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RESPONDING TO HEALTH AND ENVIRONMENTAL ISSUES THROUGH BIOCHEMISTRY AND MOLECULAR BIOLOGY

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The Philippine Journal of Biochemistry and Molecular Biology (PJBMB) (formerly Bulletin of the Philippine Biochemical Society) is a peer-reviewed journal published biannually by the Philippine Society of Biochemistry and Molecular Biology (PSBMB) which cover original research articles and research communications in biochemistry, molecular biology and/or other related disciplines, biochemical education articles, biochemical literature reviews, special interest articles with biochemical applications, how-to articles on useful activities relating to biochemistry, letters and book reviews.

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Editorial

Moving through the CoVID19 pandemic via the Virtual platform.

In the past year and onwards, the CoVID19 pandemic continued to ravage the world with millions of people getting sick and dying including Southeast Asia and the Philippines. Although, we see hope in the multiple interventions coming from the different sectors including WHO, the government, the private sector and various groups, we have experienced the new normal drastically inserted into our lives to install the much-needed protection from the SARS-CoV2 virus. In our neighborhood, we experienced the recurrent, granular or perhaps the "circuit breaking" lockdowns as the new variants emerged, more so with the highly infectious Delta variant that has entered the boarders of our country and triggering an upsurge of cases not only in the "NCR bubble" but in more than nine regions of the Philippines. We see the occurrence of SARS-CoV2 variants as the new evolving trend to be addressed by the multiple interventions including the different EUA approved vaccines used in the attempt to achieve herd immunity. Furthermore, we see the grim and devastating effects of this pandemic not only globally but in the immediate vicinity of lives as we see some of our colleagues, our dear friends and relatives fight, continue to fight and some fall and become casualties of this conflagration, to them we dedicate this issue for their glory!

Our self-confinements, which are necessary intermediary interventions combined with face mask, face shield, social distancing, handwashing, created in us, a kind of social isolation that limited our abilities for physical interaction. Of course, in the scientific community and the academe in particular, we see the need to go on, find ways to continue our activities, more so our scientific discussions. Thus, we see the emerging trend of virtual platforms as venue for Scientific Conferences.

The Philippines Society of Biochemistry and Molecular Biology in its 2020 Annual Convention has adapted this virtual platform with a theme, "Responding to health and environmental issues through biochemistry and molecular biology", an aptly conceived theme in this time of crisis. Hosted by the PSBMB Central Luzon Chapter, this virtual conference yielded a remarkable turn-out of virtual presentations and poster papers. Despite the challenges in this electronic platform, we can commend the Organizers, the host and the participants for the successful conduct of this Virtual Scientific Conference, an overwhelming kudos to you all!

In this issue of PJBMB, we featured the abstracts of these virtual presentations. There were nine plenary lecture papers, four Young Scientist Forum extended abstracts, and forty-three poster papers.

We are very much indebted and grateful to the authors for their willingness and support for publishing the abstracts of their work in this issue. We are also thankful to the reviewers for their time as well as the Editorial Board for their undying efforts to keep us moving forward. This compilation will not be possible without the generous efforts of everyone. As happy memories are captured in these compiled scientific proceedings, short they maybe, these are replete with gems and reflections of the compact scientific endeavors that each one has done at this trying time. Goodluck and happy reading to all!

Francisco M. Heralde III, RN. PhD.
Editor-In-Chief

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Message from the Convention Chair

On behalf of the officers and members of the Philippine Society of Biochemistry and Molecular Biology (PSBMB), I warmly congratulate the Philippine Journal of Biochemistry and Molecular Biology (PJBMB), formerly the Bulletin of the Philippine Biochemical Society, for publishing its 2nd volume since its re-launch in 2019.

Being the National Organizing Committee Chair of last year's 47th PSBMB Annual Convention, I am overjoyed to witness this issue dedicated to the convention coming into realization. The convention's theme was "Responding to Health and Environmental Issues Through Biochemistry and Molecular Biology." Therefore, I want the readers to look forward to published papers related to the health, agri-fisheries, and environment sectors.

By reading this PJBMB issue, I hope that many will be inspired to take on further studies that will be used to improve the country's socio-economic and health conditions and preserve natural diversity.

Mudjekeewis D. Santos, Ph.D.
National Organizing Committee Chair
Vice-President, PSBMB Board of Directors (2018-2019)

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Plenary Session—Oral

Pandemic Preparedness through Biochemistry and Molecular Biology

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Abstract: In the past two decades, several important zoonotic pathogens have emerged and circulated globally, including highly pathogenic avian influenza viruses (HPAI) and Zika virus (ZIKV). A novel coronavirus, now named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged last December 2019 in Wuhan, China that subsequently spread to the rest of the world and caused a pandemic of severe pneumonia, now called coronavirus disease (COVID-19). As of 29 September 2020, more than 33 million cases worldwide were reported to the World Health Organization (WHO), with more than 100 million deaths. The factors contributing to its emergence and circulation are complicated and multi-factorial, involving the interface between ecology and human behaviors, such as farming, transportation, or wildlife wet market, which coupled with climate change and landscape fragmentation. Therefore, it also requires multiple aspects of technologies for the pandemic preparedness currently and in the future. As WHO urges all countries to scale up the testing capacity, the global efforts to develop a vaccine are racing against the clock. From the biochemistry and molecular biology point of view, the ideal diagnostic test should follow ASSURE criteria, including accurate, sensitive, specific, rapid, economically affordable, and easy to scale up. Similar concepts, such as efficacy, deliverability, and economical-affordability, can be applied to vaccine development, which also needs to accommodate low and middle-income countries. Three basic principles for diagnostic kit and vaccine/ treatment development can be applied for the preparedness of pandemic: (1) priority-pathogen based: each country need to prioritize the pathogens based on the disease burden; (2) platform development: various molecular approaches aim to reduce the time of diagnosis and easily implemented in a clinic for point-of-care use. These approaches should be standardized as the platform for the development of diagnostic kits and vaccines based on the priority-pathogen evaluated; (3) prototype-pathogen approach: Similar approach for emerging pathogens from the same family of the pathogen can be directly applied for the development of diagnostic kit and vaccine. In summary, continuously identify and prioritize the priority pathogen, procurement of biochemical/ molecular technologies and implementation of evidence-informed health delivery systems will enhance our capability for pandemic preparedness.

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Plenary Session—Oral

Viruses from Philippine Bats and an Appeal to PSBMB to Work with Bat Ecologists in Search of Potentially Zoonotic Viruses

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Abstract: For my talk, I will first report the researches that we have been conducting on bat-borne viruses in the Philippines which started in 2007. Our research group, composed of virologists and bat ecologists from the University of the Philippines Los Banos and several Japanese universities, sampled bats from 12 provinces and islands all over the country. We found serological and sequence-based evidence for the presence of eight virus groups belonging to families known to be zoonotic and detrimental to human health. These virus groups include Filoviridae (*Reston Ebolavirus*), Gammaherpes, Coronavirus (*alpha*- and *beta*-), Hantavirus, *Pteropine orthoreovirus*, Flaviridae (Dengue Virus), Henipavirus, and Poxvirus. These viruses were uncovered from 11 bat species, all of which are widespread across the Philippines and for some bat species, inhabiting human-associated habitats (e.g. buildings, orchards, villages), As such, this can pose a ground-zero event for possible spill-over of potentially zoonotic and harmful pathogens to humans. For the second part of my talk, I will be appealing to PSBMB members to re-channel some of their research efforts and look into many exciting yet pressing fields of bat-borne virology studies in tandem with bat ecologists and wildlife biologists. For this, I present four main research topics, following a framework of the Bat/One Health Research Network (BOHRN), a global-scale multi-disciplinary consortium that aims to characterize the threat of bat pathogens under the One Health research approach. These are (1) Molecular detection and distribution of viruses among Philippine bat species; (2) Pathogen surveillance and diagnostic capacity; (3) Virus ecology/dynamics in bats, domesticated animals, and wildlife interface; and (4) Risk characterization and forecasting in human-bat interaction vis-a-vis virus spill-over dynamics. In taking on the aforementioned topics, the unique expertise of molecular biologists and biochemist members of PSBMB will prove to be pivotal in mitigating the threat of emerging zoonotic infectious diseases in the Philippines.

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Plenary Session—Oral

Navigating the Maze of COVID-19 Diagnostics

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Abstract: The COVID-19 outbreak has had a major impact on clinical microbiology laboratories in the past several months. This talk covers a systematic review of the current diagnostic platforms and challenges for the laboratory diagnosis of infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Diagnostic challenges based on the stage of sample collection and processing intrinsically affect test accuracy. In the pre-analytical stage, collecting the proper respiratory tract specimen at the right time from the right anatomic site is essential for a prompt and accurate molecular diagnosis of COVID-19. Adherence to biological safety measures is necessary to keep laboratory staff safe while producing reliable test results. In the analytic stage, real-time reverse transcription-PCR (RT-PCR) assays remain to be the diagnostic test of choice for the etiologic diagnosis of SARS-CoV-2 infection while antibody-based techniques are currently being considered as a supplemental tool. Careful interpretation and appropriate clinical correlation of results in the post-analytical stage for both molecular and serological findings. Lastly, this talk will also provide an overview of emerging diagnostic platforms including random-access, integrated devices available at the point of care with scalable capacities that will facilitate the rapid and accurate diagnosis and monitoring of SARS-CoV-2 infections and other future emerging infectious disease epidemics and pandemics.

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Plenary Session—Oral

Reshaping Agricultural Research and Development in a Time of Pandemic: Integrating Biotechnology to Agricultural Value Chain

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Abstract: To secure food for the world's growing population even prior to the pandemic, agriculture already needs to produce more with less which means more in terms of yield, income, and social inclusivity, and less in terms of unnecessary inputs, energy consumption, and environmental impacts. It suggests the importance of the demand and the supply chain in research and development (R&D) specifically in biotechnology. On the demand side, we should ask what people would really need from R&D in biotechnology. On the supply side, we should ask what we should expect to be generated from biotechnology. This talk will present the proposed key priority areas for research in agricultural biotechnology and allied fields to accelerate the transformation of the agriculture sector and to strengthen its contribution to food security and socio-economic development, particularly toward long-term resilience against pandemics. This talk will also suggest a process in implementing a modern breeding program that considers practical factors like developing a crop master plan where breeding strategies will be laid out while taking into account the logistical constraints in regulatory issues. In the implementation proper, an integrated breeding platform towards speeding genetic gain must be in place. This platform includes the creation of an interdisciplinary crop breeding team and automation, shortening the breeding cycles (e.g., genomic selection strategy, gene-editing), and strengthening collaboration between the academic community and commercial crop breeders. We are the key players in society's ability to achieve the aspired food and nutrition security and economic development. But we can aspire to contribute beyond economic development that is sustainable, inclusive, environment-friendly, and most importantly, resilient to current and future pandemics and other unanticipated disruptions. Thus, we proposed an active engagement in Academe-Industry- Government (AIG) interconnectivity models on biotechnology research collaboration and co-sharing of financial resources to shorten the gap between research and knowledge utilization, including contextualizing research projects within the agricultural value chain.

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Plenary Session—Oral

Emerging Disease in Livestock: Understanding African Swine Fever thru Molecular Biology and Biotechnology

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Abstract: Before the onset of COVID-19 pandemic, African Swine Fever (ASF) had threatened the global swine industry and is still a threat to this day. African Swine fever (ASF) is a viral disease affecting domestic and wild pigs that is responsible for serious production and economic losses with mortality rates close to 100%. It is first reported in Kenya in 1921 following a high mortality in imported European pigs. For many years, the disease has been confined to Sub-Saharan Africa and few European countries. However, in 2018, It spreads to Asia as China reported its first case of ASF, and from 2018-2020 most of ASEAN countries, including the Philippines, have reported positive cases of ASF. ASF spreads thru 3 main routes: (1) direct contact with infected pigs, (2) ingestion of contaminated materials (food waste, feed or garbage) and (3) fomites and biological vectors (Ornithodoros spp.). Currently, there is no effective and approved vaccine for ASF as compared to other viral diseases of swine like classical swine fever (CSF), porcine respiratory and reproductive syndrome (PRRS), porcine epidemic diarrhea (PED), etc. The current diagnostic tests used for ASF detection are thru viral isolation, fluorescent antibody test (FAT), ELISA (antigen detection) and Polymerase Chain Reaction (Conventional or Real-time PCR). The advent of molecular biology and biotechnology may prove to be an exceptional tool to further understand the behavior of numerous pathogens including ASF. Furthermore, this may help to further develop products and tools that can be used to improve the ASF control and prevention. As ASF was confined in Sub-Saharan Africa and some European countries, there is a need to optimize and harmonize protocols for sample selection, sequencing, bioinformatics workflow and data documentation. Furthermore, Asfarviridae contains only one member and that is ASFV. There is a need to discover other viruses under this viral family, not only for evolutionary analysis but also for possibility to be used to prevent and control ASFv. Molecular biology and biotechnology can give us the glimpse and hints on the structural and behavioral characteristics of ASF virus (ASFv). This additional information may help regulatory disease agencies in crafting the best possible control and prevention measures to be applied in the field. Another, molecular biology and biotechnology are tools to be used to develop diagnostic test and kits that can help for a rapid, accurate, specific, sensitive and economical tests that can be used in the early course of disease to place proper measures to prevent further spread of disease from one place to another. This can be also used to further improve data collection and collation for molecular epidemiology of ASFv. Lastly, one of the goals to further prevent and control ASF is to develop effective vaccine. Thru molecular biology and biotechnology, new generations of vaccine can be developed to increase the efficiency and effectivity of the vaccine.

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Plenary Session—Oral

Molecular Aspects of Emerging Bacterial and Viral Diseases of Tilapia and Barramundi Cultured in ASEAN Countries

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Abstract: Tilapia and Barramandi are the major ecomomic fish species cultured in ASEAN countries. They were commonly cultured in intensive farming system. Based on geographical areas and climates of ASEAN countries, the emerging diseases of Tilapia and Barramundi are usually warm water diseases such as Streptococcosis, Flavobacteriosis, Aeromonas septicemia, Edwardsiellosis, Mycobacteriosis, Vibriosis, Tilapia Tilapinevirus infection, scale drop disease virus (SDDV) infection, infectious spleen and kidney necrosis virus (ISKNDV) infection, and etc. The molecular biology was applied in many aspects for research in emerging bacterial and viral diseases of Tilapia and Barramandi such as molecular characterization, genome comparison, molecular pathogenesis, molecular diagnosis, molecular epidemiology, molecular mechanisms of antimicrobial resistance, and etc. The techniques used in molecular research of emerging bacterial and viral diseases of Tilapia and Barramundi are including both basic and advanced molecular techniques such as restriction fragment lenge polymorphisms (RFLP), polymerase chain reaction (PCR), quantitative real-time PCR, DNA or genome sequencing, Loop mediated isothermal amplification (LAMP), Recombinase polymerase amplification (RPA), and etc. The molecular and genome study of those bacterial and viral pathogens provides a fruitful information to the better understanding about thier virulence, pathogenesis, diagnosis, and epidemiology. Additionally, the information is very useful to apply in the prevention and control of the emerging diseases such as use in vaccine design and development.

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Plenary Session—Oral

Wastewater-Based Surveillance for SARS-CoV-2: Promise, Progress, and Problems

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Abstract: Wastewater represents a pooled sample from a community- where everyone in the sewer shed contributes fecal samples to the wastewater/sewage. Wastewater and virus researchers have hypothesized that this pooled sample can be used to determine the quantity of SARS-CoV-2 in the community, and in turn be used to determine COVID19 prevalence, track trends in infection, determine hotspots, or determine changes in virus strains. In this talk, I will summarize the approaches being used by researchers around the world, the associated potential and challenges, and the current state of research in wastewater-based surveillance for SARS-CoV-2.

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Plenary Session—Oral

Philippine Marine Sediment-Derived Actinomycetes: The New Frontier in Antibiotic Discovery

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Abstract: The marine sediments in the Philippine archipelago harbors diverse actinomycetes that potentially yield new core structures with antibiotic activities against multidrug-resistant pathogens. Since 2017, the Center for Chemical Biology and Biotechnology (C2B2) of the University of San Agustin, Iloilo City explored the Philippine marine environment for actinomycetes thriving in the seafloor. Currently, the C2B2 has a library of more than 3,000 marine sediment-derived actinomycetes, which now comprise the biobank for antibiotic discovery in the country. This lecture highlights the discovery of these biodiverse actinomycetes as well as the antibiotics they produce utilizing various experimental techniques that meld the disciplines of genomics, molecular biology, chemical biology, biotechnology, natural products chemistry, and metabolomics.

Keywords: marine sediment-derived actinomycetes biodiversity, antibiotic discovery, melding multi-disciplinary technologies

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Plenary Session—Oral

Molecular Genetic Approaches to the Assessment of Dam Impact on Stream Macroinvertebrates and Dengue Virus Surveillance of Vector Mosquitoes

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Abstract: Molecular genetic techniques are used in wider study areas including evolutionary ecology, biodiversity conservation, and epidemiological surveillance. The recent advancement and use of molecular genetic technologies including next-generation sequencing (NGS) are accelerating this trend. In my lecture, I will introduce recent research projects in my lab related to the areas of freshwater biodiversity (stream macroinvertebrates) and eco-epidemiology (Dengue vector mosquitoes). The topics will cover 1) Ecological Assessment of the influence of sediment bypass tunnels on macroinvertebrates in dam-fragmented rivers using NGS-based DNA metabarcoding, and 2) Dengue Virus Surveillance of Dengue vector mosquitoes Aedes aegypti for the inferences of spatial viral transmission in Tarlac City, Philippines using real-time RT- PCR. I would like to also provide general information about my research group, Molecular Ecology and Health (MEcoH) Laboratory in Ehime University, Japan, and an available Ph.D. position in MEcoH for a student from Southeast Asian countries.

Keywords: Molecular genetic techniques, Ecological Assessment, Dengue Virus Surveillance

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Young Scientists' Forum

Comprehensive Virtual Screening of Anti-HIV Reverse Transcriptase Phytochemicals Against SARS-CoV-2 Non-Structural Proteins: Accelerating Anti-COVID-19 Drug Discovery by Repurposing Dietary Polyphenolics and Terpenoids

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1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has tallied over 43,500,000 cases and taken over 1,160,000 lives worldwide (World Health Organization, 2020). Despite its having been declared a pandemic by the World Health Organization (WHO), COVID-19 has continued to spread and has infected at least 20 million individuals, reaching a death toll of over half a million. In the Philippines alone, there are above 360,000 cases and 7,000 deaths, which are second in Southeast Asia behind Indonesia. Continuous deaths due to COVID-19 are coupled with drug unavailability, thereby reflecting the need for drug discovery.

Coronavirus disease 2019 (COVID-19), which is caused by the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has tallied over 43,500,000 cases and taken over 1,160,000 lives worldwide (World Health Organization, 2020). Despite its having been declared a pandemic by the World Health Organization (WHO), COVID-19 has continued to spread and has infected at least 20 million individuals, reaching a death toll of over half a million. In the Philippines alone, there are above 360,000 cases and 7,000 deaths, which are second in Southeast Asia behind Indonesia. Continuous deaths due to COVID-19 are coupled with drug unavailability, thereby reflecting the need for drug discovery.

Among the most targeted SARS-CoV2 enzymes are the non-structural proteins (nsps) which play an important function in post-translational modification, replication, and host immunity evasion (Wu et al., 2020). Nsps include 3-chymotrypsin-like protease (3CLpro/nsp5) and papain-like protease (PLpro/nsp3), which are cysteine proteases that cleave the polyprotein and release remaining nsps; RNA-dependent RNA polymerase (RdRp/nsp12) and helicase (nsp13) of the replicase-transcriptase complex; and S- adenosylmethionine-dependent methyltransferase (nsp16) with cofactor nsp10, which functions in mRNA capping. Nsps are conserved more than structural proteins, thereby presenting as drug targets. Most drugs including remdesivir and favipiravir that completed clinical trial phase 3/4 target RdRp (Sahebnasagh et al., 2020).

Since Human Immunodeficiency Virus (HIV) is a single-stranded RNA virus and translates a precursor polyprotein involved in pathogenesis like SARS-CoV-2, we virtually repurposed anti-HIV dietary plant compounds such as polyphenolics and triterpenoids with previously reported activity against HIV reverse transcriptase to against SARS-CoV-2 nsps.

2. OBJECTIVES

3CLpro, PLpro, RdRp, helicase, nsp10, and nsp16 of SARS-CoV-2 were targeted in silico to discover multitargeting compounds from published anti-HIV RT dietary plant secondary metabolites. Specifically, the following were aimed:

1. To determine the binding affinity of 104 anti-HIV RT compounds toward nsps through molecular docking
2. To analyze receptor-ligand interactions between the nsps and anti-HIV compounds
3. To predict the drug-likeness and absorption, distribution, metabolism, and excretion (ADME) properties of top 10 compounds of each nsp

3. METHODOLOGY

Fig. 1. *In silico framework in screening potential SARS-CoV-2 nsp inhibitors*

4. FINDINGS

4.1. Cysteine proteases

PLpro and 3CLpro cleave polyprotein PP1ab 3 sites at the N-terminus and 11 sites at the C-terminus, respectively, to release nsps (Figure 2A) (Wu et al., 2020). This initiates the cascade of replication. Top 10 compounds against PLpro exhibited strong binding affinities of -10.1 to -10.8 kcal/mol. The biflavonoid amentoflavone exhibited the highest affinity and bound Lys711 with pyrone and hydroxyphenyl moieties through H-bonding and *pi*-anion interactions, respectively (Figure 2BI). Hydroxyphenyl participated in H- bonding with Arg712 and Hi342, *pi*-anion interaction with Asp339, *pi*-cation interaction with Arg558, and *pi*-alkyl binding with Ala579 and Leu742. Ile310 and Ile580 were occupied by a chromene ring through *pi*-alkyl.

Top compounds against 3CLpro had binding affinities from -7.9 to -8.6 kcal/mol. Amentoflavone and volkensiflavone showed the highest affinity (Figures 2BII and 2BIII). Amentoflavone chromene moieties showed stacked amide-*pi* and *pi-pi* T-shaped interactions with His41. These were also demonstrated by hydroxyphenyl to Asn142. Its chromene linked to Met165 through *pi*-alkyl interaction. A chromene hydroxyl had H- bonds with Cys44, Val186, Arg188, and Glu166. Volkensiflavone bound the 3CLpro catalytic dyad, His41 and Cys145, through chromene and another chromene hydroxyl in *pi*-anion interaction and H -bonding, respectively. A chromene hydroxyl bound Glu166 through H-bonding while hydroxyphenyl bound Met165 through pi-sigma interaction.

4.2 Replication-Transcription Complex

RdRp replicates and transcribes RNA while helicase unwinds synthesized RNA from the template (Chen et al., 2020). Top 10 compounds against RdRp showed binding affinities of -8.6 to -9.5 kcal/mol. Ellagitannin punicalin exhibited the highest affinity (Figure 2BIV). Its galloyl hydroxyl bound Asn497 and Gly590 through H-bonding. Glucose and carbonyl oxygen were in H-bonds with Asp684 and Tyr689, respectively. Glucose participated in carbon-hydrogen bonding with Ala685 and Asp684. Ellagic acid moieties were in *pi*-alkyl interactions with Ile494 and Lys577.

Top compounds against helicase had binding affinities of -8.4 to -9.4 kcal-mol. Biflavonoid morelloflavone exhibited the strongest affinity (Figure 2BV). Its dihydroxyphenyl moieties bound Glu341 and Ala313 through H-bonding and *pi*-alkyl interaction, respectively. These also participated in *pi*-alkyl interaction with Val340 and Ala312 and *pi*-sigma interaction with Ala312.

4.3 SAM-dependent Methyltransferase Complex

SAM-dependent methyltransferase with nsp10 functions in 2'-hydroxyl group methylation for RNA capping, protecting RNA from host immunity (Figure 2A) (Viswanathan et al., 2020). Top compounds against nsp10 showed binding affinities of -6.9 to -7.7 kcal/mol. Biflavonoid robustaflavone had the highest affinity (Figure 2BVI). Its pyrone was in carbon-hydrogen bonding with Ile4334. Chromene hydroxyl formed H- bonding with Asp4335. Carbon atoms of pyrone and hydroxyphenyl formed salt bridges with Lys4346.

Top compounds against nsp16 exhibited affinities from -9.4 to -10.6 kcal/mol. Alkaloid michellamine B had the greatest affinity (Figure 2BVII). An isoquinoline moiety was in H-bonding with Asp6928 and Asp6897 and in carbon-hydrogen bonding with Gly6869. Another isoquinoline was in *pi*-anion interaction with Asp6931. A naphthalene moiety participated in *pi-pi* T-shaped interaction with Phe6947 and in *pi*-sulfur interaction with Cys6914. A methyl group connected to naphthalene manifested alkyl interactions with Met6929, Leu6898, and Cys6913.

4.4 Drug-likeness and ADME

Eleven of the top-scoring compounds manifested drug-likeness (Table 1). PLpro- and RdRp-targeting pomolic acid, 3CLpro- and nsp10-targeting digitoxigenin-3-O-glucoside, and nsp10-targeting artemisinin exhibited high gastrointestinal absorption. Artemisinin was also a blood-brain barrier permeant.

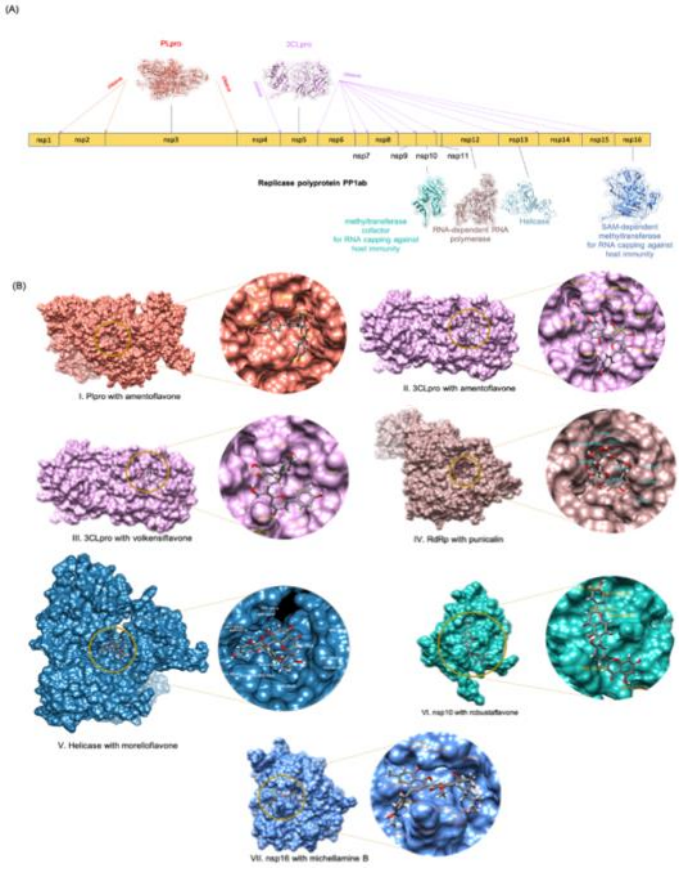

Fig. 2. (A) Nsps function in autoproteolytic cleavage, viral replication, and host evasion; and (B) docked poses of top-scoring compounds per nsp

Table 1. Druggable anti-HIV compounds against SARS-CoV-2 according to Lipinski's Rule of Five

Compound	MW <500	#H-bond acceptors <10	#H-bond donors <5	Liphopilicity MLogP<5	Lipinski violations	Drug-likeness	Target
artemisinin	282.33	5	0	2.21	0	Yes	nsp10
betulinic acid	456.70	3	2	5.82	1	Yes	PLpro
digitoxigenin-3-O-glucoside	520.65	8	4	1.95	1	Yes	3CLpro, nsp10
friedelin	426.72	1	0	6.92	1	Yes	PLpro, RdRp
garcisaterpene A	498.74	4	1	5.97	1	Yes	3CLpro
garcisaterpene C	454.68	3	1	5.63	1	Yes	Helicase
hinokiflavone	538.46	10	5	0.52	1	Yes	3CLpro, RdRp, helicase, nsp10
lupenoic acid	440.70	2	1	6.7	1	Yes	RdRp
oleanolic acid	456.70	3	2	5.82	1	Yes	PLpro, RdRp, nsp16
pomolic acid	472.70	4	3	4.97	1	Yes	PLpro, RdRp
ursolic acid	456.70	3	2	5.82	1	Yes	PLpro, RdRp

5. CONCLUSION / IMPLICATIONS

Anti-HIV RT dietary plant secondary metabolites present as therapeutic candidates against SARS-CoV-2. The fruit flavonoids amentoflavone and robustaflavone targeted all tested nsps. The anticancer terpenoid pomolic acid and CNS-active sterol glycoside digitoxigenin-3-O-glucoside were found to be multitargeting, druggable compounds while the antimalarial diterpenoid artemisinin was noted to target nsp10. This study provides a relevant scientific foundation for drug repurposing and development of phytochemicals/nutraceuticals against SARS-CoV-2.

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Young Scientists' Forum

Antimicrobial Activity and COX-2 Modulatory Effects of Tetrahydrobisbenzylisoquinoline Alkaloids from *Phaenthus ophthalmicus:* Validation of Ethnomedicinaluse from *in vitro* and *in silico* Perspectives

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1. INTRODUCTION

Under certain circumstances, microorganisms gain entry to the eye allowing infections to occur. Common pathogenic bacteria that cause eye infections are *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Hemophilus influenza, and Klebsiella species* [1]. Among inflammatory bacterial infections, conjunctivitis (red eye) is often caused by staphylococcal or streptococcal bacteria from the skin or respiratory system. Drug development of efficient and cost-effective treatment modalities for bacterial infections remains an interesting field in disease drug discovery due to the rapid emergence of drug-resistant strains. Therefore, the development of safe and effective natural products is warranted to curb the emergence and re-emergence of these pathogens.

COX-2 constitutes a minor cyclooxygenase (COX) isoform that is highly expressed following the stimulation of pro-inflammatory factors such as cytokines or endotoxins. It is responsible for the conversion of arachidonic acid into prostaglandin H2, which is then transformed into prostaglandin D2, prostaglandin E2, prostacyclins, or thromboxane A2. Prostaglandins possess immunomodulatory characteristics that can worsen or improve bacterial clearance. Cyclooxygenase-2 has been shown to exhibit different effects during several bacterial and viral infections [2] but little is known about its role in other bacterial infections.

The leaves and bark of the medicinal plant *Phaeanthus ophthalmicus* (Roxb. ex G.Don) J.Sinclair (Annonaceae) are traditionally used in the Philippines to treat pink eyes. With an objective to validate its ethnomedicinal value, the inhibitory activity of *P. ophthalmicus* extracts and tetrahydrobisbenzylisoquinoline alkaloids tetrandine (1) and limacusine (2) (Fig. 1) against drug-resistant strains causative to bacterial conjunctivitis and COX isoforms (1 and 2) were explored. In addition, molecular docking studies and molecular dynamics simulation virtual

experiments were also conducted to probe the stable binding affinities of the two alkaloids on COX isoenzymes *in silico.*

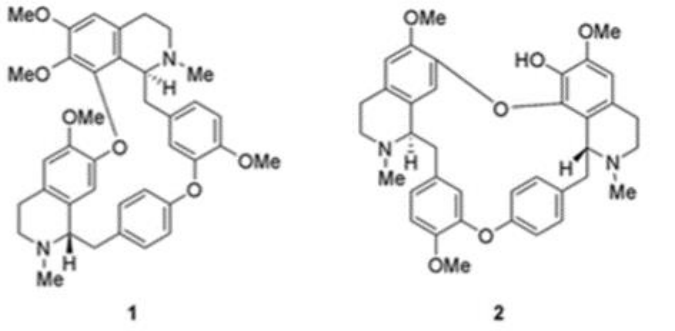

Fig. 1. Tetrahydrobisbenzylisoquinoline alkaloids from *Phaeanthus ophthalmicus*

2. METHODOLOGY

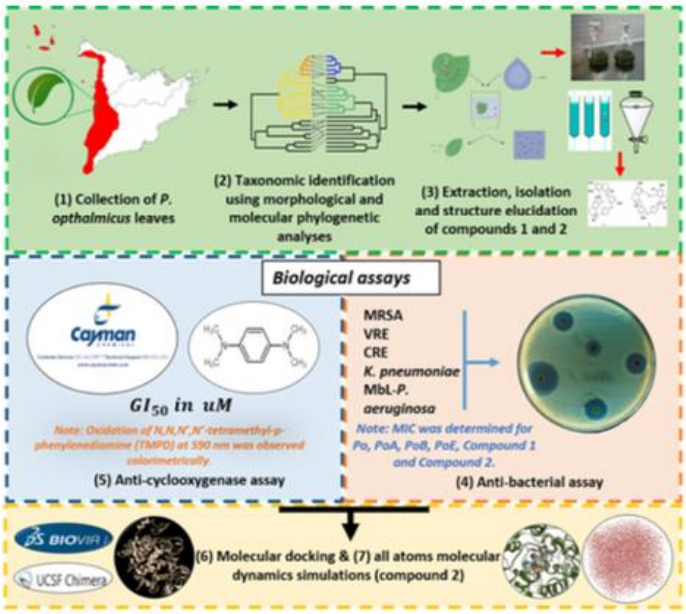

Fig. 2. General methodological workflow of the study

2. METHODOLOGY

3. MAJOR FINDINGS AND DISCUSSION

3.1 Results of antibacterial assay suggest the potential of tetrandine (1) and limacusine (2) against Gram-negative bacteria.

- Crude DCM-methanol extract (Po) exhibited the following MIC values against the test bacterial organisms: MRSA (275µg/ml), *K. pneumoniae* (137.5µg/ml), VRE (137.5µg/ml), and *P. aeruginosa* (137.5µg/ml).
- Fractionation of the crude extract using gradient pH acid-base partitioning yielded alkaloid-rich sub-extracts PoA, PoB and the non-alkaloid extract PoE. Sub-extract PoB showed broad-spectrum, and two-fold improvement of MIC (68.75µg/mL) against the Gram-negative MDR bacteria *K. pneumoniae* and *P. aeruginosa*, demonstrating selectivity against these test organisms.
- Both compounds had MIC at 137.5µg/ml for MRSA and VRE, and 68.75µg/ml for *K. pneumoniae* and *P. aeruginosa*.
- Alkaloids in general have proven reputation in the development of new antibacterial and chemotherapeutic compounds. In the case of tetrahydrobisbenzylisoquinoline dimers, presence of rigid aromatic rings and centrally locked nitrogen atoms are fundamental to their purported antibacterial activities [3].
- Mechanism of action is thought to rely on synergistic effects through inhibition of bacterial efflux pumps. Relevant to this study and its results, anti-MRSA synergistic effects between dimeric tetrahydrobisbenzylisoquinoline alkaloids such as tetrandrine (1) and commonly used antibacterial drugs have been observed to enhance in vitro inhibitory efficacy of the antibiotic drug cefazolin [4].

3.2 Results of anticyclooxygenase assay reveal selective COX-2 inhibition of limacusine.

- Limacusine (2) showed selectivity against the COX -2 (GI_{50} =68.8µM) isoform compared to tetrandrine (1) (GI_{50}>100µM)
- Both compounds exhibited modest COX-1 (GI_{50}>100µM for both compounds) and -2 activities compared to the positive drug control celecoxib (GI_{50} =6.9µM)

3.3 *In vitro* anti-COX assay for limacusine (2) correlates with in silico analysis showing selectivity towards COX-2 over COX-1.

- The attachment of alkaloid 2 is not on the hydrophobic channel but rather on a larger neighboring hydrophilic side pocket.
- Compound 2 is stabilized to the said hydrophilic pocket mostly *via pi-pi* (T-shaped) stacking interactions with His351 and His356 against the isoquinoline A and phenoxide moieties, respectively.
- Compound 2 is stabilized to the said hydrophilic pocket mostly *via pi-pi* (T-shaped) stacking interactions with His351 and His356 against the isoquinoline A and phenoxide moieties, respectively.
- The presence of hydroxyl substituent in 2 and the fact that it is more polar than 1 (based on the topological polar surface area, TPSA, estimated using Osiris Property Explorer) could be the primary reason for the stronger binding affinity of limacusine (2) with the hydrophilic binding pocket of COX-2.
- Tetrandine (1) exhibited a weaker affinity towards the said enzyme (BE=-7.4kcal/mol) compared to limacusine (2)(BE=-8.8kcal/mol).

3.4 All atoms molecular dynamics simulation confirmed limacusine stably and efficiently binds with and inhibits COX-2 active site functionalities.

- Root mean square deviation (RMSD) results

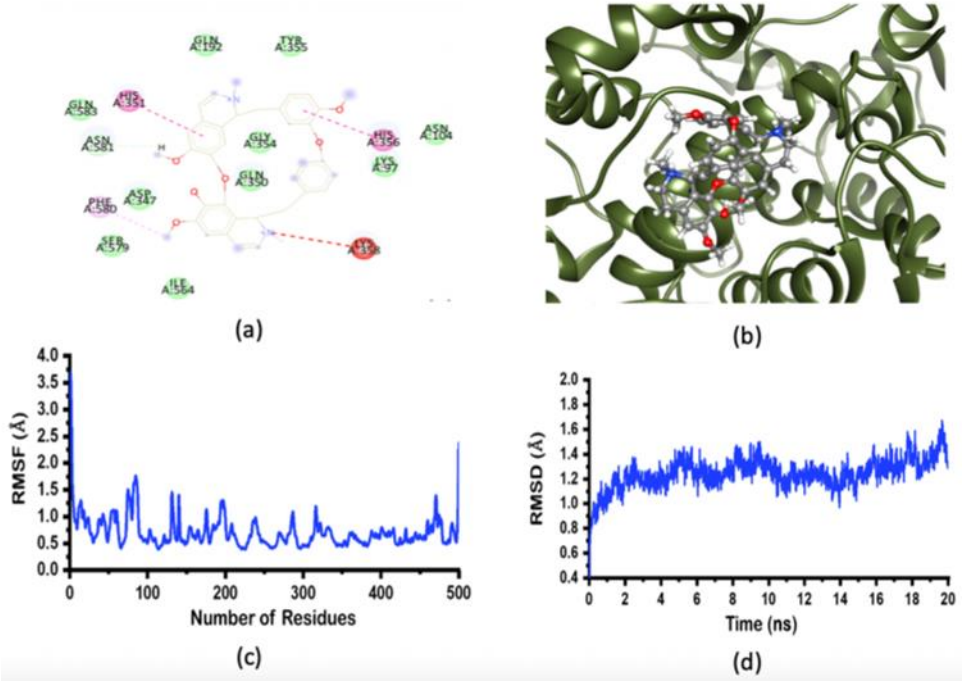

Fig. 3. Docked poses of limacusine (2) against COX-2 (PDB ID: 4M11) shown as (a) 2D binding diagram and (b) ribbon representation. RMSD of the docked poses of limacusine (2): (c) The x-axis is showing time in nanoseconds while the y-axis is showing RMSD in Å; (d) The x-axis is showing the total number of residues while the y-axis is showing RMSF in Å.

revealed complex attained stability after reaching 2ns at1Å (RMSDave=1Å) (Fig. 3c).

- Regions 80-100, 140-150, and 180-220 fluctuated a little higher than the others, while the flexibility of other residues remained lower (Fig. 3d).
- The binding affinity analysis revealed stronger binding for alkaloid 2 in the active site (ΔGtotal=-41.26kcal/mol).
- Electrostatic energy was reported at -16.42kcal/mol while vdW at -36.24kcal/mol
- Using OSIRIS Property Explorer, both compounds were predicted to show no to low toxicity risks.

4. CONCLUSION

The present study demonstrated the antibacterial and anti-inflammatory potentials of tetrahydrobisbenzylisoquinoline alkaloids especially limacusine (2) as well as the crude extracts and alkaloid sub-extracts of *Phaeanthus ophthalmicus*. This study corroborates with the results of other studies which implicates the modulatory roles of the COX-2 pathway during bacterial infection in *Pseudomonas aeruginosa* [5]. The selective inhibition of COX-2 by alkaloid 2 correlates to its observed biological activity against bacteria that causes inflammatory conjunctivitis. Future studies should confirm whether COX-2 inhibition will cause significant bacterial clearance *in vivo*. Taken together, our results suggest that COX-2 inhibition is crucial to control bacterial conjunctivitis and other bacterial infectious diseases. The antibacterial activities along with a report on the selective COX-2 inhibitory activity of alkaloid 2 support and validate the traditional use of *P. ophthalmicus* in treating conjunctivitis.

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Young Scientists' Forum

Effect of *Garcinia rubra* Merr. Leaf Extracts on Mammosphere Formation and Development of MCF7 Cancer Stem Cells

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1. INTRODUCTION

Recent developments in cancer research have been intensely focused on understanding cancer stem cells (CSCs) – tumorigenic cells that have indefinite proliferative potential and are resistant to traditional radio- and chemotherapy. Developing drugs that can selectively target these CSC's is pivotal in the prevention of cancer progression and relapse.

2. OBJECTIVES

This study analyzed the effects of *Garcinia rubra* Merr. ethyl acetate (GrEA) and hexane (GrH) fractions on undifferentiated mammospheres – cell clusters enriched with cancer stem/progenitor cells.

3. METHODS

In vitro propagation of mammospheres in serum-free media and non-adherent conditions became the basis for evaluating the cytotoxicity of the extracts against putative MCF7 CSCs. Extracts were added either at seeding (day 0) or day 4 to determine their effects on mammosphere formation and development, respectively.

4. MAJOR FINDINGS

The extracts successfully inhibited mammosphere formation (GrEA at ≥ 25 µg/mL; GrH at ≥ 12.5 µg/mL) and partially dissociated peripheral cells of the sphere (GrEA at ≥ 50 µg/mL; GrH at ≥ 12.5 µg/mL), unlike doxorubicin which failed to do both. Resazurin reduction assay was done to quantify the cytotoxicity (IC50) of doxorubicin (10.00 µg/mL), GrEA (32.93 µg/mL), and GrH (11.17 µg/mL).

5. CONCLUSION

The findings reveal the inhibitory potential of GrEA against CSCs at a concentration below its known cytotoxicity, as well as the effectivity of GrH as an anti-cancer treatment due to its ability to inhibit and dissociate spheres at a concentration similar to its known cytotoxicity. Bioactive compounds present in GrEA and GrH may, therefore, be further studied as chemotherapeutic agents that selectively target CSCs.

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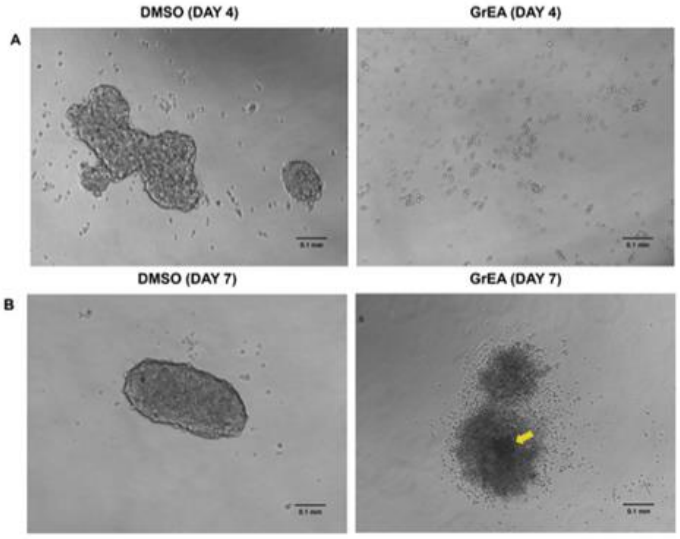

Fig. 1. (A) Inhibition of mammosphere formation by *G. rubra* ethyl acetate extract (GrEA; 25 µg/mL) after 4 days (x100). Treatment was added upon seeding (day 0). No mammospheres were formed when treated with GrEA and cells remained in single-cell suspension. (B) Morphology of untreated and GrEA-treated (50 µg/mL) mammospheres at day 7 (x100). Treatment was added to existing mammospheres at day 4 to determine the effect of the drug on mammosphere growth and development. Scale bars representative of 0.1 mm. A dark cell death region was observed in GrEA-treated spheres (yellow arrow), with cortical cells dissociating from the mammosphere.

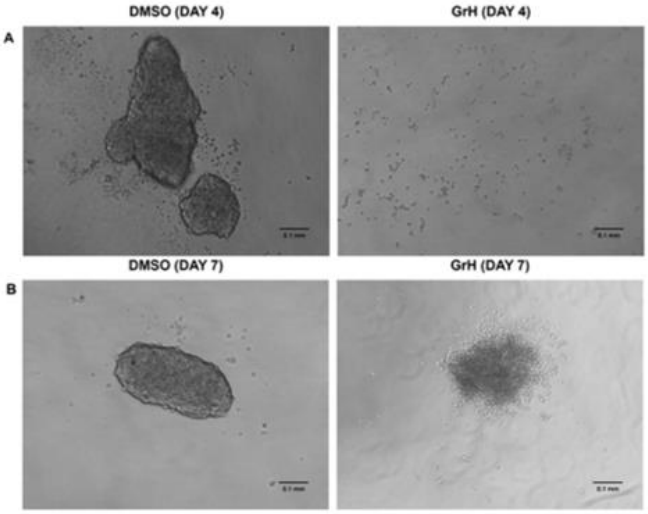

Fig. 2. (A) Inhibition of mammosphere formation of *G. rubra* hexane (GrH; 12.5 µg/mL) after 4 days (x100). Treatment was added upon seeding (day 0). No mammospheres were formed when treated with GrH and cells remained in single-cell suspension. (B) Morphology of untreated and GrH- treated (12.5 µg/mL) mammospheres at day 7 (x100). Treatment was added to existing mammospheres on day 4 to determine the effect of the drug on mammosphere growth and development. Scale bars representative of 0.1 mm. An overall darkened sphere was observed with cortical cells dissociating from the mammosphere at the highest concentration (12.5 µg/mL).

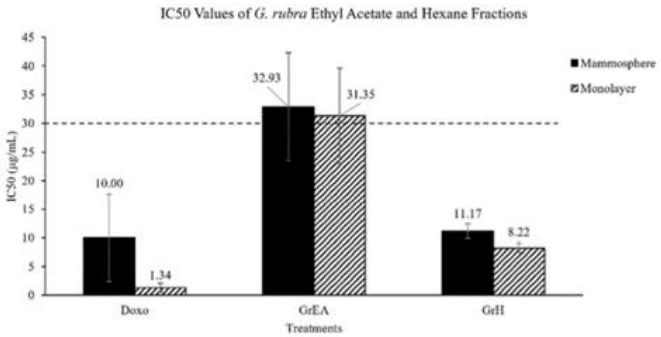

Fig. 3. IC50 values of *G. rubra* ethyl acetate and hexane fractions on mammospheres and monolayer MCF7 cells. The latter is based on the data obtained by Limbo & Jacinto (2019) using the MTT assay. Treatments with IC50 values below 30 µg/mL (dashed line) are considered active as established by the American National Cancer Institute (Jokhadze et al., 2007).

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Young Scientists' Forum

Discovery of Dual-Disease Targeting Phosphodiesterase Inhibitors from *Uvaria alba* DCM Sub-Extract with Potential Anti-Cancer and Anti-Neurodegenerative Therapeutic Properties: Insights from *in vitro* and Consensus Virtual Screening

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1. INTRODUCTION

Many complex diseases such as cancer and neurodegenerative disorders are related to multiple pathological manifestations. Moreover, drugs for their treatments often have serious side effects. Thus, it is important to discover multi-indication therapeutics that can both target a wide array of interest diseases and limit side effects. However, classic one-drug-one-disease protein drug discovery paradigms and budding polypharmacology approach rarely solve the hurdles of multi-indication drug design.

Inhibition of the major cAMP-metabolizing enzyme phosphodiesterase 4 (PDE4) has shown potential for the discovery of drugs for cancer, inflammation, and neurodegenerative disorders such as Alzheimer's disease (AD). As a springboard to explore new anti-cancer and anti-Alzheimer's chemical prototypes from rare Annonaceae species, the present study evaluated the anti-PDE4B along with antiproliferative and anticholinesterase activities of the extracts of the Philippine endemic species Uvaria alba using *in vitro* assays and framed the resulting biological significance through computational binding and reactivity-based experiments against the molecular targets ubiquitin-specific peptidase 14 (USP-14) and Kelch-like ECH- associated protein 1 (KEAP-1) in addition to PDE4 B2B and acetylcholinesterase (AChE).

2. OBJECTIVES

- Evaluate the *in vitro* antiproliferative, anticholinesterase, and anti-phosphodiesterase activities of the sub-extracts of *Uvaria alba* and;
- Discover the putative binding mechanisms of its chemical constituents *in silico.*

3. METHODOLOGY

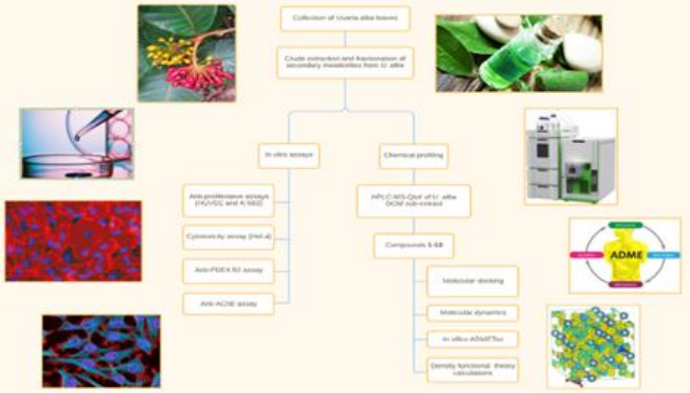

Fig 1. Schematic flow of combined in silico and in vitro determination of the biological activities of the secondary metabolites from the dichloromethane sub-extract of *Uvaria*

4. MAJOR FINDINGS

- The PDE4 B2B inhibiting dichloromethane sub-extract (UaD) of *U. alba* elicited antiproliferative activity against chronic myelogenous leukemia (K-562) and cytostatic effects against human cervical cancer (HeLa).
- The extract also profoundly inhibited AChE, an enzyme involved in the progression of neurodegenerative diseases. Chemical profiling analysis of the bioactive extract allowed the identification of eighteen putative secondary metabolites.
- Molecular docking and molecular dynamics simulations showed strong free energy binding mechanisms and dynamic stability at 50-ns simulations in the catalytic domains of PDE4 B2B, USP-14 and KEAP1-BTB domain for the benzylated dihydroflavone dichamanetin (16), and of AChE and KEAP1 for 3-(3,4- dihydroxybenzyl)-3',4',6-trihydroxy-2,4-dimethoxychalcone (8) and grandifloracin (15).
- DFT calculations demonstrated Michael addition reaction of the most electrophilic metabolite and kinetically stable grandifloracin (15) to Cys151 of the Keap1-BTB domain by forming a beta-addition adduct. The top-ranked compounds also conferred favorable in silico pharmacokinetic properties.
- Our findings suggest that the DCM sub-extract of U. alba can be a promising herbal-based therapeutic for the development of effective anti-cancer and anti- neurodegenerative therapeutic. This study demonstrates the potential of interplaying in vitro and in silico methods in discovering bioactive compounds from plants with a defined biological mechanism using computational approaches. It may facilitate transforming the conventional one-drug-one-gene-one-disease drug discovery process into a new one-drug-multi-target-multi-indication paradigm for complex diseases.

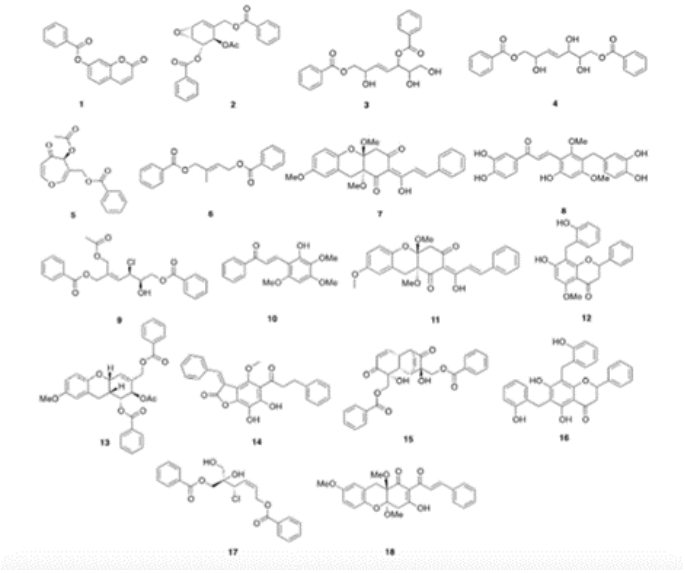

Figure 2. Structure of secondary metabolites 1-18 from *Uvaria alba*.

5. CONCLUSION

This is the first report highlighting the anti-phosphodiesterase potential of U. alba along with its anticholinesterase and cytotoxic properties. In addition, we also demonstrate for the first time the potential of grandifloracin (15) to initiate a Michael addition reaction with the active nucleophilic Cys151-containing tripeptide in the Keap1- BTB domain. With favorable pharmacokinetic properties of top-ranked ligands, these results suggest further investigations on the secondary metabolites of U. alba against their respective target proteins for the discovery of new drug leads against cancer and AD.

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Poster Session - Agri-Fisheries

Amplification and Sequence Analysis of dhaS, One Component of the INDOLE-3-Pyruvic Acid Synthetic Pathway of the Phytohormone INDOLE-3-Acetic Acid

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Abstract: Several species of *Bacillus* are plant growth-promoting rhizobacteria that can produce the phytohormone Indole-3-acetic acid (IAA) which regulates plant growth and development, and in some species, protects the host plant from pathogen invasion. Previous studies reveal several pathways for IAA biosynthesis in various bacterial species including genes and enzymes that take part in the biosynthetic pathway. In this study, we aim to amplify *dhaS*, one of the component genes from the indole-3-pyruvic acid pathway (IPyA) for IAA synthesis, and to conduct 16S rRNA sequence analysis from two *Bacillus* spp. isolated from Fermented Plant Juice biofertilizer. Genomic DNA extraction was performed in isolate 1 (*B. amyloliquefaciens*) using CTAB method followed by *dhaS* gene amplification through polymerase chain reaction using designed gene-specific primers. Sequenced DNA was then analyzed and BLASTn results showed 98% identity with *B. velezensis* strain FJAT-45028 and a translated BLAST hit with an aldehyde dehydrogenase protein, having a percent identity of 96%. The secondary structure of the protein coded by *dhaS* gene in isolate 1 was also examined using Phyre2 and Protein Data Bank (PDB) displayed models of proline dehydrogenase and aldehyde dehydrogenase. Furthermore, 16s rRNA sequences from two *Bacillus* isolates were analyzed. 16S rRNA from Isolate 1 showed a top BLAST hit with *Bacillus* sp. strain 1CY1 (99.67%) while Isolate 2 showed a top BLAST hit with *B.subtilis* strain GX S-11 (95.65%). Phylogenetic trees were generated using the Maximum likelihood method to reveal the relationship of the two isolates to their top five BLAST hits. To further understand the potential roles of *dhaS* in the IPyA pathway for IAA synthesis, transcriptional responses to l-tryptophan and functional genomic studies must be done. These will help us further understand the physiological bases of biofertilizers towards sustainable agriculture in reducing problems associated with the use of chemicals fertilizers

Keywords: IPyA, dhaS, *Bacillus* spp.

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Poster Session - Agri-Fisheries

Membrane Lipid Unsaturation Confers Cold Germination Ability in Fatty Acid Mutants of Upland Cotton *(Gossypium hirsutum)*

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Abstract: Seed germination is a highly regulated process that begins with the imbibition of a mature, dry seed and ends with the protrusion of the radicle from the seed coat. During imbibition, cell membranes reorganize from a hexagonal to a lamellar phase to restore the normal metabolic functions of the seed. Perturbations in the cell membrane during this transition result in cytoplasmic leakage. Under cold stress, leakage of solutes out of the cell is exacerbated, leading to membrane damage and eventually, to embryo death. In this study, we determined the effects of fatty acid composition on the germination under cold stress (12°C and 15°C) of upland cotton mutants with higher unsaturated: saturated fatty acid (FA) ratios. Results of the study showed a more uniform and higher germination (60-80%) of the FA mutants compared to the wild type (0-20%) under the critically low temperature of 12°C and cardinal temperature of 15°C. Consistent with the reported benefits of hydropriming on seed germination under cold stress, the treatment significantly improved the germination rate, mean germination time, mean daily germination, peak value, and germination index of all the experimental materials even under low temperatures. Biochemical assays showed that lipid peroxidation, an established measure of oxidative stress, was generally higher in the FA mutants after imbibition at 12°C and 15°C for 8 hours compared to the wild types. Conversely, electrolyte leakage was higher in the wild types than in the mutants after imbibition at 12°C and 15°C for 8 hours. Results suggest that the higher proportions of unsaturated fatty acids in the mutants enhanced the fluidity of the cell membrane during reorganization, facilitating the rapid restoration of cellular functions and allowing the faster and higher germination of the FA mutant seeds even under cold stress.

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Poster Session - Agri-Fisheries

Genotyping by Sequencing Based QTL Mapping for Yield, Grain Zinc and Iron Concentration of Rice *(Oryza sativa L.)*

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Abstract: Rice (*Oryza sativa* L.) is one of the most important food crops and serves as the principal source of calories for more than half of the world's population. Development of high-yielding rice varieties with improve grain Zn and Fe concentration is essential to help solve micronutrient malnutrition. This study was conducted at the ZES, Strategic Innovation Platform of the International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. Genotyping-by-sequencing (GBS) based high-density linkage map of rice was developed using two recombinant inbred lines (RILs) mapping populations namely IR95040/Kaliboro (2018 SNPs covering 1232.22 cM) and IR95097/ Kaliboro (2213 SNPs covering 1071.53 cM) with an average marker density of 0.61 cM and 0.48 cM, respectively. The parental lines IR95040 and IR95097 have irrigated lowland breeding lines while Kaliboro is an *Aus*-type rice variety known to have high grain Zn and Fe concentrations. RILs were grown in an alpha lattice design with two replications each during the 2017 dry and wet seasons, and 2018 dry seasons. In total, five QTL for YLD were identified on chromosomes 4, 6, and 7, with phenotypic variance, explained (PVE) ranged from 7.07-14.86%. For Fe, three QTL (*$qFe_{4.1}$, $qFe_{6.1}$, and $qFe_{6.2}$*) were identified on chromosomes 4 and 6. The favorable donor allele Kaliboro has contributed to the high Fe concentration in *$qFe_{4.1}$* and *$qFe_{6.1}$*. A total of 12 QTL associated with Zn were identified on chromosomes 3, 4, 5, 6, 7, 8, 10, and 12, which accounted for 5.17-18.33% of PVE. Four QTL were consistently detected on chromosomes 3, 7, and 10 in IR95040/Kaliboro and on chromosome 4 in IR95040/Kaliboro viz. *$qZn_{3.1}$*, *$qZn_{7.3}$*, *$qZn_{10.1}$*, and *$qZn_{4.1}$*. Validation of the genomic regions reported in this study will help accelerate the development of SNP chips for breeding healthier rice varieties and will be beneficial in the rapid development and deployment of biofortified rice varieties.

Keywords: rice, zinc, GBS, Quantitative Trait Loci

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Poster Session - Agri-Fisheries

Validation of Chalcone Synthase Gene Single Nucleotide Polymorphism for Red Color in Mango *(Mangifera indica L.)*

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Abstract: The Carabao mango is the Philippines' vital and premium export variety because of its nutritive value and its distinctive sweet taste compare to other varieties. Carabao variety is one of the top fruit exports that has been essential in the growth of the Philippine economy. According to PSA (2015), mango ranks third in terms of production volume of 885,000 metric tons. Its export potential is, however, hampered by industry problems and a growing preference for mangoes with red blush. Red blush trait correlates to plant resistance against cold and pathogens and has a potential benefit to human health. Ten newly designed primers based on SNP of chalcone synthase gene derived from the GBS data of 341 mango genotypes were used to amplify chalcone synthase from sixteen mango genotypes with red and yellow skin color. This was an attempt to develop SNP markers for a red blush that can be utilized for mango breeding programs. Only four out of ten primers were successfully optimized for the amplification of chalcone synthase namely FLVS, Chs2, DRPRGA3, and At4g. The optimum annealing temperatures for each of the markers were 51.6°C, 49°C, 47.9°C, and 46°C, respectively, resulting in amplification of desired DNA bands after PCR. The primers were able to differentiate mangoes with red peels from those with yellow peels. After sequencing of selected PCR products followed by sequence analysis, it was found that red and yellow varieties exhibited highly conserved sequences for the chalcone synthase. However, different insertions and deletions within the conserved region were also observed. The phylogenetic tree based on Chs2 sequence of sixteen mango genotypes showed that the same-colored mangoes tended to cluster together in one clade, showing the usefulness of chalcone synthase SNPs for identifying red skin color in mango.

Keywords: Carabao variety, SNP marker, chalcone synthase, red blush

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Poster Session - Agri-Fisheries

Identification of Linamarase-Producing Lactic Acid Bacteria and Yeasts for Cassava *(Manihot esculenta Crantz)* Sourdough Fermentation

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Abstract: Cassava (*Manihot esculenta* Crantz) is a tropical root crop considered a staple food to millions of people worldwide. However, one of the drawbacks of this root crop is the presence of toxic cyanogenic compounds used by the plant as a defense mechanism against pests. Fortunately, the toxic compound can be degraded during fermentation, facilitated by linamarase which is a hydrolytic enzyme for cyanide degradation and can be produced by lactic acid bacteria (LAB) and yeasts. Thus, this project aimed to screen LAB and yeasts for their ability to produce linamarase which can then be utilized for cassava sourdough fermentation. Previously, we have screened a total of 166 LAB from an existing collection of cultures and from cassava sourdough, and 113 yeast isolates from the forest canopy for their ability to degrade cyanide. To ensure that the isolates are safe to be used for the fermentation of cassava sourdough, the isolates were identified through 16S and 18S rRNA sequencing. The high linamarase-producing LAB isolates were that from papaya flower, identified as *Enterococcus faecalis,* and that from cassava sourdough, identified as *Leuconostoc mesenteroides.* Moreover, the three yeast isolates with high linamarase activity were identified as *Cyberlindnera mrakii.* The HCN degrading abilities of these isolates were characterized during sourdough and bread preparation.

Keywords: Cassava, Lactic Acid Bacteria, Yeast, Linamarase, sourdough

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Poster Session - Agri-Fisheries

Sequencing and Identification of WRKY Transcription Factors in Abaca *(Musa textilis Neé)*

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Abstract: The abaca plant (*Musa textilis* Neé) is a fiber crop indigenous to the Philippines and is a close relative of bananas. Diseases due to viral pathogens such as the abaca bunchy top virus (ABTV), banana bract mosaic virus (BBrMV), and sugarcane mosaic virus (SCMV) can be detrimental to the quality and yield of fiber produced. Though of great economic importance to the country, there has been only a few published works that delve into the molecular genetic mechanisms that underlie processes such as fiber production and disease resistance. This study is focused on the WRKY genes, which are a family of transcription factors ubiquitous to green plants that are identified by the conserved WRKYGQK motif and a C2H2 or C2HC zinc-finger binding motif. These transcription factors have been implicated in a variety of biological functions dealing with plant development and stress responses, among others. Total DNA was extracted from *M. textilis* var 'Abuab' and used as a template to amplify WRKY genes with PCR using degenerate primers targeting the WRKY motif. Amplified fragments of 250 bp, 300 bp, 800 bp, and 1000 bp were purified, cloned, and sent for sequencing. Comparison of the DNA and predicted translated protein sequences obtained to online databases with BLAST revealed 27 sequences contained WRKYGQK and partial C2HX domains that were closely related to predicted *Musa acuminata* and *Musa balbisiana* WRKY transcripts. Mapping and phylogenetic analysis revealed that the sequences clustered with either Group II or Group III WRKY domain types. This is the first reported study on sequence identification of WRKY transcription factors in abaca and is the first step to identify genes for targeted genetic engineering for increased disease and stress tolerance in abaca.

Keywords: abaca, PCR, WRKY, transcription factor

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Poster Session - Agri-Fisheries

Isolation and Characterization of Biotin Synthase Gene in Coconut *(Cocos nucifera L.)*

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Abstract: Coconut, *Cocos nucifera* L., is an important oil crop in the Philippines. Recently, there had been growing interest in increasing its oil content through metabolic engineering. The first committed step in the biosynthesis of lipids is catalyzed by the tightly regulated enzyme, Acetyl-CoA carboxylase (ACCase) which significantly controls the carbon flux in this pathway (Ali and Tyagi, 2016). Biotin acts as a cofactor for ACCAse and its synthesis involves four significant enzymes. One of which, biotin synthase, is considered as the rate-limiting step in the biotin synthesis pathway. Increasing the concentration of biotin on its maximum level as the co-factor of ACCAse would also increase the rate of lipid biosynthesis. This study aimed to isolate and characterize the biotin synthase gene from coconut towards understanding its utility in increasing oil production through biotechnology. The isolated 749-base pair nucleotide sequence revealed significant homology with the biotin synthase of various plant species such as *Elaeis guineensis*, *Phoenix dactylifera*, *Musa acuminata*, *Asparagus officinalis*, *Ananas comosus*, *Sorghum bicolor*, and *Zea mays* with 76- 85% identity. The deduced amino acid sequence consisted of 244 residues and shared 59-73% homology with the Radical S-adenosylmethionine (SAM) region and the Biotin and Thiamin Synthesis (BATS) associated domain of other oil crops. Phylogenetic analysis showed that the coconut biotin synthase gene was closely similar to those of the oil palm and the date palm. Sequence analysis of the isolated gene fragment from coconut ascertained its identity to published biotin synthase gene sequences. This characterized partial coconut biotin synthase gene sequence could be used to further isolate the full-length gene.

Keywords: Coconut, lipid biosynthesis, biotin, biotin synthase

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Poster Session - Agri-Fisheries

Developing Morphological and Simple Sequence Repeats Markers for Putative Drought Tolerant Papayas

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Abstract: Drought as a result of climate change has adverse effects on plant production. It is known to negatively impacted food production. One important crop in the Philippines which is affected by drought is papaya. Papaya ringspot virus (PRSV) and bacterial crown rot (BCR) are two other pressing problems of this crop. Under drought conditions in the field, papaya showed decrease productivity as it produces smaller and fewer fruits but had sweeter flesh. Ten papaya genotypes were selected as putative drought tolerant. The basis for drought tolerance is their relative growth rate in terms of plant height, stem diameter, the chlorophyll content of leaves, and a number of closed stomata during the imposition of drought. Compared to drought susceptible genotypes, putative drought-tolerant papaya genotypes have more closed stomata, higher chlorophyll content, bigger stem, and crown diameter, and have a greater number of leaves. To mark the putative drought-tolerant selections, Screening of 21 simple sequence repeats (SSR) primers to reveal polymorphism among 15 genotypes was done. Polymorphic bands were consistently revealed by 5 SSR primers across 15 genotypes. From the 21, 2 SSR primers have been selected to mark putative drought tolerant genotypes.

Keywords: *Carica papaya* L. chlorophyll, drought, simple sequence repeats, stomata

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Poster Session - Agri-Fisheries

Perturbation of Rice *(Oryza sativa L.)* Leaf Architecture Through Ectopic Overexpression of Polyadenylate Binding Protein (PABP)

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Abstract: Functional genomics through the use of gain of function approach such as full-length cDNA overexpression is often utilized to potentially identify gene function. The gain-of-function approach offers an advantage over loss-of-function mutants since most species have a large proportion of redundant genes, and the function of such genes is impossible to identify using a gene-knockout approach because the redundant copy just compensates for the loss of function created. In this study, full-length cDNA was generated from RNAs derived from rice seedling root tissues. A rice overexpression mutant manifesting debilitating dwarfism was generated, however, closer examination of the fl-cDNA revealed it to be a polyadenylate binding protein of *Schizosaccharomyces cerevisiae* origin. The fl-cDNA is 2,254 bp with the longest predicted ORF of 2,013 bp, representing 89.31% of the total length. The predicted polypeptide is 670 amino acid residues. Analysis of the protein sequence revealed a dominant RNA recognition motif (RRM) at amino acid positions 134-204, 227-297, and 330-400. RNA recognition motif (RRM) is characterized by 90 amino acid residues with two conserved sequences of eight and six amino acids, called RNP-1 and RNP-2, respectively, in the protein primary structure. Proteins with RNA recognition motif have multitudes of functions which include the heterogeneous nuclear ribonucleoproteins (hnRNPs) that function in the processing of hnRNAs into a mature mRNA as well as a *trans-regulatory* factor in gene expression proteins involved in the regulation of alternative splicing, RNA stability, and translation and protein components of small nuclear ribonucleoprotein.

Keywords: full-length cDNA; RNA recognition motif*; Schizosaccromyces cerevisiae;* heterologous overexpression

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Poster Session - Agri-Fisheries

Improvement of Asian Rice Cultivars through Marker-Assisted Introgression of Yield QTLS Grain Number 1A (GN1A) and Wealthy Farmer's Panicle (WFP)

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Abstract: Food insecurity is one of the major problems of our growing population. With the threats of climate change and an exponential decrease in arable land, crop productivity improvement is needed more than ever. The advent of molecular markers has given the field of plant breeding the ability to fast track plant selection with target genes for selected traits in shorter generations. Introgression of abiotic and biotic related QTLs has been a common practice for crop improvement; however, utilizing yield-related QTLs has remained limited. In this study, major QTLs *Grain number 1a* (*Gn1a*), for an increase in spikelet number, and *Wealthy Farmer's Panicle* (*WFP*), for an increase in primary branching, were introgressed in four Asian rice cultivars (Kinandang patong, IRAT 109, Silewah, Basmati) from the donor parents ST12 and ST6. The rice microsatellite (RM) markers RM3360, RM 5493, and RM3452 were used to confirm the introgression. Introgressed lines at BC_3F_5 and BC_3F_6 generation from each genetic background were evaluated for phenotypic traits, focusing on primary branching per panicle (PBPP) and total spikelet per panicle (TSPP). Improvements in TSPP and PBPP were observed in selected lines representing four genetic backgrounds. Improved lines with introgressed *Gn1a* or *WFP* were comparatively similar to its recurrent parent in yield-related traits such as heading date (HD), plant height (PH), hundred-grain weight (HGW). This study demonstrates the success of utilizing yield QTLs for improving the yield performance of our existing rice cultivars.

Keywords: Rice, Molecular Breeding, Marker-assisted breeding, *Gn1a*, *WFP*, QTL, Crop Improvement

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Poster Session - Agri-Fisheries

The Genetic Diversity of Brown and Red Rice Samples and their Antioxidant Activities, Anticancer, and Low Glycemic Properties

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Abstract: The double burden nutrition problem (i.e. lack of essential nutrients and calorie-rich food) is a growing epidemic in Asia and around the globe, where cancer and diabetes are part of the major causes of death. Hence, bringing diet-based nutritional intervention by identifying rice varieties with grains rich in nutrient density is becoming increasingly significant. In this research, a metabolome-wide association study (mWAS) was conducted by linking the metabolites with ultra-dense genotyping data of 1.7 million single nucleotide polymorphisms (SNPs). It revealed novel genetic regions and/or candidate genes influencing the levels of various amino acids and catechin as pivotal molecules in determining the nutritional quality of brown and red rice. Importantly, the novel genetic variants identified through mWAS which was found to affect catechin accumulation on chromosome 7 (6.06-6.43 Mb region), co-located with GWAS peaks of red color and amylopectin composition and thereby the glycemic index. Contrasting lines resulted from mWAs analysis were used to extract the free and bound phenolic components. Red rice extract has higher phenolic content thereby, effective antioxidant activities than brown rice extract in concordance with its potent antiproliferative property against different cancer cell lines. The extract of red rice exhibited strong anti-proliferative activity with mean IC50 values of 8.25, 17.13, and 24.47 mg/mL while the brown rice extract with mean IC50 values of 1580.57, 23007.56, and 55400.58 mg/mL against HT-29, MCF-7, and HepG2, respectively. Further, *in vitro* GI analysis has demonstrated a lower glycemic index in milled red rice with high resistant starch. The selected rice samples may be used as a functional food to address diabetes and certain forms of cancer.

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Poster Session - Agri-Fisheries

TGW6 Knockdown Causes Pleiotropic Effects in Elite Rice Varieties Increasing Yield

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Abstract: An estimated 4 billion people depend on rice as their staple food and this number increases rapidly. As such, increasing its production is a major challenge. A suggested solution for this is gene editing yield-related regions of interest in elite indica rice varieties such as IR64 and Samba Mahsuri (SM) since these varieties are already widely accepted in the market. One promising region of interest is the *THOUSAND GRAIN WEIGHT 6 (TWG6)* because it has shown promising influence in grain weight when knocked out in japonica varieties. In this study, *TGW6* was knocked down in IR64 and SM rice varieties using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cpf1 system. A CRISPR Cpf1 vector construct (IRS-1466) was assembled to target *TGW6* in both IR64 and SM. The resulting knockout IR64 and SM were analyzed through PCR screening for the presence of CRISPR gene while cutting efficiency and mutational changes in the region of interest were analyzed using T7 assay and sequencing, respectively. Knockout SM had 81.7% cutting efficiency while knockout IR64 had 94.34% cutting efficiency. Sequence analysis revealed minimal single-nucleotide insertion-deletions (indels) in the region of interest. Phenotypic data showed a significant increase in total grain weight of both knockout SM (increased up to 33.5%) and IR64 (105.11%) compared to the wild-type controls. In addition, an increase in grains per panicle was also observed in both SM and IR64 knockouts. These results reveal that targeted knockdown via CRISPR-Cpf1 of *TGW6* increases grain weight thus, increasing rice grain yield which has great potential as a possible solution in the foreseeable rice production challenges ahead.

Keywords: CRISPR-Cpf1, Gene editing, Rice, and Yield.

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Poster Session - Biodiversity

Microencapsulation of *Pediococcus acidilactici* In Chitosan/Polyaniline Composite

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Abstract: Microencapsulation is among several methods and strategies that are being developed to protect probiotic bacteria against adverse environmental conditions in the stomach and increase their recovery rates. This study aims to determine the potential microencapsulation and delivery of *Pediococcus acidilactici* in Chitosan/ Polyaniline composite, wherein ionic gelation through the extrusion method of microencapsulation is used. It also determined the shelf life and viability of the microencapsulated probiotic. Results show that the optimal CS/PANI ratio that could encapsulate *Pediococcus acidilactici* is 3%/0.5% extruded in 1% sodium citrate. The number of probiotic cells that have been entrapped per microbead is 1.61 x 10^6 Cfu/ml ± 0.08. The microencapsulated probiotic was subjected to simulated gastrointestinal (GI) conditions to determine survival through GI transit. The observed cell release of entrapped probiotics in the simulated gastric fluid is considerably higher than expected ranging from 10^5-10^6 cumulative value due to the immediate swelling of the CS/PANI microbeads. However, at the end of exposure to the simulated intestinal fluid, the cumulative release is 10^6-10^7, indicating potential to be released in the gut. While it was found that the cell viability of microencapsulated (46.23%±0.02) probiotics is low as compared to the free cells (69.64%±0.04) after 30 days of storage at 4°C, the results at 30th day of storage at room temperature showed reciprocal findings for free cells (25.92±0.16) and microencapsulated (33.10±0.13). Therefore, microencapsulation of *P. acidilactici* can be a considerable means to achieve higher cell viability both in the course of gastrointestinal delivery and storing at room temperature.

Keywords: Microencapsulation, *Pediococcus acidilactici,* chitosan, polyaniline

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Poster Session - Biodiversity

Construction and Phenotypic and Molecular Assessment of a Bidirectional Plasmid Vector Incorporating the *Oryza sativa* L. spp. japonica BIP1 Bidirectional Promoter and Antibiotic Resistance Genes

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Abstract: Bidirectional promoters are promoters that allow dual-direction gene expression. Often, plasmid vectors with unidirectional promoters are available thus, it would be more advantageous if plasmid vectors can drive gene expression in both directions. This study aims to construct a bidirectional plasmid vector utilizing antibiotic resistance genes and a reported bidirectional *Oryza sativa* L. spp. japonica *(OsBIP1)* promoter. The *OsBIP1* promoter obtained from the Nipponbare variety and the ampicillin (*amp*) and chloramphenicol acetyltransferase (*cat*) antibiotic resistance reporter genes were amplified using primers appended with different restriction sites to facilitate directional ligation and employed optimized PCR conditions for annealing temperature (T_m) and $MgCl_2$ and dNTP concentrations. The amplified *OsBIP1* bidirectional promoter and these antibiotic resistance genes were restriction digested and directionally-ligated (*OsBIP1-sense* and *OsBIP1-antisense* orientations) into the pBI121 plasmid backbone using various insert: vector ratios to assess for promoter activity and directionality. The ligation products were transformed into competent *E.coli DH5α* and grown in media supplemented with ampicillin and/or chloramphenicol to phenotypically assess the developed bidirectional plasmid.

Cell growth was observed in the ampicillin-only and in the ampicillin-chloramphenicol plates. Maximal bacterial colony growth for *OsBIP1-sense* and *OsBIP1-antisense* on these double antibiotic plates was detected using the 1:1 insert: vector (v/v) ratio. PCR confirmation detected the expected amplicon for *OsBIP1* promoter*::amp* using the extracted plasmid DNA from randomly picked bacterial clones. The growth of the transformed bacteria in the double antibiotic plates and the detection of the expected *OsBIP1* promoter*::amp* amplicon verify the construction of the bidirectional vector. This bidirectional vector may be used as a baseline for future studies.

Keywords: bidirectional promoters, antibiotic resistance genes, plasmid vector, *Oryza sativa*, *OsBIP1*

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Poster Session - Biodiversity

Oligosaccharide Profiling of Milk Colostrum from Two Breeds of Porcine

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Abstract: Colostrum oligosaccharides are important components of milk with multifunctional health benefits to newborn children. There is an increasing interest in porcine milk because of its similarity to the milk components in human milk. However, there is limited data on the oligosaccharide profile in porcine colostrum. The study focuses on the identification and annotation of colostrum oligosaccharides in two breeds of porcine using liquid chromatography-mass spectrometry and NIST mass spectral library search methods. One-hundred sixty-six oligosaccharides were identified and used to build a glycan mass spectral library of porcine milk. Comparing PMO (Porcine milk oligosaccharides) profile using NIST glycan library repository, 63 is found unique to porcine, 79 are oligosaccharides that shared a common structure with mature human milk and 24 are common to other mature mammalian milk. The PMO displayed different patterns of variation between black and white breeds. Of which, PMO content is highest in the black breed, giving 12 unique oligosaccharides that are not found in the white breed. In general, porcine milk contains both acidic (sialylated) and neutral (fucosylated) oligosaccharides, but oligosaccharides containing sialic acid predominate the PMO profile in both breeds. Among the identified oligosaccharide, pLNnH (neutral) and 3'-Sialyllactose (acidic) is the most abundant in both breeds. In summary, colostrum from the black breed has the best oligosaccharide profile and diversity.

Keywords: *Milk oligosaccharides, Colostrum, Porcine Milk Oligosaccharides, NIST MS search, Hybrid search*

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Poster Session - Biodiversity

Microbial Community Diversities Across Hyporheic Zones of Gravel Bars in a River: Taxonomic and Functional Distributions

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Abstract: Gravel bars are a geographical component in rivers and known to introduce habitat heterogeneity in river ecosystems. Among the gravel bar characteristics, the hyporheic zone, the area where surface water and groundwater meets, is known to largely affect the microbial communities. Microbial communities in gravel bars are important to the biogeochemical cycle in the riverine ecosystem. In this study, we aimed to investigate the spatial distribution of microbial diversities (α and β diversity) and the biological functions in three different sampling points (downwelling, upwelling, and their intermediate point) within three gravel bars in the Tenryu River, Japan using 16S rRNA amplicon sequencing. The NMDS plots showed differences in bacterial community structures (β-diversity), with a clear separation of three points, but the α-diversity was constant. Among the microbial organisms, the Proteobacteria were found to be the most abundant taxa throughout three points. Functional Annotation of Prokaryotic Taxa (FAPROTAX) was used to identify the potential biological function. We found the chemoheterotrophy was the most abundant function throughout the three points, suggesting the importance of primary energy metabolism for the microbial community. Overall, our study highlights the changes of microbial communities' composition among gravel bars spatial differences and future studies assessing river management.

Keywords: α-diversity, β-diversity, hyporheic zone, microbial communities, 16S rRNA amplicon sequencing

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Poster Session - Biodiversity

Purification and Characterization of Antioxidant Proteins from Rice Bean (*Vigna umbellata*)

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Abstract: Higher intake of plant protein is associated with lower risk in degenerative diseases caused by oxidative stress, thereby places enormous demand in the identification of plant-based sources of protein like legumes. Rice bean (*Vigna umbellata*) is known to be a great source of low-cost and good nutritional quality protein for utilization in food products. This study characterized the proteins from rice beans reflecting their nutritional quality and potential as a food additive. The total soluble proteins of rice beans were extracted using 50 mM Tris-HCl (pH 7.2) and 35mM potassium phosphate buffer (pH 7.2) containing 0.40 M NaCl. Major storage protein was purified using ammonium sulfate fractionation, selective precipitation, and gel filtration chromatography. Pepsin digestion was done for 2 hours followed by multiple digestions of trypsin, chymotrypsin, thermolysin digests on the purified protein for 1 hour, 2 hours, and 4 hours, respectively. *V. umbellata* protein concentrate, hydrolysates (1 hr, 2 hrs, 4hrs), and dialysate were subjected to DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay to confirm antioxidant activity. *V. umbellata* crude protein extract and enzyme-digested protein extract provided (4-hr incubation) the highest antioxidant activity.

Keywords: *Vigna umbellata*, antioxidant activity, protein, hydrolysate

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Poster Session - Biodiversity

Identification of Non-Peptidic Venom Components of Philippine Tarantula Species

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Abstract: There is a growing interest in the non-peptidic components of spider venom because of the neurotoxic and cytotoxic activities of this group of compounds. In this study, we aimed to identify the non-peptidic venom components of tarantula species collected from the Municipality of Wao in Lanao del Sur in Mindanao.

Spider venom was extracted by electrostimulation for untargeted metabolomics analysis. Initial separation of the components of the venom was done by reverse-phase high-performance liquid chromatography (RP-HPLC). A fraction previously reported to contain non-peptidic bioactive components were further analyzed by Ultra Performance Liquid Chromatography – Quadrupole Time of Flight Data Dependent Analysis (UPLC-QTOF DDA) to structurally identify the components.

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Poster Session - Biodiversity

Ampalaya (*Momordica charantia*) and Bayabas (*Psidium Guajava*) Extracts' Synergistic Effect on Immortalized Lung Tumor Spheroids (Gl001) Verified in Rt-Pcr and *In Silico* Modelling

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Abstract: In the Philippines, lung cancer is the leading cause of cancer-related deaths in males and the fourth among females. Thus, there is a need for the development of accessible and effective treatments for the disease. *Momordica charantia* and *Psidium guajava* have been found to be cytotoxic to A549 lung cancer cells, but the potential of these natural products as a source of anti-cancer bioactive components remain untapped. This study aimed to determine the synergistic effects of *M. charantia* and *P. guajava* extracts on spheroidal cultures of GL001 lung cancer cells. Through the hanging drop method, the study was able to generate spheroidal GL001cells, which provided a more appropriate representation of a tumor microenvironment in a Filipino patient-derived lung cancer cell. Furthermore, RT-qPCR analysis revealed that combinatorial extracts treatment induced upregulation of *CASP8*, a gene involved in Bid cleavage essential in apoptosis activation, and downregulation of *MDM2*, a gene involved in p53 degradation promoting tumorigenesis. This suggested that the anticancer activity of the combined extracts is through promoting apoptosis and cell cycle arrest. Verification with the *in silico* molecular docking analysis showed that metabolites found in ampalaya and bayabas may synergistically act by binding to specific sites in CASP8 and MDM2 protein. In particular, the synergistic binding of certain ampalaya and bayabas metabolites to CASP8 potentially stabilizes Bid-CASP8 interaction and promotes apoptosis. On the other hand, the synergistic effect of certain ampalaya and bayabas compounds on MDM2 led to either a decreased p53-MDM2 binding or a potential stabilizing effect. Both scenarios possibly disrupt p53-MDM2 interaction, preventing p53 degradation and thereby promoting cell cycle arrest. Overall, this study showed through gene expression and molecular docking analysis that *M. charantia* and *P. guajava* have synergistic anticancer effects on lung cancer cells.

Keywords: *Ampalaya, Bayabas, GL001 spheroidal cells, CASP8, MDM2,* metabolites, molecular docking

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Poster Session - Biodiversity

The Discovery of "Philippine Cherry" *Syzygium Lineatum* for Diabetes: *In Vitro* and *In Silico* Studies

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Abstract: Diabetes mellitus is growing to epidemic proportions, leading to devastating complications if left untreated. This study investigated the enzyme inhibitory activity, LC-MS analysis of the leaf extracts of *S. lineatum* (DC). Merr. & L.M. Perry and computational-based studies on the binding mechanisms of the putative bioactive compounds. The dichloromethane-methanol crude extract was subjected to solvent partitioning to yield petroleum ether, dichloromethane and n-butanol sub-extracts. Enzyme inhibitory assays against α-glucosidase and α-amylase were conducted. Identification of putative compounds were conducted through LC-HRMS analysis. Molecular docking was conducted to identify the best-suited receptor sites. In the α-glucosidase inhibitory assay, the n-butanol sub-extract of *S. lineatum* displayed an IC_{50} value of 48.31 ± 0.890 ug/mL whereas, the crude extract has shown an IC_{50} value of 13.60 ± 0.050 ug/mL in the α–amylase inhibition assay. LC-HRMS analysis of the crude and n-butanol sub-extract yielded staphylionoside J (1), quercetin-3-glucoside (2), myricetin (3), quercitrin (4), kaempferol-3-rhamnoside (5), arjunolic acid (6), and asiatic acid (7). Molecular docking studies revealed strong binding affinities (ca. -9 kJ/mol) on the catalytic site of α-glucosidase for the flavonoids myricetin (3), quercitrin (4), kaempferol-3-rhamnoside (5). Thus, the plant-derived bioactive compounds contribute to the inhibition of the enzymes in the *in vitro* assays complemented by in silico experiments. This is a pioneering study of *S. lineatum* leaf extracts which presents potential benefits on inhibition of intestinal enzymes.

Keywords: *Syzygium lineatum, α-glucosidase, α-amylase,* diabetes, euglycemic

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Poster Session - Biodiversity

Suitability of ITS2, nad1 and ycf1b as DNA Barcodes for the Ten Medicinal Plants of the Philippines

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Abstract: The Department of Health had listed ten herbal medicines commonly used in the Philippines for different ailments referred to as "Sampung Halamang Gamot" or 10HG[1]. Ensuring proper identification of these herbal medicines is important to prevent numerous adverse reactions. DNA barcoding, a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species[2], can be used to augment the current methods of identification. ITS2, *nad1*, and *ycf1b* are three of the most commonly used genetic loci for DNA barcoding[3,4].

This study determined the suitability of these genetic markers as DNA barcodes for the identification of 10HG by comparing their PCR success rate, sequence quality, and discriminatory power. For PCR success rate, the 10 HG gave 100%, 80%, and 40% amplification using ITS2, *ycf1b*, and *nad1*, respectively. With regard to the sequence quality, high-quality sequences were given by *A. sativum, P.guajava, Q. indica,* and *E.microphylla* using ITS2 while high-quality sequences were given by *C. alata*, *M. charantia*, *P. guajava*, *V. negundo*, and *Q. indica* using *ycf1b.* Finally, for the discriminatory power, six out of the 10 HG gave species-level discrimination using ITS2 and two out of the ten for *ycf1b. Nad1* data for sequence quality and discriminatory power was inconclusive.

From the data gathered, it can be inferred that ITS2 is the most suitable DNA barcode for the 10HG. The utilization of ITS2 as a standard DNA barcode for 10HG is supported by the study of Chen et al.[5] wherein they concluded that the ITS2 region can be potentially used as a standard DNA barcode to identify medicinal plants and their closely related species. For the suitability of *nad1* and *ycf1b* as 10HG DNA barcodes, however, further studies are recommended to make a conclusion.

Keywords: DNA Barcoding, *ITS2, nad1, ycf1b,* Medicinal plants

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Poster Session - Biodiversity

Trichoderma reesei Rad51 Tolerates Mismatches in Hybrid Meiosis with Diverse Genome Sequences

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Abstract: Most eukaryotes possess two RecA-like recombinases (ubiquitous Rad51 and meiosis-specific Dmc1) to promote interhomolog recombination during meiosis. However, some eukaryotes have lost Dmc1. Given that mammalian and yeast *S. cerevisiae* (*Sc*) Dmc1 have been shown to stabilize recombination intermediates containing mismatches better than Rad51, we used the Pezizomycotina filamentous fungus *Trichoderma reesei* to address if and how Rad51-only eukaryotes conduct interhomolog recombination in zygotes with high sequence heterogeneity. We applied multidisciplinary approaches (next- and third-generation sequencing technology, genetics, cytology, bioinformatics, biochemistry, and single-molecule biophysics) to show that *T. reesei* Rad51 (*Tr*Rad51) is indispensable for interhomolog recombination during meiosis and, like *Sc*Dmc1, *Tr*Rad51 possesses better mismatch tolerance than *Sc*Rad51 during homologous recombination. Our results also indicate that the ancestral *Tr*Rad51 evolved to acquire *Sc*Dmc1-like properties by creating multiple structural variations, including via amino acid residues in the L1 and L2 DNA-binding loops.

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Poster Session - Biodiversity

Screening and Identification of Alkaliphilic Bacteria Producing Cyclodextrin Glucanotransferase and Proteases from Manleluag Hyperalkaline Spring

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Abstract: Manleluag hyperalkaline spring is a unique spring in Pangasinan, Philippines. In this study, alkaliphilic bacteria were screened for the presence of industrially important enzymes – cyclodextrin glucanotransferase (CGTase) and proteases. From a total of 826 bacterial isolates, 54 isolates exhibited significant CGTase production evaluated using the Phenolphthalein Assay. The highest recorded CGTase activity is at 46.01 U/ml. Using two pH conditions (pH 7 and pH 10), 428 isolates showed protease activity when tested using Skim Milk Assay with an activity reaching up to a clearing zone ratio of 0.2056. Based on whole-genome sequence assemblies, the top CGTase producer was found to be a *Bacillus lehensis* isolate while the top protease producer was a putative *Bacillus gibsonii* isolate. Hence, this study showed that the Manleluag hyperalkaline spring served as a good source of alkaliphilic bacteria producing industrially important compounds.

Keywords: Manleluag hyperalkaline spring, alkaliphilic bacteria, protease, cyclodextrin glucanotransferase

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Poster Session - Bioinformatics

Automatic Recognition of Central Vein and Sinusoids in Rat Liver Histopathological Images for Damage Assessment Caused by Alcohol

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Abstract: Chronic binge alcohol consumption is a leading cause of chronic liver disease worldwide. This disease could lead to cirrhosis and hepatocellular carcinoma. Prolonged alcohol drinking significantly affects the liver, kidney, pancreas, heart, lungs, Central Nervous System (CNS), and other organs, as reported by recent work in literature. Building a tool for assessing and quantifying these damages could provide a different perspective on medical diagnosis. This work is part of the effort of infusing computational biology in medical diagnosis. In this research, we investigated how to detect the Central Vein and Sinusoids automatically by a computer. Based on the literature, the Central Vein and Sinusoids are a few of the liver morpho-anatomical parts affected by alcohol. Detecting these parts automatically could revolutionize the damage estimation in the liver. We composed our dataset from the Michigan Histology and Virtual Microscopy Learning Resource. One hundred histopathological images were collected and were processed for analysis. These histopathological images were manually cropped using 40x.svs (Scan Scope Virtual Slide) magnification and were manually labeled and annotated for ground truth comparison and performance analysis. Sixty percent (60%) of the dataset was used for training, and the other forty percent (40%) were used for testing. Gaussian and thresholding filters were used for the recognizer. Our initial results show that the model could meet 90%-pixel accuracy in detecting the Central Vein and Sinusoids in the Histopathological images. This finding shows promising development in the field of medical diagnosis.

Keywords: Alcohol, Damage Assessment, Hepato, Sinusoids, Central Vein, Computer Vision, Artificial Intelligence, Machine Learning

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Poster Session - Bioinformatics

Microarray Analysis of MR7-3, High Amino Acid Rice, During Seed Development

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Abstract: MR7-3, a stable mutant line from mature Korean cultivar Donganbyeo (WT) subjected to 70 Gy, was known to have a 20 times greater amino acid content but chalky grain quality. To elucidate the transcriptional changes in MR7-3, microarray data generated from RiceXPro using 5, 10, 15, and 20 days after pollination (DAP) grain developmental stages of WT and MR7-3 were analyzed. Differentially expressed genes (DEGs) were filtered using the limma package for R using the following conditions: p-value 0.05, fold change (FC) ≥ 2, and false discovery rate 0.05 using a Benjamini-Hochberg correction. There were 43, 245, 338, and 661 DEGs found in 5, 10, 15, and 20 DAP, respectively. Gene ontology of DEGs using Panther classification showed that most of the DEGs belong to catalytic activity (GO:0003824) in terms of molecular function, metabolic (GO:0008152) for the biological process, and most of the DEGs belong to cells (GO:0005623). DEGs were mapped in the KEGG database for pathway analysis to show changes in the starch and sucrose metabolism pathways as well as in the biosynthesis of amino acids. Coexpression analysis was conducted to show possible connections between the high amino acid content and chalkiness in rice grains using a weighted correlation network analysis via the WGCNA package for R. Through coexpression analysis, it was revealed that known gene related to chalkiness, AGPase, found in the starch and sucrose metabolism pathway, was highly correlated to threonine aldolase and chorismate mutase, both found in the biosynthesis of amino acids pathway. Connecting and related pathways between the two are glycolysis and gluconeogenesis, suggesting it is worth looking into these pathways for more concrete explanations between the relationship of amino acid content and chalkiness. These insights on the gene expression of chalky rice with high

Keywords: rice microarray, gene ontology, network pathways, coexpression analysis, amino acid content, chalkiness, grain development

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Poster Session - Bioinformatics

Comparison of Affinities Between Two Integrin α6 Subunit Binding Partners through *in silico* Analysis

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Abstract: Integrins are a family of cell-surface receptors that mediate cell-matrix and cell-cell adhesion. Each member is a heterodimer comprised of non-covalently associated alpha and beta subunits, whose identities in each integrin direct ligand specificities and function. In vertebrates, 18α and 8β subunits associate to form 24 unique heterodimers. The α6 integrin subunit pairs with either β1 or β4, but both heterodimers are receptors for laminin. Neither α6β1 nor α6β4 have deposited crystal structures. In this study, the structures of the ligand-binding domains (LBDs) of integrins α6β1 and α6β4 were predicted, and these models were analyzed computationally. *In silico* Alanine substitutions across the protein sequence predicted hotspots of interaction primarily at Tyr, Asp, and Arg residues for both β1 and β4. To compare the two α6 integrins, the models of the LBDs of α6β1 and α6β4 were assessed based on inter-subunit binding affinity. This was predicted to be higher for α6β4, which may be attributed to its role in promoting cellular adhesion and maintaining stability in the intermediate filament system. To determine the behavior of α6β1 and α6β4 in different environments, the models were equilibrated in NaCl and $CaCl_2$. In agreement with binding affinity predictions, steered molecular dynamics (SMD) yielded higher rupture forces for α6β4 than for α6β1 in both environments. The rupture force of α6β1 was higher in NaCl (4658.61 kJ mol^{-1} nm^{-1}) than in $CaCl_2$ (4349.82 kJ mol^{-1} nm^{-1}), which may be explained by the greater number of H-bonds and larger inter-subunit binding interface exhibited by α6β1 in NaCl. In contrast, α6β4 separates later and with a greater rupture force in $CaCl_2$ (5931.96 kJ mol^{-1} nm^{-1}) than in NaCl (4662.51 kJ mol^{-1} nm^{-1}). These results suggest that α6β4 dimerizes more readily than α6β1, and that their binding affinities change in different ion environments.

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Poster Session - Bioinformatics

Concerted Virtual Screening of Myxobacterial Natural Products Reveal Dual Inhibitors of SARS-CoV-2 Spike Proteins

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Abstract: The coronavirus disease 2019 (COVID-19) is a major public health concern caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that resulted in a pandemic causing more than fifty million cases including approximately eight-thousand Filipino mortalities. In this study, we exploited the potential of secondary metabolites from Myxobacteria – a known producer of structurally and functionally diverse metabolites with broad-spectrum antiviral activity. Thus, the inhibitory prospects of 74 antiviral Myxobacterial secondary metabolites were assessed through *in silico* molecular interaction-based approaches against mechanisms underlying SARS-CoV-2 viral entry. Ligands were prepared using Avogadro and the three-dimensional structures of the proteins were derived from RCSB PDB. Virtual screening of the prepared library was performed following the Broyden-Fletcher-Goldfarb-Shanoo algorithm of AutoDock Vina and the binding affinity of the enzyme–ligand complex conformation was determined using UCSF Chimera, visualized, and analyzed using BIOVIA Discovery Studios. The 74 secondary metabolites were docked against the receptor-binding domains (RBDs) of the SARS-CoV-2 spike protein to angiotensin-converting enzyme 2 (ACE2) and glucose-regulated protein 78 (GRP78). Molecular dynamics simulations of the spike protein complex of SARS-CoV-2 were studied in YASARA dynamics software package with the aid of AMBER14 force field. SwissADME software and OSIRIS property explorer program were also used to predict *in silico* the pharmacokinetic and toxicity profile of the compounds, respectively. Among the metabolites screened, the chondramide group of metabolites – chondramide C3 and chondramide C9 – showed highest affinity to ACE2 (-8.6 kcal/mol) and GRP78 (-8.9 kcal/mol) RBD of the viral spike, respectively. Protein-protein docking experiments also showed repulsive interactions of inhibitor-spike protein complex with ACE2 and GRP78 receptors. Selectivity of chondramide C3 towards the ACE2 RBD of the spike was demonstrated by docking the ligand to ACE2 receptor itself. When the binding energies (BEs) of chondramide C3 towards ACE2 RBD (-8.6 kcal/mol) and ACE2 receptor (-7.6 kcal/mol) were compared, it displayed a stronger affinity towards the spike protein. The top fifteen strong binding Myxobacterial secondary metabolites were also docked against SARS-CoV-2 mutants A475V, L452R, V483A, and F490L wherein chondramide A9 consistently demonstrated high affinity towards each variant with a BE of -9.1 kcal/mol. These variants of the RBD of the spike to ACE2 exhibit resistance to neutralizing antibodies. The secondary metabolites were also screened to the globally prevalent mutation D614G of the spike protein with a co-occurring mutation, I472V, at the RBD of ACE2. Chondramide C emerged with the strongest BE towards the D614G-I472V variant at -8.5 kcal/mol. Molecular dynamics simulations demonstrated the stability of chondramides C, C3, and C9 when complexed with their target RBDs of the viral spike in a 120ns simulation. Finally,

the top ligands were predicted to confer favorable pharmacokinetic and toxicity properties. Thus, Myxobacterial chondramides are promising compounds to develop drugs against SARS-COV-2 entry. and *P. guajava* have synergistic anticancer effects on lung cancer cells.

Keywords: SARS-CoV-2, spike proteins, Myxobacterial secondary metabolites, molecular docking

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Poster Session - Bioinformatics

Molecular and *in silico* Structural Characterization of Viral Genome-Linked Protein (VPg) of the Banana Bract Mosaic Virus Infecting Abaca

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Abstract: Abaca is an important agricultural crop in the Philippines due to the high commercial value of its fibers. The abaca industry; however, is plagued with multiple viral diseases, one of which is the banana bract mosaic virus (BBrMV). The viral protein genome-linked (VPg), one of the protein components of the BBrMV genome, is known to play significant roles in the potyviral life cycle. VPg interaction with the host eukaryotic translation initiation factor 4E (eIF4E) is required for successful potyviral infection. The study aimed to clone and characterize BBrMV VPg and determine the structural and protein-protein binding properties through *in silico* analysis. The full coding sequence of BBrMV VPg was isolated from BBrMV-infected abaca, expressed in *E. coli,* and analyzed through *in silico* docking analysis with predicted eIF4E proteins of abaca. SDS-PAGE analysis showed a 27 kDa protein corresponding to 6xHis-tagged BBrMV VPg which was confirmed by immunoblot analysis with anti-6xHis antibody. The protein was found both in the soluble and insoluble fractions. Homology modeling through the I-TASSER server showed BBrMV VPg had high structural similarity with the potato virus Y (PVY) VPg. Protein docking analysis between the BBrMV VPg and three putative abaca eIF4E structures via HADDOCK showed the docking of the loop α2-α3 region of BBrMV VPg to the cap-binding pocket of MteIF4E which has been previously observed for PVY VPg-eIF4E interaction. Binding affinity values and dissociation constants derived from PRODIGY analysis showed the high binding affinity between BBrMV VPg and the three abaca eIF4Es. The predicted dimeric BBrMV VPg structure showed the interface to be at the loop β4-β5 region which suggests multiple functionalities of BBrMV VPg. These findings will be significant for further elucidating the mechanism of virus-host interaction specifically in BBrMV and abaca.

Keywords: potyvirus, VPg, recombinant protein expression, immunoblot, homology modeling, protein-protein interaction

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Poster Session - Bioinformatics

In silico Characterization of GSPXII: A Novel γ-Conotoxin-Like Turritoxin Targeting the Cardiac Pacemaker Channel

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Guevara, MGAC; Bascos, NAD; Saloma, CP. 2021. *In silico* Characterization of GSPXII: A Novel γ-Conotoxin-Like Turritoxin Targeting the Cardiac Pacemaker Channel. PJBMB. Vol. 2, No. 1, 2021, pp. 49. doi:

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Abstract: Turritoxins are venomous peptides from the Family Turridae. Gene mining performed within our laboratory revealed that *Gemmula speciosa* has conotoxin-like and non-conotoxin-like peptides that may similarly target ion channels and receptors with high specificity similar to that of the Conus peptides which have been harnessed for use in pharmacology. One of the peptides from this study was GspXII, a 46 AA turritoxin that shares the γ-conotoxin framework XII present among published γ-conotoxins and γ-conotoxin-like peptides based on multiple sequence alignment. γ-conotoxins target the pacemaker channels; however, none of these have elicited a phenotype in vertebrate models. The intravenous and intracranial injection of GspXII in mouse models resulted in a decrease in heart rate and back stiffening. Based on previous experiments, GspXII may target the cardiac pacemaker channel, HCN4. To test this hypothesis, *in silico* based docking studies were done using structural models of the peptide, and the target receptor (HCN4). Structures of the GspXII peptide were modeled through I-TASSER (zhanglab.ccmb.med.umich.edu/I-TASSER/) with and without disulfide constraints predicted by DiANNA (clavius.bc.edu/~clotelab/DiANNA/). These models, together with the prototype of the γ-family of conotoxins, PnVIIA, were used to dock to HCN4 (PDB ID: 6GYO, 6GYN) via ClusPro 2.0 (cluspro.bu.edu/). GspXII was observed to target HCN4 in both hyperpolarized and cAMP-bound states. The predicted binding of GspXII to the HCN4 pore is expected to prevent the flow of ions through the channel. Notably, PnVIIA was not observed to dock onto the pore of the HCN4 channel; hence, this data supports that PnVIIA is unable to elicit a phenotype in vertebrates (Fainzilber et al., 1998). GspXII was also not observed to dock to the pore of the HCN1 channel (PDB ID: 5U6O, 5U6P, 6UQF) despite the similarity of HCN4 and HCN1, suggesting a specific GspXII-HCN4 interaction. GspXII remains the only member of the γ-conotoxin family of peptides that elicits a phenotype in vertebrate models. Our results suggest this mechanism involves the specific targeting of the HCN4 channel by this pore-blocking peptide.

Keywords: Turritoxin, Ion Channels, Molecular Docking

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Poster Session - Bioinformatics

Designing A Multi-Epitope Vaccine Using Epitopes from The Structural Proteins Of SARS-CoV-2: An Immunoinformatics Approach

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Abstract: The rapid transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in hundreds of thousands of deaths worldwide. It has severely affected the economy and the healthcare system in many countries, making it crucial to accelerate vaccine development against SARS-CoV-2. Thus, this work utilized immunoinformatics in designing a novel multi-epitope vaccine that can potentially induce immune response through mapped epitopes from immunogenic, and abundantly expressed structural proteins in SARS-CoV-2.

Epitopes were screened and evaluated using various immunoinformatics tools and databases. Antigenicity, allergenicity, and population coverage were assessed. Epitopes were adjoined to form a single vaccine construct (Cvax), linked with 50S ribosomal protein as an adjuvant. Physicochemical properties, cross-reactivity, antigenicity, and allergenicity of Cvax were evaluated. The tertiary structure of Cvax was modeled, refined, and validated for docking with toll-like receptor 4 (TLR4). The binding affinity of Cvax-TLR4 was estimated and compared with TLR4-adjuvant as control. Lastly, the immune response with Cvax was simulated, and compared with adjuvant alone.

A total of 33 epitopes from S (21), E (3), M (5), and N (4) proteins were merged in Cvax. These include epitopes on the receptor-binding motif (RBM) of S protein known to be essential in viral attachment. *In silico* evaluations classified Cvax as stable, antigenic, and non-allergenic. Epitopes were estimated to have large worldwide population coverage, especially in areas with high infection rates, indicating the broader efficacy of Cvax as a vaccine for the most affected populations. Results in this work showed that Cvax can bind to TLR4, indicating immunogenicity, and superior properties necessary for a successful vaccine.

Overall, this work efficiently minimized time, effort, and cost in urgently designing a vaccine against SARS-CoV-2. *In vitro* and *in vivo* studies on Cvax are anticipated.

Keywords: Epitopes, immunoinformatics, *in silico*, receptor-binding motif, SARS-CoV-2, vaccine

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Poster Session - Bioinformatics

Structural and Molecular Docking Analysis of Gibberellin Insensitive Dwarf1 (Gid1) Receptors of Abaca

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Abstract: The abaca is an economically important fiber crop; however, knowledge of the molecular mechanisms behind fiber development is limited. Gibberellins or gibberellic acids (GA) are plant growth regulators known to regulate fiber cell development in various fiber crops. GA perception in land plants is mediated by the GA receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1). GA binds to GID1 via the GA binding pocket and activates the GA-response pathway through the association of the GA-GID1 complex with the DELLA inhibitor protein. The degradation of the DELLA protein activates the transcription of GA-response genes. Recently, three abaca GID1 (MtGID1) genes were cloned and sequence analysis revealed them to be GA receptors. The study, therefore, aims to predict the 3D protein structures of the three MtGID1 proteins (MtGID1-1, MtGID1-2, and MtGID1-3) through homology modeling and to conduct protein docking analysis with DELLA protein and GA ligands. Homology modeling revealed the top structural analogs to be *Arabidopsis thaliana* GID1 (AtGID1a) receptor with the conservation of GID1 core domain structure and N-terminal extension lid structure. Superimposing the three MtGID1 structures showed the location of the loop variable region that connects the N-terminal lid to the GID1 core domain with MtGID1-3 having the shortest loop region among the MtGID1 proteins. Docking of MtGID1 proteins with AtDELLA showed interaction of the N-terminal lid of the three MtGID1 proteins to the DELLA motifs and resulted in negative binding affinity values indicating the spontaneous formation of the MtGID1-AtDELLA complex. Protein-ligand docking analysis of MtGID1 proteins with bioactive GA molecules showed the residues that are canonically determined to be involved in GA-binding pocket and N-terminal lid closure. These findings suggest that the identified GID1 proteins are bona fide GA receptors of abaca and are capable of activating the GA-GID1-DELLA signaling pathway in abaca.

Keywords: *Musa textilis*, gibberellin receptor, homology modeling, protein-protein docking, protein-ligand docking

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Poster Session - Bioinformatics

Model Prediction and Molecular Docking Simulations of a Novel Cone Snail Toxin, tcon-1

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Abstract: Venom from terrestrial and aquatic animals has always been a source of fear for mankind, but studies have shown that this cocktail of toxins is a rich source of potential pharmacological molecules. Predatory marine snails are known to harbor potent toxins in their venom that elicit an array of physiological phenomena, one of which has already been developed into a commercial analgesic. A novel cone snail toxin, tcon-1, was discovered through the transcriptomic analysis of three marine snails (*C. cerithina, U. bisaya,* and *G. speciosa*) and was subsequently characterized *in silico* and expressed *in vitro*. The initial *in silico* analysis revealed that tcon-1 is a structural analog of a previously described *Conus striatus* toxin called con-ikot-ikot. In this study, a protein model of tcon-1 was predicted by I-TASSER and subsequently docked unto the con-ikot-ikot target receptor. A dimerized model of tcon-1 was utilized in the molecular docking simulations against the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) using ClusPro. The docking results show that tcon-1 binds to similar regions as con-ikot-ikot in its putative target receptor, the AMPAR. Succeeding *in vitro* experiments relating to these observed similarities will shed light on the validity of the predicted functional mechanism of tcon-1 action.

Keywords: Molecular docking, Protein modeling, Turritoxin

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Poster Session - Bioinformatics

The Possible Role of Selected Antidepressant Metabolites in Antitumor Immunity: A Molecular Docking Study of Granzyme B

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Abstract: Medical researches on cancer treatment and prevention has been in the highest demand for many years. Many patients who undergo such treatments also suffer from clinical depression. The role of secondary metabolites on tumor growth and depression may open up a spectrum of possibilities in the development of an effective treatment against cancer.

Secondary metabolites including alkaloids, phenolic acids, and terpenes with antidepressant properties were examined for their potential role in antitumor immunity. To determine their possible role in antitumor immunity, a molecular docking study was performed on twelve selected antidepressant metabolites with Granzyme B by using the Autodock Vina software. Parameters such as the binding affinity, bond distance, as well as amino acid interactions, were measured.

Results showed that chlorogenic acid had the highest binding affinity among the 12 selected antidepressant metabolites, while mauritine A displayed the highest number of interactions with the active site of the enzyme. Gallic acid showed the shortest bond distance with the enzyme.

The study suggests that chlorogenic acid, mauritine A, and gallic acid have the highest possibility among the selected secondary metabolites of exhibiting antitumor immunity with antidepressant effects which may help in the oncologic treatment of cancer patients who also suffer clinical depression. Further study is encouraged involving other parameters, other classes of secondary metabolites, the compounds' mechanisms of action, and *in vivo* researches.

Keywords: Granzyme B, molecular docking, antidepressant metabolites, antitumor immunity

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Poster Session - Health

Probing the Role of Different Membrane Repair Mechanisms During Necroptotic Cell Death

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Abstract: Necroptosis is a caspase-independent form of regulated cell death involved in numerous pathophysiological conditions. This mechanism of cell death is inherently immunogenic due to the release of damage-associated molecular patterns upon plasma membrane rupture. During the execution of necroptosis, the cell activates various counterbalancing repair mechanisms that serve to delay cell death and allow proper cell-to-cell communication to occur. Here, we show that CHMP4B, an ESCRT-III component previously implicated in membrane repair, is activated, and forms punctae concomitant with an increase in cytosolic Ca^{2+} concentration. Previous data showed that damaged membranes were shed during necroptosis, which we similarly observed but only in a very small proportion of cells. Instead, most of the CHMP4B punctae appeared intracellular in nature, suggesting the possible involvement of other pathways in membrane repair. Shedding light on how these repair mechanisms work during necroptosis will be important towards understanding the potential means by which we can control this type of cell death, and possibly exploit it for some therapeutic benefit.

Keywords: necroptosis, ESCRT-III, CHMP4B, plasma membrane repair

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Poster Session - Health

Antibacterial Potential of Locally Formulated Disinfectant/Antiseptic from Nipa Bioethanol

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Abstract: Ethyl Alcohol, 70% was formulated from Nipa Bioethanol, "Nipahol", to augment the dwindling supply of alcohol to fight COVID-19. Nipahol is high-grade bioethanol produced from nipa sap using the proprietary fermentation and distillation technologies developed by a group of researchers at the MMSU-NBERIC. For more than a decade now, MMSU has been implementing RDE for the production of fuel-grade ethanol from non-traditional sugar feedstocks like nipa sap in support of the Biofuel Act of 2006. To ensure the quality and efficacy of the formulated product, the present study was set to explore the antibacterial potential of the locally formulated nipa disinfectant/antiseptic as an alternative alcohol formulation amid the COVID-19 pandemic using standard microbiological assays. Susceptibility test revealed that 70% and 80% nipa alcohol formulations showed inhibitory activity against *Staphylococcus aureus* (6.25 mm and 4.25 mm zone of inhibitions). The 95% nipa alcohol concentration showed a bactericidal effect against *Escherichia coli* and *S. aureus*. High percent (%) bacterial cell reduction (90-99.9% log reduction) was observed when alcohol concentration and time increases. A confirmatory antimicrobial susceptibility test conducted by DOST-RO1 Microbiology Division reported that 95% nipa alcohol showed an active inhibitory effect to test organisms while partial active observed in 70% nipa alcohol formulation. Glo-Germ Test revealed nipa disinfectant/antiseptic is as effective as commercial alcohol, thus, it can be utilized as an alternative intervention to prevent the spread of infectious microorganisms. The effectiveness of nipa disinfectant/antiseptic formulations is heightened with proper handwashing, strictly following proper hygiene, and health protocols. In conclusion, the formulated nipa disinfectant/antiseptic possesses the antibacterial potential to inhibit the multiplication of *E. coli* and *S. aureus*.

Keywords: nipa bioethanol, nipa disinfectant/antiseptic, percent bacterial cell reduction, antimicrobial susceptibility, Glo germ test

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Poster Session - Health

Activity of Putative Bacteriocins from *Lactobacillus Plantarum* Bs25 and *Pediococcus acidilactici* S3 Against Antibiotic-Resistant *Vibrio spp.*

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Abstract: *Vibrio spp.* with antibiotic resistance phenotypes were previously isolated from Manila Bay and is considered a possible threat to public health. Lactic acid bacteria (LAB), the commonly used microorganisms in food fermentation and a natural, sustainable flavor enhancer, secrete metabolites that have antimicrobial activities. It is then worth exploring the potential of these metabolites against pathogens from environmental reservoirs. This study determined the inhibitory effects of crude cell-free supernatant (CFS) and ammonium sulfate precipitate (ASP) preparations from *Lactobacillus plantarum* BS25 (LP) and *Pediococcus acidilactici* S3 (PA) against antibiotic-resistant *Vibrio alginolyticus* (P3) and *Vibrio fluvialis* (0405-W4-01-02) isolates. Resazurin assay showed that CFS and ASP from LP and PA can inhibit antibiotic resistant strains of *Vibrio spp.* isolates with the highest recorded activity against *Vibrio fluvialis*. Preparations of putative bacteriocins of 123 mg/mL from 1.94 x 10^{12} CFU/mL PA (CFS) exhibited 92% inhibition while 86 mg/mL from 1.703 x 10^{12} CFU/mL LP (ASP) showed 99% inhibition against 1.87 x 10^{12} CFU/mL of *Vibrio fluvialis* (50 uL and 40uL, respectively). The activities observed were higher compared to the control (225 ppm) tetracycline by 6% and 66% ($a=0.05$, $p < 0.00001$), respectively. Total protein content from ASP preparation of LP (0.012 mg/mL) and PA (0.016 mg/mL) were determined using Bradford assay. Putative class I and III bacteriocins were observed in SDS-PAGE bands of LP (10kDa, 37kDA to 75 kDa) and PA (10kDa, 37 to 100kDa), respectively. Putative bacteriocins from LP and PA have inhibitory effects against antibiotic-resistant *Vibrio spp.* Results of the study can be used in the development of postbiotics against antibiotic-resistant pathogens isolated from environmental reservoirs.

Keywords: LAB, *Lactobacillus plantarum* BS25, *Pediococcus acidilactici* S3, *Vibrio spp.,* Postbiotics

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Poster Session - Health

Anticancer Potential of *Eleusine indica* Methanolic Leaf Extract via RAS- and Wnt-Related Pathways Evaluated in Transgenic *Caenorhabditis elegans* Strains

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Abstract: In the Philippines, many accounts have resurfaced claiming different herbal and therapeutic advantages of *Eleusine indica* (*Indian goosegrass),* such as antiviral, anti-plasmodial, antidiabetic, antioxidant, antidiuretic, anti-inflammatory, and antibacterial properties [1-3]. One of these advantages is its anticancer potential. Despite some studies claiming that the crude extract has cytotoxic and pro-apoptotic activity, it is still insufficient [4-5]. Hence, further scientific evidence is needed to support this claim. In this study, we evaluated the anticancer potential of *E. indica* methanolic leaf extract (EMLE) by focusing on two cancer-related pathways, Ras and Wnt pathways. Dysregulation in the Wnt pathway has been linked to colorectal cancer, ovarian cancer, and breast cancer. Meanwhile, irregularities in the Ras pathway are associated with myelomonocytic leukemia, ovarian cancer, colorectal cancer, and cervical cancer. We used wild-type and transgenic *Caenorhabditis elegans* strains, which have irregular Ras or Wnt signaling. Humans and *C. elegans* share comparable Ras and Wnt signaling pathways, especially their downstream targets. We determined the average number of eggs laid of each strain and the multi-vulva development of the Ras-mutant strain. Our findings show that EMLE does not affect the number of eggs laid of the wild-type, Ras-mutant, and Wnt-mutant worms. Furthermore, EMLE was not able to reduce the Ras-mutant population demonstrating multi-vulva. Taken together, our data suggest that the anticancer potential of EMLE may be independent of Ras and Wnt signaling pathways.

Keywords: Anticancer, *Eleusine indica*, Ras pathway, Wnt pathway

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Poster Session - Health

Antibacterial Effect of *Vernonia cinerea* Root Extract Compared with Mupirocin Against *Staphylococcus aureus*- Induced Wound in Mice

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Abstract: Wounds, if not properly managed, become one of the leading causes of morbidity and mortality in the country. That being said, the use of herbal drugs to explicate their potential in wound management and as natural remedies is growing due to their cheaper price and greater accessibility. *Vernonia cinerea*, also known as Tagulinaw, is a potential medicinal plant. The study aims to determine the antibacterial effect of Vernonia cinerea root extract against *Staphylococcus aureus*-induced wounds in mice. Vernonia cinerea dried roots were pulverized and the extract was made into an ointment. The study lasted for 16 days and applications of Mupirocin (positive control), Paraffin base (negative control), and Vernonia cinerea ointment was done once a day. The percent wound reduction in size was measured using a ruler and bacterial colony count was done. Results revealed that the Mupirocin and *Vernonia cinerea* ointment were comparable in eradicating *Staphylococcus aureus* on the wound of the mice based on the colony count done between the groups. On the other hand, the percent of wound reduction in size increases from the first day (4th) of measurement till the last day (16th) of observation. It was concluded that *Vernonia cinerea* ointment was effective in healing wounds and in the prevention of bacterial infections.

Keywords: Mupirocin, *Staphylococcus aureus*, Tagulinaw, *Vernonia cinerea*

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Poster Session - Health

CTTNBP2 and Zinc, as Brothers in Arms in Autism Spectrum Disorder

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Abstract: Autism spectrum disorder (ASD) is a highly prevalent psychiatric disorder. Both genetic and environmental factors are involved in ASD etiology. Recently we reported the molecular and physiological role of an ASD high-risk gene, Cortactin-binding protein 2 (CTTNBP2) from mouse genetic models. We showed that *Cttnbp2* deficiency leads to aberrant social interaction, one of the core symptoms of ASD. Proteomic and immunoblotting analyses further revealed that a set of ASD-associated genes, including SHANKs and NMDAR, are down-regulated in *Cttnbp2* deficiency synaptosomal fraction. Administration of D-cycloserine (DCS, an NMDAR coagonist) improves social behavior defects of CTTNBP2 deficiency models further validates the involvement of NMDAR signaling in CTTNBP2 regulated social behaviors. In addition, zinc association is also one of the convergent features in differentially expressed proteins identified in *Cttnbp2* deficient synaptosomal fractions. The concentration of zinc in *Cttnbp2* deficient brain tissue is also reduced. Seven days of zinc supplementation improved social behaviors of *Cttnbp2* deficient models. These results suggest, as a genetic factor, *Cttnbp2* interacts with zinc, an environmental factor in the regulation of social behaviors. Our model provides an example of genetic and environmental factor interaction in ASD etiology.

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Poster Session - Health

Diet Composition and Gut Microbiome of Healthy Adults in Albay and Manila

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Abstract: Gut microbes interact with their host in maintaining health or in the development of disease. Of the many factors that affect its structure, diet is one that can be classified by geographic location (rural vs. urban). Since health and disease also vary across locations, the study of gut microbiota, as an interface between diet and health status, can provide insights into the understanding of disease states. Therefore, this study will determine differences in the diet and the gut microbiome of healthy adults from rural Albay and urban Manila. A total of 61 healthy adult participants was recruited: 37 from Albay and 24 from Manila, with the given number of participants per group allowing ≥90% power to detect an effect size less than the set type I error (α=0.05). Their demographic profile, dietary information, and stool samples were collected. Fecal bacterial DNA was extracted from the stools and the 16 rRNA gene was sequenced on MiSeq. The gut microbiome profile was determined using QIIME/USEARCH. Based on the dietary information, Albay has a significantly higher consumption of rice and porridge and carbohydrates intake than Manila ($p<0.05$) while protein and fat intake are the same between groups. Based on the sequencing data to date, Manila has a significantly higher abundance of *Bifidobacteriaceae* ($p<0.05$), a driver of BB-type microbiota expected in a high-fat, high-protein diet. The *Prevotella* abundance that is associated with vegetarian and high-plant-polysaccharide diets was inversely proportional to *Bacteroides* abundance, another driver of the BB-type microbiota. Lastly, gut bacterial diversity represented by the number of detected OTUs per sample in Albay is significantly greater than that of Manila ($p<0.05$). This shows that the abundance of certain bacterial signatures such as *Bifidobacteriaceae and Prevotella* and gut bacterial diversity vary between urban and rural healthy adult individuals who consume different diets.

Keywords: diet, gut microbiome, adults

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Poster Session - Health

Neuroprotective Effects of The Oxindole Alkaloids Isomitraphylline and Mitraphylline in Damaged Human Neuroblastoma SH-SY5Y Cells

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Abstract: Alzheimer's disease (AD) is a dominant neurological disorder characterized by cognitive impairments and synaptic dysfunctions affecting mostly the elderly. There are only five drugs approved to minimize the progression of symptoms related to AD. Hence, the challenge of finding an alternative treatment or therapy from natural products is warranted. The purified oxindole alkaloids, isomitraphylline, and mitraphylline from *Uncaria perrottetii* (locally known as "sungay kalabaw"), revealed their ability to break amyloid aggregates *in vitro* suggesting their therapeutic potentials in Alzheimer's disease (AD). Thioflavin-T assay for assessing amyloid-*beta* (Aβ) aggregation of these alkaloids exhibited inhibitions at 60.32% ± 2.61 (50 μM) for isomitraphylline and 43.17% ± 3.48 (50 μM) for mitraphylline. Neuroprotective effects were elaborated against Aβ-induced SH-SY5Y cells at 20 μM and 10 μM for isomitraphylline, and 20 μM for mitraphylline. In addition, both alkaloids attenuated and protected the H_2O_2-induced SH-SY5Y cell cytotoxicity at 20 μM. The intracellular ROS levels of SH-SY5Y cells from H_2O_2-induced oxidative stress were reduced at 20 μM and 10 μM, and the mitochondrial membrane potentials of Aβ-induced SH-SY5Y cells were protected at 20 μM. The overall results suggested the potentials of both alkaloids to target certain pathological biomarkers of AD and could be further investigated as therapeutic or preventive drug leads against AD.

Keywords: Alzheimer's disease; Oxindole Alkaloids; SH-SY5Y cells; *Uncaria perrottetii*

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Poster Session - Health

Prevalence of Pks+ *Escherichia coli* in Colorectal Cancer Among Selected Filipino Cases

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Abstract: Colorectal cancer (CRC) ranked 4th cause of cancer-related death in the world and 3rd leading cause of malignancy in the Philippines in 2018. Among the established risk factors are advancing age, family history of cancer, high fat diet, sedentary lifestyle, smoking, and alcohol. Over the past few years, there has been a growing interest in the role of the members of the gut microbiota, including *Escherichia coli*, in colorectal cancer development. Some *E. coli* strains may carry the *pks* pathogenicity island, which encodes the compound colibactin that is believed to alkylate DNA on adenine residues and induce double-strand break. Thus, this study aimed to determine the possible association of *pks*+ *E. coli* with CRC development among selected Filipino cases. A total of 62 formalin-fixed paraffin -embedded (FFPE) colorectal tissues positive for cancer cells (cases) matched with 62 cancer-free tissues (controls) were analyzed for the presence of *uidA*, *clbB, clbN* and *clbA* genes using an in-house developed real-time qPCR. Results show that there is no significant difference (p>0.05) between the cases and controls in terms of the prevalence of the *uidA* gene, which is an important target for the detection of *E. coli*. As to the markers of the *pks* pathogenicity island, *clbN* gene was more prevalent (p<0.05) in cancer-free tissues than in malignant colorectal samples. No significant difference (p>0.05) was observed between cases and controls in terms of the prevalence of the *clbA* and *clbB* genes. Therefore, pks+ *E. coli* may not be a risk factor in CRC development among Filipinos.

Keywords: colorectal cancer, *pks*, *Escherichia coli*, real-time qPCR, formalin fixed paraffin embedded (FFPE) colorectal tissues, gut microbiota

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Poster Session - Health

Assessment of the Prebiotic Activity of Arabinogalactans Isolated from *Zea mays* on *Bacteroides acidifaciens* and Secretory IgA levels in BALB/c Mice

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Abstract: Arabinogalactan is a prebiotic that can be extracted from Larch trees, carrots, wheat, and corn. Various clinical studies associated arabinogalactan supplementation with increased immune defenses. *Bacteroides acidifaciens*, a resident microflora of both mouse and human colon, has been demonstrated to induce sIgA levels and promote B cells in the colon of gnotobiotic mice. Larch arabinogalactans and *B. acidifaciens* had been separately shown to increase sIgA expression levels. However, there is no study that correlates the increase in sIgA levels with the increase in *B. acidifaciens* population due to a prebiotic. The study aims to utilize corn as an alternative source of arabinogalactans since the Larch tree is not easily grown in tropical countries. The effect of corn (*Zea mays*) arabinogalactans on *B. acidifaciens* is being investigated to support the sIgA immune response. Arabinogalactans were extracted from fresh corn kernels. The growth curve of *B. acidifaciens* reference culture on culture media containing various arabinogalactan concentrations was used to determine the optimum prebiotic dosage. The prebiotic activity of corn (*Zea mays*) arabinogalactan granules on *Bacteroides acidifaciens* was determined *in vitro* using batch culture fermentation. It was found that the optimum prebiotic dosage is 1.0 mg/mL. Using Eggert-Gangnon broth, enrichment with Larch and corn arabinogalactan showed bacterial growth of 6500 ± 80 cells and 7200 ± 150 cells, respectively, as compared to 3000 ± 90 cells of the negative control and 4800 ± 200 of the inulin treatment (positive control) after 6 hours of fermentation. In an *in vitro* set-up, it is observed that corn arabinogalactan enrichment can reach a maximum prebiotic score of 2.2. A preliminary study of BALB/c mice treatment with corn arabinogalactan indicates an increase in total protein content in the fecal samples. qPCR analysis of fecal DNA sample from mice treatment showed an increase in *B. acidifaciens* population after 30 days of supplementation with 0.1 mL 1.0 g/mL corn arabinogalactan treatment. The data from batch culture fermentation suggests that medium enrichment with arabinogalactan has a positive effect on the population growth of *B. acidifaciens*. The maximum prebiotic score of 2.2 implicates that *B. acidifaciens* has enzymes that can utilize arabinogalactan which results in an increase in growth rate. The increase in total protein content may indicate an increase in sIgA expression; to confirm this hypothesis, a sIgA ELISA is needed. In the next step of the study, *in vivo* prebiotic activity of corn arabinogalactan and the mucosal sIgA expression levels from fecal and colon swab samples collected will be compared among different treatment groups of BALB/c mice.

Keywords: *Zea mays*, *Bacteroides acidifaciens*, prebiotics, sIgA immune response, arabinogalactans

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Typing. Manuscript should be encoded, double-spaced, using the font Arial, size 12, with a 2.5 cm margin around. When applicable, each selection should begin on a new page (title page, abstract, introduction, methods, results, acknowledgements, references, tables, figures). Each page should be numbered, starting with the title page.

General Rules on Style. All symbols, abbreviations, and acronyms should be defined. All acknowledgements should be gathered into a brief statement at the end of the references and notes. Tables should supplement, not duplicate, the text. They should be numbered according to the order of their citation in the text.

Title Page. This page should contain the title of the manuscript, names, addresses, and telephone numbers of the authors, and the laboratory where the work was done. The author who is responsible for correspondence should be indicated. Titles of general articles should have no more than 50 characters.

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There are seven categories of papers that are published.

Biochemical Education Articles. A biochemical education article (up to 3000 words) is expected to describe a personal approach to teaching a concept, or to review current trends in the teaching of biochemistry. A class laboratory experiment that students have found especially helpful maybe submitted. The use of illustration is encouraged, but should be limited to three. Figures and tables together should not exceed four. References should be included when applicable, but are limited to 20.

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