Newsletter

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Agriculture | Innovation | Life

Faculty of Science is pleased to present the fifth issue of thrice-yearly e-Science Putra Newsletter. This e-newsletter intended to disseminate and highlight the latest research findings, activities, and contribution to the community by the Faculty members. We hope that you enjoy reading this newsletter.

HIGHLIGHTS

- Javanese Medaka
- Waste to Resource
- Hydroponic Fodder System
- Wollastonite Foam Glass Ceramic
- Smoothing method for Modelling Statistics Data

Javanese Medaka: Model in the Making

Javanese medaka (*Oryzias javanicus* Bleeker, 1854)

Malaysia native ricefish species, Javanese medaka (*Oryzias javanicus* Bleeker, 1854) has its own potential and capability to be reliable model species for biomonitoring activities in assessing the health of aquatic ecosystem. It is a close relative to Japanese medaka (*Oryzias latipes*) which is a well-established model fish species for multidisciplinary studies for over 100 years. Javanese medaka is euryhaline and adaptive to a wide range of salinity (freshwater to brackish/marine water). It is well distributed in the estuarine areas along the west coast of Peninsular Malaysia, and reported also found in Indonesia, Singapore, Thailand and Western Borneo. Javanese medaka is a small tropical fish and they are hardy as well as have short life cycle makes them suitable to be cultured in the laboratory. The body and its eggs are translucent, while their eyes are silvery can be the prominent characteristics of Javanese medaka for field identification. They are reported feed on small crustaceans, insects and protozoans in natural habitat. In the laboratory culture, and their diet is brine shrimp (*Artemia salina*) and micro-algae.

In Malaysia, series of scientific studies utilizing Javanese medaka started more than 20 years ago and continuously progress conducted by a team of researchers from Universiti Putra Malaysia (Prof. Dr. Ahmad Ismail and co-workers). International counterparts for sharing knowledge and findings include researchers from Japan (The University of Tokyo, National Institute for Basic Biology, Kyoto University, Kagoshima University and etc.), Indonesia (Indonesian Institute of Sciences (LIPI), Hasanuddin University and etc.) and Thailand (Mahasarakham University and etc.). Research activities at the Ecotoxicology Laboratory and Aquatic Laboratory UPM focuses on the ecological and ecotoxicological responses of Javanese medaka towards some of environmental stressors of aquatic environment, including manipulation of water parameters, exposure to various organic pollutants (biocides, alcohols and etc.), inorganic pollutants (dissolved and particulate metals), harmful bacteria, and microplastics. The genetics and molecular studies related to Javanese medaka are currently conducted. Several scientific articles have been published in various international reputable journals. It is hopeful that this small fish species with enormous potentials can be a perfect model organism for various purposes.

Figure 1: The visit of fellow scientists from Japan who collaborated in the study of Malaysian native fish species, Javanese medaka, in wild habitat.

Figure 2: Laboratory conditions in Medaka Mass Culture Satellite Laboratory at the Department of Biology, Faculty of Science, Universiti Putra Malaysia.

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Waste to Resource: Green Production of Standardized Oil Palm Leaf-Flavonoid Enriched Extracts as Potential Therapeutic Agent in Regenerative Medicine (PI2019004359)

Oil palm (*Elaeis guineensis* Jacq.) is a major commodity in many countries. In Malaysia, 5.8 million hectares of oil palm plantation produce 43.4 million tonnes palm oil annually. In the wake of large-scale cultivation of the crop, a huge amount of biomass is produced that leads to a great challenge to agricultural waste management. With an annual production of 19.5 million tonnes, oil palm leaves (OPL) are considered as an underutilized oil palm biomass with low value. Interestingly, OPLs contain large amount of flavonoids in particular luteolin and apigenin derivatives which are known to possess useful biological properties such as antioxidant, anti-inflammatory and wound healing properties.

Conventional methods of separation and purification of phytochemicals, which often start with solid-liquid extraction, followed by liquid-liquid extraction and adsorption chromatography, are not only time-consuming and have low efficiency, but also consumes high amounts of organic solvents and energy. At present, there is no method of efficiently harnessing OPLs for production of useful fine chemicals for health and other applications. Effective extraction and enrichment protocols to produce flavonoid enriched extracts from OPLs are needed in order to turn this agriculture biomass into a useful resource. With this aim in mind, we embarked on our research to develop and optimize a process for extraction and enrichment of OPL flavonoids to produce standardized OPL extracts for potential applications as botanical therapeutic agents in regenerative medicine. FlavoRich was thus produced by integrating ultrasonic-assisted green solvent extraction for high efficiency in flavonoid liberation, chemical pretreatment for target specificity, and macroporous resin for selective entrapment.

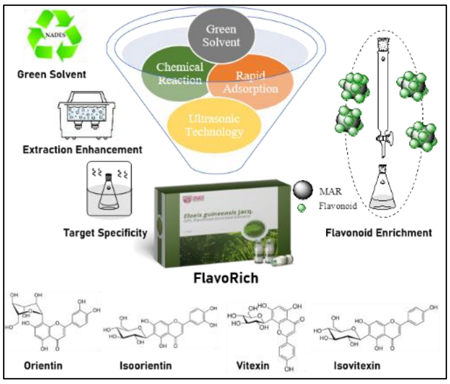

Figure 1: Integrated process for production of standardized oil palm leaf-flavonoid enriched extract (FlavoRich) and its chemical markers.

The chemical properties of FlavoRich were analysed qualitatively and quantitatively, using state of the art UHPLC and LCMS/MS techniques, while the toxicity, antioxidant, anti-inflammatory and wound healing properties were evaluated using *in vitro* and *in vivo* models. FlavoRich contains 3.5-fold total flavonoids as compared to the original OPL extract and exhibited enhanced antioxidant, anti-inflammatory and wound healing properties without showing any toxicity effect at tested concentrations. FlavoRich was standardized to its four bioactive chemical markers i.e orientin, isoorientin, vitexin and isovitexin.

This waste to resource research project was the PhD research of Mohamad Shazeli Che Zain from Natural Medicines and Products Research Laboratory, Institute of Bioscience, UPM under a supervisory team comprising Prof Khozirah Shaari in the lead, and Prof Sharida Fakurazi, Dr Nadiah Mad Nasir and Dr Soo Yee Lee as co-supervisors/co-researcher.

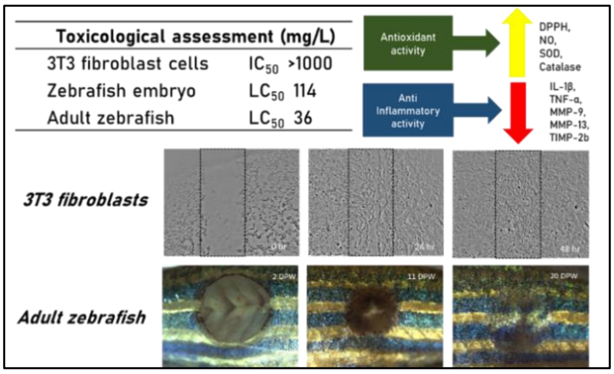

Figure 2: Toxicity profiles and wound healing properties of FlavoRich using *in vitro* and *in vivo* models.

The project was also carried out in collaboration with Prof Cheol-Hee Kim and Prof Mahanama De Zoysa. of Chungnam National University, Daejeon, Republic of Korea, specifically on *in vivo* studies using zebrafish model. The research has received several recognitions such as Diamond, Gold, Silver, Best Innovation, Best Invention, Best Video and Merit awards at both international and local exhibitions. The research has resulted in 1 patent and 1 utility innovation (UI) applications and produced 5 peer-reviewed journal papers of high impact (Q1/Q2).

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Hydroponic Fodder System: A Solution to the Feeding Management Problems (Labor-Intensive Cut-and-Carry System) Faced by Smallholders of Goat Industry in Malaysia

Nutrition is a significant factor to achieve successful goat production such as meat, milk, fibre and work. A good and balanced nutrition will prevent health, reproduction and production problems. However, two main challenges faced by the livestock industry especially the smallholder are the cost of animal feeds and lack of land for fodder cultivation. The smallholder of goat farming is burdened with the constraints of conventional method to cultivate fodder, for instance this method is more time and space consuming, higher labour works, requirement of manure and fertilizer and fencing to prevent fodder crops from wild animals and gain less profit due to high cost of animal feed. Therefore, hydroponics technology is found as an alternative to grow green fodder for animals. Recently, an automated and mechanized intensive production system such as hydroponics technology is coming up as an alternative to grow fodder for farm animals such as ruminants whether in small- or large-scale production.

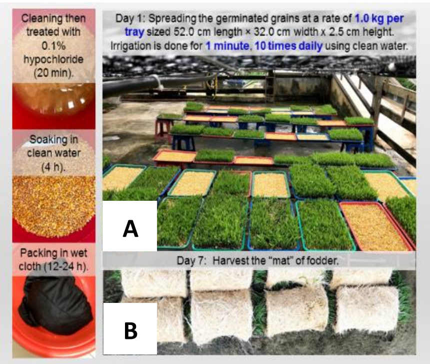

Figure 1: A used cattle pen was used as the site of open-air hydroponic fodder growing system where germinated (A) and (B) the mat of hydroponic maize fodder which was grown for 7 days.

The hydroponic technology embodies the concept of "owner-operator" which means a smallholder who typically also runs the day-to-day operations of the farm. Hence, many researchers have implemented the suggestion of growing different fodder crops through hydroponic technology such as barley (Reddy, Reddy and Reddy, 1988); oats, wheat (Snow, Ghaly and Snow 2008); sorghum, alfalfa, cowpea (Al-Karaki and Al-Hashimi, 2012) and maize (Naik, Dhuri, Swain and Singh, 2012). This system has been highlighted as a cost-effective method due to its ability to increase production of fodder with the utilization of limited land or space, water and labor. High digestibility and crude protein contents make the hydroponic fodder a suitable feed for ruminants.

In Malaysia, a commercial hydroponic fodder system, hailed as "landless fodder production" had been introduced by a foreign company and was brought to Universiti Putra Malaysia (UPM) in 1996 to produce feed for horses and ruminants that require high energy feed (Abdullah, 2001). The system consists of an imported environmentally controlled cabin for hydroponically germinating and growing barley grass for feeding. However, the commercial marketability of the imported system is directed mainly at high value animals, such as equines and livestock producing high value products, including high producing dairy cows, milk goats, deer and ostriches, resulting in the limited literature published that solely address hydroponic fodder for small and large ruminant such as goat and cattle.

Figure 2: Blood sample collection was performed with the assistance of trained staff from Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Although research in hydroponic fodder has increased, however, more research is required to focus on the development of low-cost devices for the hydroponic fodder production using locally accessible materials on different categories of livestock, which is still lacking in Malaysia. Moreover, the recent study in 2015 did not reveal the effect of hydroponic fodder on growth hormone level in meat goats. Most of the smallholder goat farms practise improper feeding regime due to poor knowledge and information, which further resulted in lower growth and reproductive performance of the goats, feed consumption and production.

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Wollastonite Foam Glass Ceramic from Food Wastes

Nowadays, there is an abundance of municipal solid wastes in the landfilled sites. In fact, the World Bank estimates waste generation in world cities is 1.3 billion tonnes / year and expected to reach approximate to 2.2 billion tonnes by 2025. In Malaysia, 80% of solid waste that characterize as a recyclable waste material are disposed at the landfilled sites, however these wastes are unable to recycle completely due to the limited source of separation. Therefore, the kind of recyclable waste materials such as paper, plastics and glass having the greatest potential to recycle to fabricate valuable products of wealth technology. Waste glasses are able to be upgraded into foam glass ceramic (FGC) materials. FGC represents a highly valuable solution for lightweight fill material, which has low density, incombustible, and good in mechanical strength.

Wollastonite or calcium silicate ($CaSiO_3$) is a single chain of silicate with repeated unit of three tetrahedral. Wollastonite presents the excellent properties such as non-toxic, anti-chemical and thermal stability and it has been commonly used in wide applications such as ceramics, cement, rubber, plastic and paint, etc. The theoretical density of wollastonite is 2.90 g/cm³. FGC is the kind of promising products that can be fabricated from recycled glass. The presence of its cellular structure is useful in a range of engineering applications. Physically, foam glass-ceramics is a lightweight glass material which have low density in the range 0.100 to 1.200 g/cm³ with 0.40 to 6.00 MPa of compressive strength. In term of structural, it contains two phase system which is solid phase and gaseous phase. The thin walls of the cells which have a several micrometers of thickness indicate the solid phase meanwhile the gaseous phase is within that cells.

Our research group has successfully managed to produce the FGC materials via solid-state conventional method. We used soda-lime-silica (SLS) glass waste as a matrix glass and food wastes such as eggshell (ES) and clam shell (CS) (Figure 1) as foaming agents.

Figure 1: Eggshell and clam shell food wastes

We found that the optimum parameter of the foam glass-ceramics was obtained at 800°C for 60 mins with the substitution of 3 to 6 wt.% ES and CS content. In fact, the optimum temperature was supported by the thermogravimetric analyzer (TGA) which indicate that the mass loss of $CaCO_3$ in ES and CS occur at the temperature of 800°C.

The samples prepared this way to provide a minimum bulk density of 0.326 – 0.421 g/cm³ with the maximum porosity of 87.2 – 83.16 %.

XRD analysis revealed that the formation and growth of cristobalite (SiO_2) and wollastonite ($CaSiO_3$) crystal phases after the heat-treatment process at 800 °C as shown in Figure 2.

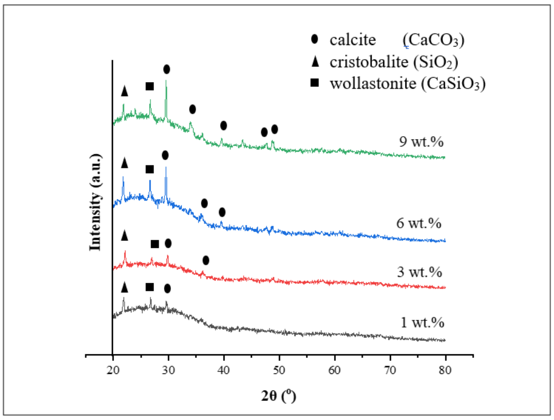

Figure 2: The typical XRD pattern of FGC at 800°C

Meanwhile, the FTIR analysis indicates the strong intensity of Ca–O bond at 620 to 650 cm⁻¹ of wavenumber associate with decreasing the intensity peak of C–O after the heat-treatment process at 800°C. Conventional, the FGC are fabricated by using solid-state sintering method or fully vitrification. The closed-cell structure is formed by the progress in the glass phase encapsulating the gas at high temperature. Initially there is small pores are formed, then the pores are continuously to grow followed by the formation of thin lamella from glass particles that surround the pores as shown in Figure 3.

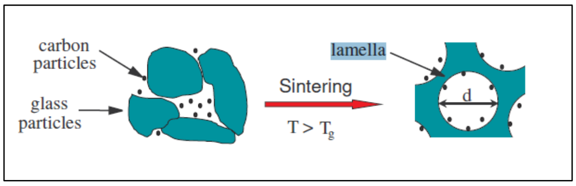

Figure 3: Schematic diagram of initial bubbles formation during sintering of a glass/carbon powder mixture

FGC is a porous material, lightweight and it is a heterophase system that consists of a solid phase and gaseous phase. It is useful and can be a candidate to be used in thermal insulation materials such as ceramic materials in the furnace, insulating for roofs, ceilings, and walls. Figure 4. reveal the microstructure of foam glass-ceramics for different content of ES at 800°C of temperature for 60 mins.

Figure 5 shows three main types of foam glass-ceramics form. Loose foam glass-ceramic aggregates are actually broken sheet of FGC, while blocks and shapes foam glass-ceramics are shaped to a specific size. Pelletization is a continuous production of spherical pellets of FGC that can apply in the manufacture of blocks, panels and slabs. The significance of this foam type material for the industrial where it can minimize heat loss or heat gain.

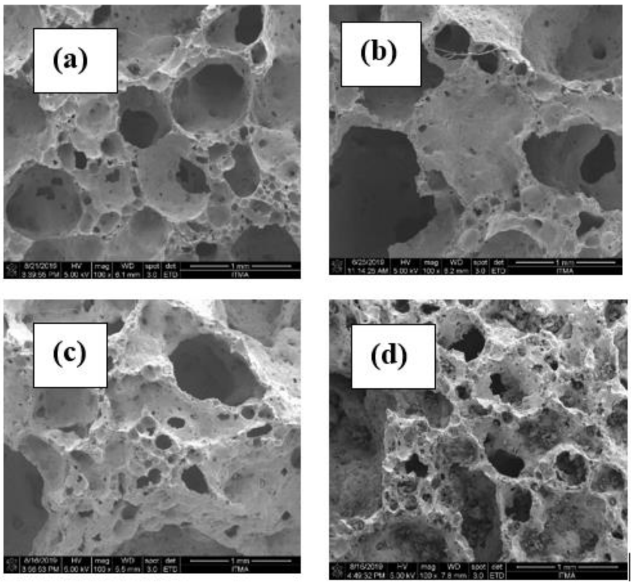

Figure 4: Microstructure of foam glass-ceramics at 800 °C for 60 mins, (a) 1 wt.% (b) 3 wt.% (c) 6 wt.% (d) 9 wt.% of ES

The preparation of foam glass-ceramics using solid wastes provide a promising way to prepare ceramic aggregate for building application as well as giving benefit to the economy and environment issue. In recent years, foam glass-ceramics have attracted great attention due to the properties and wide range of applications. The main function of FGC is to reduce the heat transfer between combustion space and molten glass due to its millions of sealed glass cells which act as thermal insulation material. FGC can be characterized as non-combustible, waterproof, impermeable to moisture and resistant to biological life. Therefore, all of these properties contribute to the constancy of thermal conductivity value of foam glass-ceramics with time.

Figure 5: Three main types of foam glass-ceramics form

In summary, the properties of foam glass-ceramics were studied underlying several objectives in this research. The type of porosity such as closed porosity, open porosity and isolated porosity can be synthesized by varying the content of foaming agent, heat treatment temperature and heat sintering duration depending on its application. Last but not least, the fundamental of foaming process depends on the balance of pressure by the gas generated and viscosity of the system. From this result, the foam glass-ceramics can be utilized in the construction sector for lightweight concrete aggregate.

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Smoothing Method for Modelling the r-smallest Order Statistics Data: Application in the Men's Olympic 100 m Freestyle Events

Let $Z_{(i,j)}$ be a series of independent r-smallest order statistics with block $i = 1, \ldots, n$ and order $j = 1, \ldots, r$. The joint density function for r-smallest order statistics for block i is

$$h(z_{(i,1)}, \ldots, z_{(i,r)}) = \exp\left\{-\left[1 - \xi\left(\frac{z_{(i,r)} - g_i}{\sigma}\right)\right]_+^{-1/\xi}\right\}\prod_{j=1}^{r}\frac{1}{\sigma}\left[1 - \xi\left(\frac{z_{(i,j)} - g_i}{\sigma}\right)\right]_+^{-1-\frac{1}{\xi}}$$

for $z_{(i,1)} < \ldots < z_{(i,r)}$ for block i (Adam, 2007). When the penalized likelihood is applied to the extreme value models, $\boldsymbol{g}$ corresponds to a smoothing through time of the location parameter. The penalized log-likelihood for Generalized Extreme Value for Minimum (GEVM) is $l_\lambda(\boldsymbol{g}, \sigma, \xi) = l(z : \boldsymbol{g}, \sigma, \xi) - \frac{1}{2}\lambda \boldsymbol{g}^T K \boldsymbol{g}$, with the log-likelihood for the r-smallest order statistics is

$$l(z : \boldsymbol{g}, \sigma, \xi) = -nr\log\sigma - \left(1 + \frac{1}{\xi}\right)\sum_{i=1}^{n}\sum_{j=1}^{r}\log\left[1 - \xi\left(\frac{z_{(i,j)} - g_i}{\sigma}\right)\right]_+ - \sum_{i=1}^{n}\left[1 - \xi\left(\frac{z_{(i,r)} - g_i}{\sigma}\right)\right]_+^{-1/\xi} - \frac{1}{2}\lambda \boldsymbol{g}^T K \boldsymbol{g}$$

where $Z_{(i,r)}$ is the rth smallest of $(X_{(i-1)m+1}, \ldots, X_{im})$, the notation $z_{(i,j)}$ means the jth smallest order statistic from block i and K is a symmetric $n \times n$ matrix of rank $n - 2$. For GEVM modelling, the parameter involved in the iteration using Fisher Scoring algorithm are σ, ξ and the smoothing functions, $\boldsymbol{g}$. The iteration is divided into two parts, the first iteration involving the smoothing functions and the second iteration involving σ and ξ. The Fisher Information for $\boldsymbol{g}$ is $\boldsymbol{I}(g) = \text{diag}(E_i)$ where $E_i = E\left[-\frac{\delta l_i^2(\sigma,\xi,g_i)}{\delta g_i^2}\right]$. The algorithm of the annual minima used is extended to the r-smallest order statistics case. This fitting method is an alternative to bayesian method (Adam & Lee, 2007).

Figure 1 (A) shows a sample of simulation data with respective parameter values. When we use smoothing parameter $\lambda = 1$, by plotting the Q-Q plot (not shown), it gives a good fit for r-smallest order statistics. Although the profile of the Olympic data is not linear, in Figure 1 (B), we have successfully fitted the non-parametric location smoothing model for the men's Olympic 100 m Freestyle from 1908 to 2004.

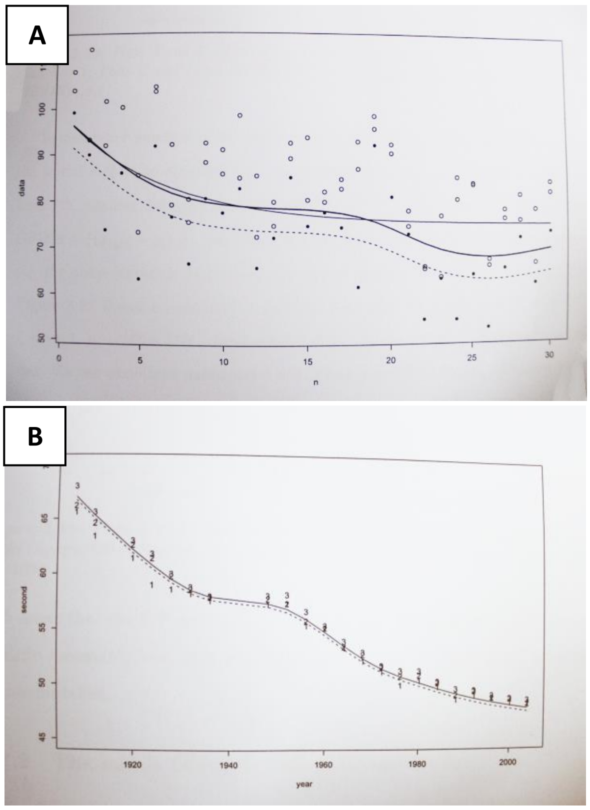

Figure 1: (A) Successful simulated GEVM($\mu_t, \sigma = 10, \xi = -0.1$) with $n = 30$ and $r = 3$. The thin line is the trend $\mu_t = \alpha - \beta\{1 - \exp(-\gamma t\}$ with $\alpha = 100$, $\beta = 25$ and $\gamma = 5$. Thick line is the penalized log-likelihood estimate of μ_t . Solid point is $x_{(t,1)}$ is the smallest data from $x_{(t,1)} < \ldots < x_{(t,r)}$ for $t = 1, \ldots, n$. The dashed line is the expected value $E(Z_t) = \hat{g}_t + \frac{\hat{\sigma}}{\hat{\xi}}[1 - \Gamma(1 - \hat{\xi})]$; **(B)** The men's Olympics 100 m Freestyle from 1908 to 2004, with the numeric labels correspond to gold (1), silver (2) and bronze (3) respectively.

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