cultivation. The optimal growth conditions, mutagenesis, and astaxanthin synthesis that will be discovered in this study will play an important role and produce fundamental knowledge for the future development of largescale production regimes. These results will be published in a local or international journal.

Research Project 6

Research Topic: Assessment of climate change risk and adaptation for the loss and damage of transportation infrastructures in Battambang and Prey Veng Provinces of Cambodia

Duration of the Sub-project: 3 years

Total Cost of the Sub-project: US$ 69,268.00

Research Team: Dr SPOANN Vin (Principal investigator), Dr SEAK Sophat, Dr NOP Sothun, Dr SAN Vibol, Dr THOUN Try, Dr YIM Mongtoeun, Mr SAT Sitak, Ms HOY Vannareth and Mr SE Bunleng

The Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) predicts that Asia will experience rises in temperature, longer summer heat spells, more intense and more frequent precipitation, increased extreme rainfall, a higher number of extreme tropical cyclones,

and rises in sea levels. The recent frequent occurrence of extreme weather events in Cambodia provides some evidence of increased intensity and frequency of climate events, which will potentially have negative impacts on urban and rural transportation infrastructure in Cambodia. Therefore, an improved understanding of loss and damage caused by extreme weather events, as well as the linkage between vulnerabilities, hazards and adaptive capacity, is critical. However, there is a shortage of practical methods for estimating loss and damage in the context of extreme climate events and transportation infrastructure (TI). Management of transportation infrastructure is a complex issue and there is limited knowledge of how to incorporate appropriate adaptation measures and strategies into rural and urban transport planning.

Therefore, this study has the following specific objectives: (i) To identify climate change-related hazards with potential impact on rural and urban TI; (ii) To characterize and classify types of urban and rural TI, identifying assets and inventories for each; (iii) To evaluate current losses and damage for TI in the context of climate change by rapid urban and rural assessments in the selected study sites of two provinces, and conduct detail vulnerability assessments (VA); (iv) To determine the loss and damage for each road infrastructure type in different climate change scenarios obtaining from the VA study; (v) To share study findings among stakeholders in the study sites through, policy discussion forum, publications and policy brief papers. This research presents a rapid assessment that will be conducted in Battambang

City and selected location in Prey Veng Province related to current losses and damages from extreme climate events.

The rapid assessment will be employed to assess current losses and damages to TI caused by main or extreme climate hazards, especially flooding, by applying an urban rapid assessment adopted from RRA, Impact Matrices and Multi-Criteria Analysis. Then, two research sites from each province will be used as a site for a vulnerability assessment (VA) at the community level. The scenarios will be created and an evaluation of assessment will be examined, as well as various adaptation measures. The NK-GIAS modelling package (GIS-based model) will be applied for estimating the economic loss and damage for TI associated with the major hazards.

This project will result in two scientific publications, two policy briefs and a manual guide on assessing climate risk on TI and will be of benefit to students and the research program of the Royal University of Phnom Penh and the Institute of Technology Cambodia. The project outputs will be shared with stakeholders through consultative workshops for further development of a set of guidelines/actions for climate change risk assessments to better understand losses and damages to TI and locally appropriate adaption measures.