UPM UNIVERSITI PUTRA MALAYSIA BERILMU BERBAKTI | KAMPUS BINTULU SARAWAK BINTULU SARAWAK CAMPUS

M-FICORD | MINISTRY OF FOOD INDUSTRY, COMMODITY, AND REGIONAL DEVELOPMENT SARAWAK

2nd ISCIC 2022

e-Proceeding 2nd International Scientific Conference on Indigenous Crops

21-24 September 2022, Waterfront Hotel, Kuching, Sarawak

Editors:
Shiamala Devi Ramaiya
Noorasmah Saupi
John Keen Chubo
Nozieana Khairuddin
Waseem Razzaq Khan

Supported by

BUSINESS EVENTS SARAWAK | TRIBE LEGACY SARAWAK CAMPAIGN | Sarawak More to Discover

http://cosafs2022.upm.edu.my

Al Falah Sedekap Melangsah Memupuk Falah

Edited by:

Shiamala Devi Ramaiya
Noorasmah Saupi
John Keen Chubo
Nozieana Khairuddin
Waseem Razzaq Khan

Published 2022

Faculty of Agriculture and Forestry Sciences,
University Putra Malaysia Bintulu Sarawak Campus

cosafs2022@upm.edu.my
http://cosafs2022.upm.edu.my

Foreword

The indigenous plant is defined as native plant species which originated from a region or was introduced from other places but has gone through natural processes or human selection for a long time. Local communities, particularly those living in rural areas, depend on much of their daily life on food sources available in their natural surroundings. Although important as food sources, indigenous plants have been considered lower-prestige crops with little monetary value and inferior quality.

Sarawak enjoys rich biodiversity and natural resources and should be at the forefront in sustainably cultivating potential indigenous plants besides managing on-ground indigenous crop issues and challenges to promote better productivity. Recognizing the potential of indigenous plants as cultivated crops and proper and sustainable management is the way forward. A multidisciplinary approach in related fields concerning indigenous plants is necessary for advocating their increased utilization and potential as commercial crops. This approach would comprise management and operations that are legal, economically viable, environmentally appropriate, and sustainable, as well as socially beneficial. Dissemination of knowledge and constant research is crucial to ensure the sustainable development of indigenous crops that may benefit mankind and the environment.

ISCIC2022 is highlighting the theme of 'People, Planet and Profit' with four main tracks; (a) Wild Indigenous Plant Resources, (b) Food and Medicinal Potential of Indigenous Plants, (c) Utilization of Indigenous plants for Food and Non-Food Uses and (d) Future Crops: Production and Commercialization. The conference is generally intended to promote the knowledge of indigenous crops to encourage their commercial production and downstream application and to provide a platform for all stakeholders, i.e., scientists, academics, industrial players, regulators, and the public, to share their knowledge concerning indigenous crops.

Reviewing and editing the proceedings of ISCIC2022 has been a tremendous task for the Scientific Committee, but it has brought about an incredible feeling of satisfaction upon its completion. We sincerely hope that the compilation of this procedure can benefit all interested in knowing more about our indigenous plants. Most of these indigenous plants still require detailed studies and exploration before their potential for commercialization can be exposed. Thus, this proceeding can surely assist as a reference to further study indigenous plants without the worry of duplicating earlier research.

Last but not least, we would like to thank the Ministry of Food Industry, Commodity and Regional Development Sarawak (M-FICORD), who has jointly contributed to the success of ISCIC2022. Our thanks also to all authors for sharing the result of their research work and ideas. We look forward to greater collaboration with all parties interested in expanding their research on indigenous crops.

Dr. Shiamala Devi Ramaiya
Head, Scientific Programme Committee 2nd ISCIC2022

Table of Contents

Foreword ii

Physicochemical Properties of Durian (*Durio zibethinus*) and Cempedak (*Artocarpus integer*) Seed Flour 3

Characterization of Indigenous Durians (*Durio graveolens* and *Durio oxleyanus*): Relationship of Physicochemical and Aroma Properties with Sensory Profiling 7

Zingiberaceae Species Used as Food and Traditional Medicine by Villagers in Kota Belud, Sabah, Malaysia 11

Production of Mycelium-Based Composite using Sago Waste Fiber for Packaging Application 14

Development and Characterisation of Noodles Fortified with Dabai Fruit Powder (*Canarium odontophyllum* Miq.) 17

The Quality of Dried Midin Fern (*Stenochlaena palustris*) After Rehydration 22

Seaweed (*Caulerpa lentillifera*): A Potential Sustainable Food Source 25

Wild Tuber Food Resources Among The Bateq and Semoq Beri Tribes in The East Coast of Peninsular Malaysia 29

Terap Seeds: A Promising Source of Nutrients and Oil with Functional Properties 33

Total Phenolic, Flavonoid, and Antioxidant Content in Five Selected Indigenous Food Flavouring Plants 37

Utilization of Indigenous Species as Crafts Among Iban People in Bintulu, Sarawak 41

Flower Development of Crystal Longan (*Pometia pinnata* J.R. Forst & G. Forst) 46

Synergistic Effects of Growth-Promoting Microorganisms on Seedling Production of Terap (*Artocarpus odoratissimus* Blanco) 50

Traditional Ecological Knowledge of Orang Asli in Malaysia: The Utilization of Plant Tubers as Food Resources 54

Effect of Storage Period and Temperature on External Quality of Durian Nyekak (*Durio kutejensis* (Hassk.) Becc.) 58

Molecular Identification of Bacteria with Quorum Sensing Inhibition Properties from Matang Mangrove Forest Reserve 62

Investigation of Local Palm Hearts (Umbut) as Potential Prebiotic Ingredients Using *In Vitro* Colon Model Experimentation 66

The Checklists of Insects Associated with The Sarawak Indigenous Eggplant, Terung Asam (*Solanum lasiocarpum* Dunal.) 70

Development of Micropropagation Protocol in Borneo Sour Eggplant, *Solanum lasiocarpum* Dunal. for Multiplication 74

Evaluation of Plant-Based Food Wrapper by Communities in Bintulu, Sarawak 78

Exploration of Plant Growth-Promoting Endophytic Bacteria from The Roots of Native Plant *Uncaria borneensis* 83

Preliminary Evaluation of *Midin (Stenochlaena palustris)*, The Edible Fern of Sarawak, Malaysia as A Potential Prebiotic Ingredient 87

Physicochemical Properties of Durian (*Durio zibethinus*) and Cempedak (*Artocarpus integer*) Seed Flour

Irine Runnie Ginjom*, Satchitanantha Sivakumaran, and Mary Elizabeth Pariamble Javapro
School of Engineering and Science, Faculty of Engineering, Computing and Science, Swinburne University of Technology Sarawak, Jalan Simpang Tiga, 93350 Kuching, Sarawak, Malaysia

**Corresponding author's email: ihenry@swinburne.edu.my*

INTRODUCTION

Fruits make up a large portion of the natural human diet. Hence, large amounts of fruit are consumed daily by the human population. Durian (*Durio zibethinus*) and cempedak (*Artocarpus integer*) are among the fruits that are commonly consumed in Malaysia (Dadak, 2019). These fruits, however, consist of 70-80% of waste parts, like the peel, rind, skin, and seeds, that end up in landfills or incinerated (Akter and Haque, 2019; Payus et al., 2021). A solution to this problem is to reutilize these fruit wastes effectively and find a value-added use for them. One common target is the seed, which commonly stores high proportions of starch to provide energy and materials for growth. The seeds could be utilised as human or animal food. Starch is a very common ingredient and additive in food. It is added for its thickening, gelling, filling, and adhesive properties. These functions depend on the starch's chemical structure and compositions, which vary with different starch sources.

In this study, the physicochemical properties of durian seed flour (DSF) and cempedak seed flour (CSF) were evaluated for their suitability to be used as a new source of carbohydrate-rich flour. Commercial multi-purpose wheat flour was also analysed for comparisons. Proximate analysis was conducted to determine moisture, ash, lipid, protein and carbohydrate contents of the seed flours. Selected functional properties such as the water and oil absorption capacity, syneresis and swelling power of the seed flours were also determined. This study provided a preliminary information on the potentials of durian and cempedak seed as source of nutritional and functional food components, while at the same time, reduce food wastages.

MATERIALS AND METHODS

One kilogram of local durian (*Durio zibethinus*) and cempedak (*Artocarpus integer*) seeds were separated from the edible flesh and rinsed with water. The cempedak seeds were soaked overnight to ease the removal of their outer coat. The seed coat of the durian seed was manually peeled with a knife or fruit peeler. The seeds were rinsed once again, allowed to air-dry for about one hour, and grated into small pieces (Figure 1). The grated pieces were then sun-dried for two days or until they reached constant weight. Both seeds contained about 45% of moisture. The seeds were then ground into powder or flour using a kitchen blender and sieved using a 0.85 mm mesh-hole flour sieve. Wheat flour was purchased from a local supermarket. All flour samples were kept in air-tight containers at room temperature for further analysis.

The chemical composition was determined according to the AOAC (2007) methods. Moisture content was determined using oven drying methods involving overnight drying at 105°C. Total protein was estimated using the Kjeldahl method, while total fat was determined using the Soxhlet method. Ash content was determined using the dry ashing method. Total carbohydrate percentage was calculated by difference through the formula 100 – (moisture % + protein % + fat %). The water absorption capacity and oil absorption capacity, syneresis, swelling power and solubility of the seed flour were determined based on the methods described by Baraheng and Karrila (2019). Except for the total protein and total carbohydrate, all determinations were examined in triplicates and results expressed as means±standard error.

(A) (B) (C)

(D) (E) (F)

Figure 1. Photos of fruit seed samples; (A) Clean durian seed, (B) Grated durian seed, (C) Durian seed powder, (D) Clean cempedak seed, (E) Grated cempedak seed, and (F) Cempedak seed powder.

RESULTS AND DISCUSSION

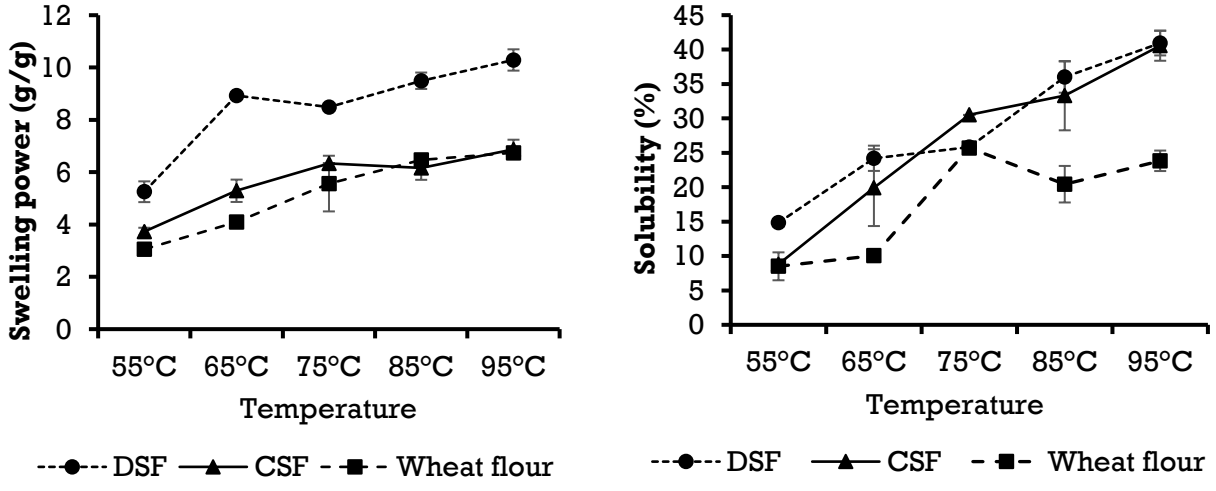
The chemical composition of the fruit seed flours was compared to those of wheat flour (Table 1). The moisture contents of the seed flours were significantly lower than those of wheat flour. However, these values are lower than 14%, which is within the permissible range of moisture content of flour as stated in Regulation 43: Wheat flour (Malaysia, International Law Book Services, 2014). Low moisture content ensures that the flour has a long shelf life. The total fat in the DSF was two times more than those reported in the CSF and in wheat flour.

Water absorption capacity (WAC) and oil absorption capacity (OAC) are the amount of water or oil taken up by food/flour to achieve the desirable consistency and good quality products. Thus, different bakery products and food formulations require different WAC and OAC. Table 1 also summarizes the WAC and OAC of DSF and CSF, in comparison to wheat flour. Both seed flours have lower WAC and OAC than those wheat flour. Substituting wheat flour with seed flour would then lead to the under-absorption of water and oil, which could result in bakery products that are crumbly, lower volume and stale more quickly (Godswill et al., 2019). Likewise, both seed flours have higher syneresis value than wheat flour, suggesting lower freeze-thaw stability properties. Syneresis is also linked to higher amylose content (Bao and Bergman, 2004).

Table 1. Chemical composition and functional properties of DSF, CSF and wheat flour.

Parameters	**DSF**	**CSF**	**Wheat flour**
Moisture (%)	2.39±0.13^{b}	1.07±0.17^{c}	8.50±0.15^{a}
Total fat (%)	2.01±0.1^{a}	0.87±0.3^{b}	1.00*
Ash (%)	3.17±0.01^{b}	4.15±0.08^{a}	2.72±0.03^{b}
Crude protein (%)	6.70*	10.30*	11.4*
Total carbohydrate (%)	85.78*	83.12*	76.47*
WAC (g/g)	3.06±0.01^{b}	2.43±0.00^{c}	6.01±0.06^{a}
OAC (g/g)	8.60±0.06^{b}	8.55±0.10^{b}	11.47±0.09^{a}
Syneresis (%)	71.11±1.11^{a}	75.00±0.96^{a}	55.56±2.22^{b}

Values are given in means±standard error. Mean values in the same row with different alphabets (a>b>c) are significantly different at $p<0.05$. *Data from single determinations. DSF: Durian seed flour; CSF: Cempedak seed flour; WAC: Water absorption capacity; OAC: Oil absorption capacity.



(A) (B)

Figure 2. Effect of temperature on (A) swelling power, and (B) solubility of durian seed flour (DSF); cempedak seed flour (CSF), and wheat flour.

Figure 2 summarises the swelling power and solubility of the three flour samples. The values of both parameters increase with the increase in temperature. The DSF showed higher swelling power than those of CSF and wheat flour. This is consistent with other findings that also compared both cempedak and durian seed starch swelling power from Thailand (Tongdang, 2008). The swelling power of a flour or starch is affected by the particle size and composition of the starch. For example, the high amylose content in jackfruit seed starch leads to its lower swelling power (Madruga et al., 2014). On the other hand, starch with higher amylopectin content, such as potato starch and sago starch, are expected to have higher swelling power.

CONCLUSION

In summary, the chemical composition of the three flour samples varies, especially in their moisture content. In terms of functional property analysis, DSF and CSF have lower water and oil absorption capacity and higher syneresis value. The swelling power of DSF is higher than both CSF and wheat flour. With an overview of all the data, it is deduced that the current form of DSF and CSF is not a suitable substitute for wheat flour. However, this opens the option of DSF and JSF being used for replacements of other types of flour or other uses.

ACKNOWLEDGEMENT

Authors acknowledge support from Swinburne University of Technology Sarawak Campus for NPS30003 Grand Challenges in Science capstone projects.

REFERENCES

Akter, F. and Haque, M.A. 2019. Jackfruit waste: A promising source of food and feed. *Annals of Bangladesh Agriculture, 23*(1): 91–102.

AOAC. 2007. Official Methods of Analysis. 18th Ed., AOAC International, Gaithersburg, MD, Washington, D.C., USA: Association of Official Analytical Chemists.

Bao, J. and J.C. Bergman. 2004. The functionality of rice starch. pp. 258-294. En: Eliasson, A.C. (ed.). Starch in food: Structure, function and applications. CRC Press, New York, USA.

Baraheng, S. and Karrila, T. 2019. Chemical and functional properties of durian (*Durio zibethinus* Murr.) seed flour and starch. *Food Bioscience, 30*:100412.

Dadak, R.A. 2019. Trends in production, trade, and consumption of tropical fruit in Malaysia. FFTC Agricultural Policy Platform (FFTC-AP). https://ap.fftc.org.tw/article/1381 (Accessed August 02, 2022).

Godswill, A.C. 2019. The functional properties of foods and flours. *International Journal of Advanced Academic Research – Sciences, Technology and Engineering, 5*(11):139-160.

Madruga, M.S. 2014. Chemical, morphological and functional properties of Brazilian jackfruit (*Artocarpus heterophyllus* L.) seeds starch. *Food Chemistry, 143*: 440-445.

Malaysia, International Law Book Services. 2014. Food act 1983 (Act 281) & regulations: (as at 5th August 2014). Petaling Jaya: International Law Book Services.

Payus, C.M. 2021. Durian husk wastes as low-cost adsorbent for physical pollutants removal: groundwater supply. *Materials Today: Proceedings, 42*: 80–87.

Tongdang, T. 2008. Some properties of starch extracted from three Thai aromatic fruit seeds. *Starch Staerke, 60*(3-4): 199-207.

Characterization of Indigenous Durians (*Durio graveolens* and *Durio oxleyanus*): Relationship of Physicochemical and Aroma Properties with Sensory Profiling

Gerevieve Bangi Sujang[1], Shiamala Devi Ramaiya[1,*] Meekiong Kalu[2], Noorasmah Saupi[1] and Shiou Yih Lee[3]

[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu, Sarawak Campus, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia

[2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak Malaysia

[3]Faculty of Health and Life Sciences, INTI International University, Nilai, Malaysia

**Corresponding author's email: shiamala@upm.edu.my*

INTRODUCTION

Sarawak is home to 16 unique *Durio* species. These local durians are diverse in shape, taste, and smell that holds great promise for future domestication. Among the wild durians, *Durio graveolens* (isu) and *Durio oxleyanus* (daun) are popular among the local communities in Sarawak and are widely sold at Tamu markets at a good price. The local people addressed both as 'isu' as they are morphologically alike. These indigenous species provide a source of nutrition and income for local people in rural areas. Considering the rising demand for local durians, factors like nutritional, phytochemical, and aroma are further investigated to explore the potential of favourable traits and increase their economic value. Therefore, the present study focuses on the physicochemical and volatile properties of two local *Durio* species, i.e., *Durio graveolens* (isu kuning, isu oren, and isu merah) and *Durio oxleyanus* (durian daun).

MATERIALS AND METHODS

Samples were collected within Sarawak; Baram, Bekenu, Bintulu, and Sibu. The flesh was divided into fresh and oven dried. The fresh samples were used directly to determine the physicochemical and sensory analysis following the AOAC (2000) and Quantitative Descriptive Analysis (QDA®) methods, respectively. The fresh samples were also used to analyse the volatile profiling using SPME in GC-MS (Peng, 2019). The oven-dried samples were used to analyse physicochemical composition following the AOAC (2000) method. The data were statistically analyzed using SAS window program 9.4. Means were compared using single-factor ANOVA. Post-hoc Tukey's (p<0.05) was performed if the ANOVA result was significant. Principle component analysis (PCA) was carried out to study the relationship between the physicochemical and aroma compound with sensory attributes.

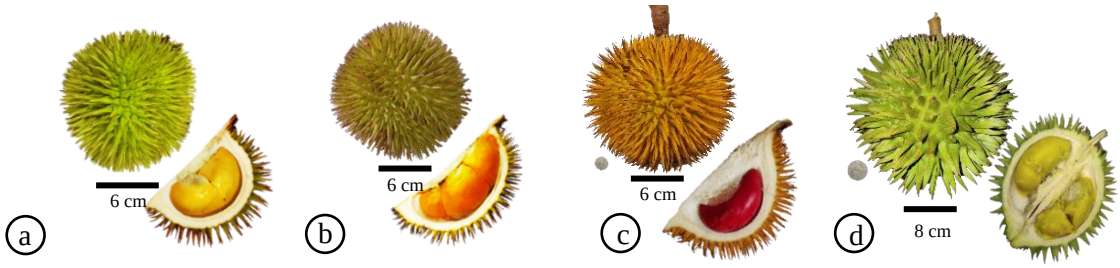


Figure 1. The fruits of *Durio graveolens* (a) isu kuning, (b) isu oren, (c) isu merah and (d) *Durio oxleyanus* (daun).

RESULTS AND DISCUSSION

The physicochemical properties of *Durio graveolens* and *Durio oxleyanus*

The result of the physicochemical analysis of the four genotypes is presented in Table 1. The pH values for the four genotypes were slightly acidic which contradicted the common *D. zibethinus*; D175 (7.51, Isa et al., 2019). Isu merah showed the lowest TSS at 12.50±0.28 °Brix but possessed twice higher fat (17.40%) and fiber (17.30%) contents among the four genotypes. The TTA values ranged 0.12 to 0.14%, which is higher than the *D*. *zibethinus* (D24 and D175), ranged from 0.05-0.06% (Isa et al., 2019). The moisture content of the four genotypes were within the range of other durians i.e., *D. kutejensis* (55.15%, Belgis et al., 2016), and *D. zibethinus* (63.06%, Belgis et al., 2016). Isu kuning, isu merah, and durian daun have high percentages of ash implying that the fruits contain high concentrations of minerals. The four genotypes have low carbohydrate content compared to the common cultivar, D24 (35.44%, Isa et al., 2019).

Table 1. The physicochemical properties of *Durio graveolens* and *Durio oxleyanus.*

Genotypes	***D. graveolens* (Isu kuning)**	***D. graveolens* (Isu oren)**	***D. graveolens* (Isu merah)**	***D. oxleyanus* (Daun)**
pH	6.89±0.02^{a}	6.30±0.01^{b}	6.22±0.01^{c}	6.85±0.01^{a}
TSS (°Brix)	21.33±0.92^{a}	20.83±0.60^{a}	12.50±0.28^{b}	22.83±1.30^{a}
TTA (%)	0.14±0.01^{a}	0.13±0.01^{b}	0.12±0.01^{b}	0.14±0.01^{b}
Moisture (%)	54.40±2.68^{b}	63.37±0.80^{a}	46.90±0.52^{b}	64.19±1.20^{a}
Ash (%)	1.90±0.18^{ab}	1.36±0.02^{b}	1.79±0.06^{ab}	2.10±0.12^{a}
Carbohydrate (%)	28.55±2.76^{a}	21.05±0.70^{ab}	18.06±0.36^{b}	19.58±1.29^{b}
Protein (%)	3.09±0.02^{b}	2.80±0.01^{b}	3.55±0.05^{a}	3.71±0.11^{a}
Fat (%)	7.35±0.09^{b}	7.82±0.19^{b}	17.40±0.19^{a}	6.64±0.12^{c}
Fiber (%)	4.70±0.01^{b}	3.61±0.05^{c}	12.30±0.14^{a}	3.80±0.03^{c}

Different superscript alphabets in the same row indicate differences at $p<0.05$ (ANOVA, Tukey's test).

The volatile profiling of *Durio graveolens* and *Durio oxleyanus*

A total of 81 volatile organic compounds (VOCs) were detected in the four genotypes comprised of 30 esters, 22 alcohols, 9 ketones, 7 ethers, 6 aldehydes, 3 alkanes, and 4 sulfur compounds. The ester and alcohol compounds presented 32-42%, and 23-33%, respectively and the remaining 3%-13% of the total peak area corresponded to another group of VOCs which included ketones, ethers, aldehydes, alkanes, and sulfur (Figure 2). Notably, alkane compounds were not present in isu oren and daun. The VOCs in the four genotypes contradicted previous study of common durian clones; *i.e,* Musang King, D24, and Black Thorn (Tan et al., 2020) where the sulfur compounds predominate. Sulfur compounds give a distinctive "onion-sulfur' odour (Chin et al., 2007), while ester compounds give a sweet, fruity aroma and pleasant odour (Brat et al., 2004), which proves that indigenous durian has a less intense aroma compared to the common cultivar.

The sensory attributes of *Durio graveolens* and *Durio oxleyanus*

The three genotypes of *D. graveolens* vary in sensory attributes, although they belong to the same species (Figure 3). According to the panelist, isu merah was the creamiest fruit indicating high-fat content, but it is the least sweet and smooth among the four genotypes. This genotype also possessed strong nutty and grassy aroma. Durian daun was perceived to have the smoothest texture as it has high moisture content. The stickiness was higher in *D. graveolens* (isu oren and isu merah). In terms of aroma, the panelists perceived that the four genotypes have less aroma compared to the common durians. *Durio graveolens* (isu oren) perceived fruity and sweet aroma among the genotypes.

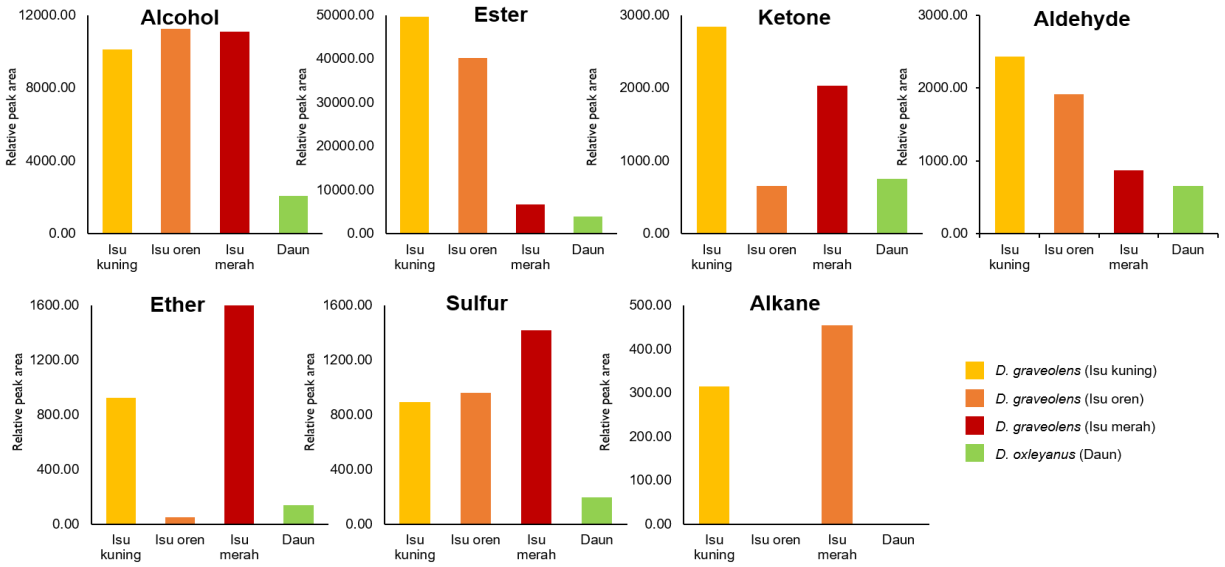


Figure 2. Distribution of volatile organic compound (VOCs) in *Durio graveolens* and *Durio oxleyanus*.

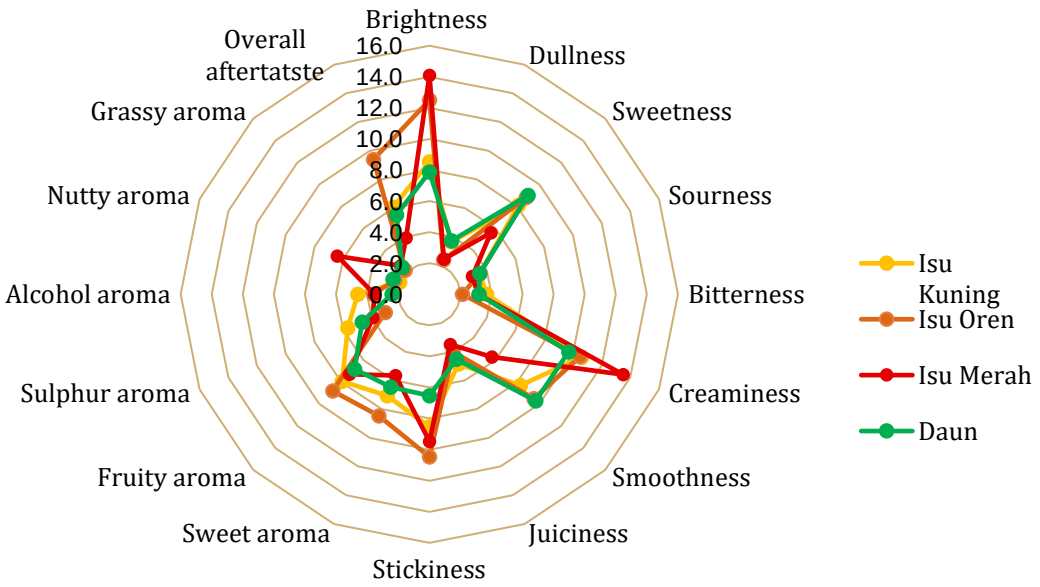


Figure 3. Sensory profile composed of average scores of 16 attributes in *Durio graveolens* and *Durio oxleyanus.*

The relationship between aroma and sensory attributes

The relationships between different aromas and sensory attributes for the four genotypes are presented in Figure 4. The first two PC's accounted for 77.22% of the total variance. The studied *Durio* genotypes were clustered into three main groups. Group 1 consisted of *D. oxyleanus*, which was predominant with ester compounds and has milder aroma. Isu oren and kuning were members of Group 2, which were highly associated with diverse compositions of volatile compounds related to sweet, fruity, and alcoholic aromas. Group 3 consisted of isu merah that exhibited grassy, sulfur, and nutty aroma, which were associated to higher alcohol content together with sulfur, ether, and alkane compounds.

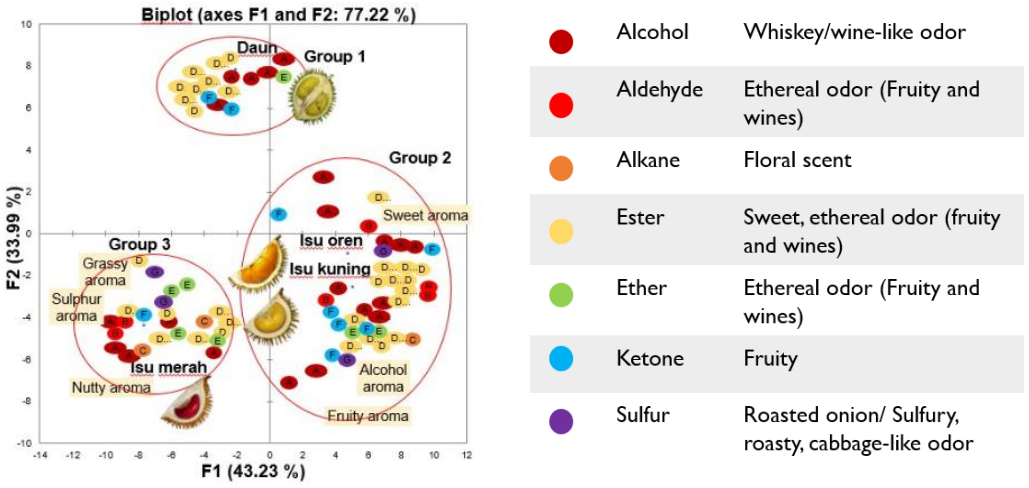


Figure 4. The Principal Component Analysis (PCA) for aroma and sensory attributes.

CONCLUSION

The present findings provide basic information for product development and breeding of new durian cultivars with milder aromas.

ACKNOWLEDGEMENT

Thank you to the Ministry of Higher Education under the Fundamental Research Grant Scheme FRGS/1/2020/STG03/UPM/02/10, and the durian growers who helped us throughout this research.

REFERENCES

AOAC, 200O. Official Methods of Analysis. 17th Ed., AOAC International, Gaithersburg, MD, Washington, D.C., USA: Association of Official Analytical Chemists.

Belgis, M., Wijaya, C.H., Apriyantono, A., Kusbiantoro, B., and Yuliana, N.D. 2016. Physicochemical differences and sensory profiling of six lai (*Durio kutejensis*) cultivars indigenous Indonesia. *International Food Research Journal*, *23*(4): 1466.

Brat, P., Hoang, L.N., Soler, A., Reynes, M. and Brillouet, J.M. 2004. Physicochemical characterization of a new pineapple hybrid. *Journal of Agricultural and Food Chemistry, 52*(20): 6170-6177.

Chin, S.T., Nazimah, S.A.H., Quek, S.Y., Man, Y.C., Rahman, R.A. and Hashim, D.M. 2007. Analysis of volatile compounds from Malaysian durians (*Durio zibethinus*) using headspace SPME coupled to fast GC-MS. *Journal of Food Composition and Analysis, 20*(1): 31-44.

Isa, I.Z.M., Mustafa, N.A.A., Shahril, M.Z., Abdul, A., Hamid, N. H.Z.A. and Dek, M.S.P.2019). Proximate composition, physicochemical and antioxidant properties of three durian (*Durio zibethinus*) cultivars. *Journal of Tropical Plant Physiology, 11*(2): 22-30.

Peng, J.S.M. 2019. Volatile esters and sulfur compounds in durians & a suggested approach to enhancing economic value of durians. *Malaysian Journal of Sustainable Agriculture*, *3*(2): 5-15.

Tan, X.Y., Misran, A., Cheong, K.W., Daim, L.D.J., Ding, P. and Dek, M.S.P. 2020. Postharvest quality indices of different durian clones at ripening stage and their volatile organic compounds. *Scientia Horticulturae*, *264*: 109169.

Zingiberaceae Species Used as Food and Traditional Medicine by Villagers in Kota Belud, Sabah, Malaysia

Fadzilah Awang-Kanak*
Preparatory Centre for Science and Technology, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia

**Corresponding author's email: akfadzil@ums.edu.my*

INTRODUCTION

Sabah is located on Borneo Island, the center for plant diversity. The local population has been using available plant resources since ancient times. Ethnobotany is further elaborated as people's dealings with plants with multidisciplinary approaches, including anthropology, botany, culture, economy, traditional medicine, sociology, and traditional knowledge (Awang-Kanak and Abu Bakar, 2020). The Sama-Bajau and Dusun people in Kota Belud have been recorded utilizing the plant resources within their surroundings as food and traditional medicines to treat various ailments (Halim et al., 2013; Awang-Kanak et al., 2018a; Awang-Kanak et al., 2018b; Adam et al., 2019)

Edible traditional plants have proven their significance as traditional plant medicine (Awang-Kanak, 2021; Awang-Kanak et al., 2020). Therefore, it is important to continue documenting ethnobotany and ethnopharmacology data from local communities in Sabah, as the availability of these data could trigger further research in drug discovery and development. This work aims to record the ginger species (Zingiberaceae) that have been used as food and traditional medicine by villagers in Kota Belud, Sabah. This research was conducted through several field surveys and semi-structured interviews with local informants. The identification key of Zingiberaceae that have been used as food and traditional medicine in Kota Belud was also proposed.

MATERIALS AND METHODS

Study area

Kota Belud district is a township surrounded by villages, paddy fields, and foraging forest, covering an area of 1385.6 km^2. This district is located about 70 km northwest of Sabah state capital of Kota Kinabalu The local people of Kota Belud are of various ethnic groups, mainly Sama-Bajau, Ubian-Bajau, Irranun, Dusun, Rungus, and Chinese. The Bajaus and Irranun usually live in villages near the coastal area, while the Dusun and Rungus live in inland villages.

Data collection

Interviews were conducted using semi-structured questionnaires among key informants who possess the traditional knowledge of ginger species that have been used as food and traditional medicine. These informants were selected using the snowball sampling technique. The selection was based on (i) the position of informants in the local community as respected headman or native chiefs, and (ii) their ability to identify traditional food condiments and explain their uses, including herbal medicine (Awang-Kanak, 2018). Plant samples were observed and identified from the informant's home garden. Other than that, plant samples were also obtained from local traders in Kota Belud market. The samples are housed in Universiti Malaysia Sabah.

RESULTS AND DISCUSSION

The Zingiberaceae family has about 50 genera and more than 1300 known species. All the species were found in the lowland area, shaded or partially shaded, with high humidity, and nearby the

housing area. There are six species of the Zingiberaceae family recorded in this study, i.e. *Curcuma caesia*, *C. longa*, *C. xanthorrhiza*, *Etlingera elatior*, *E. punicea*, *Zingiber zerumbet*. All six species have been consumed as food and four of the six species, namely *C. caesia, C. longa, C. xanthorrhiza*, and *Zingiber zerumbet* are used as traditional medicine. *Cucurma longa, C. xanthorrhiza*, and *Zingiber zerumbet* were taken fresh as traditional medicine during the postpartum recovery period. Leaves and tuber of *C. caesia*, the tuber of *C. longa*, and young leaves of *C. xanthorrhiza* were prepared as decoction before being used as traditional remedies to treat cough.

Curcuma caesia plant has white to yellow-white or pale coloured tubular flower and deep blue to black coloured tuber. Meanwhile, *C. longa* or turmeric (Malay: "kunyit") is the most recognized member of *Curcuma genus* that has deep yellow to orange coloured tuber. *Cucurma xanthorrhiza* plant has long stalk of flower that can reach up to 35 cm upright. It is known as "temulawak" by the locals, and is also known as the Javanese ginger. *Zingiber zerumbet* is locally known as "lempoyang", it has inflorescence at the base and separates from the leaf shoot. Its young flower is usually green in colour and matured flower developed a very bright red colour. It is also known to have anti-microbial, antipyretic, and analgesic properties (Trimanto, 2017).

Identification key of Zingiberaceae species used as food and traditional medicine by villagers in Kota Belud, Sabah

1. Inflorescence at the base and separate from leaf shoot……***Zingiber zerumbet***

1. Inflorescence at the terminal on a central of leafy shoot……2

2. Flower tubular in white to yellow-white or pale colour……3

2. Flower and inflorescence in varying shades of pink to red or bright colour……5

3. Long flower stalk, hairy, length up to 37cm upright……***Curcuma xanthorrhiza***

3. Shorter flower stalk……4

4. Yellow-orange coloured tuber……***Curcuma longa***

4. Deep blue to grey black tuber……***Curcuma caesia***

5. Receptacle of inflorescence elongating and during blooming form cone shaped flower head……***Etlingera elatior***

5. Receptacle of inflorescence is shorter, and during blooming form a flat or bowl shaped flower head……***Etlingera punicea***

CONCLUSION

Zingiberaceae family have been used as food condiments and traditional medicine for various ailments and included as a remedy for postpartum women in Kota Belud. This indicates that these species are safe to be consumed as food and have the potential for drug discovery research. Due to Zingiberaceae traditional usage, the commercialization of Zingiberaceae species could bring economic benefits to the local people in Kota Belud, as many downstream food and beverage products are utilizing the Ginger family as the main ingredient. However, from the conservation point of view, efforts should be concentrated on conserving wild ginger species that may have novel bioactive compounds that could provide pharmaceutical benefits.

REFERENCES

Adam, A.A., Othman, N., Halim, A.A., Ismail, S.R. and Samah, A.A. 2019. The Practice of Biodiversity –

related indigenous knowledge in Kota Belud, Sabah: A preliminary study. *Pertanika Journal of Social Sciences and Humanities*, *27*(S1): 215–225.

Awang-Kanak, F. 2021. Plant parts and preparation of edible plants by indigenous Sama-Bajau and Dusun people in Kota Belud, Sabah. *IOP Conference Series: Earth and Environmental Science*, *756*(1).

Awang-Kanak, F. and Abu Bakar, M.F. 2020. Traditional vegetable salad (ulam) of Borneo as source of functional food. *Food Research*, *4*(1).

Awang-Kanak, F., Abu Bakar, M.F.and Mohamed, M. 2018a. Ethnobotanical survey on plants used as traditional salad food (ulam) in Kampung Taun Gusi, Kota Belud Sabah, Malaysia. *AIP Conference Proceedings*, *2002*.

Awang-Kanak, F., Bakar, M.F.A., Mohamed, M. and Norazlimi, N.A. 2018b. Utilization of natural resources: Preliminary study on ethnopharmacological application of "ulam" or traditional vegetables among Sama-Bajau of Kampung Menunggui, Kota Belud, Sabah. *AIP Conference Proceedings*, *2016*.

Awang-Kanak, Fadzilah, Abu Bakar, M.F. and Matawali, A. 2020. Ethnobotanical Indices for Traditional Vegetable and Herbal Medicine Species Consumed in Kota Belud, Sabah, Malaysia. *IOP Conference Series: Earth and Environmental Science*, *549*(1), 0–4.

Halim, A.A., Othman, N., Ismail, S.R., Jawan, J.A. and Ibrahim, N.N. 2013. Indigenous Knowledge and Biodiversity Conservation in Sabah, Malaysia. *International Journal of Social Science and Humanity*, *January*, 159–163.

Trimanto. 2017. *Ginger species in Besiq Bermai forest , East Borneo : Inventory and collection Ginger Species in Besiq Bermai Forest, East Borneo : Inventory and Collection*. *050002*(May).

Production of Mycelium-Based Composite using Sago Waste Fiber for Packaging Application

Muthukumaaran Manickam and Muhammad Huzaifah Bin Mohd Roslim*
Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia

**Corresponding author's email: muhammadhuzaifah@upm.edu.my*

INTRODUCTION

Plastic waste pollution is a parallel pandemic of COVID-19, threatening the welfare of humans, the environment, and biodiversity. Moreover, online shopping has substantially increased. According to Bulk Bag Reclamation (2019), Amazon, which is a profound e-commerce company, uses prime packages of about 5 billion per year and 13 million packaging per day. Research by World wildlife Fund (WWF) in 2019 reported that Malaysia tops the list of plastic utilization per annum comparable to other Asian countries like Indonesia and Vietnam, where each citizen constitutes 16.78 kg. Besides, Malaysia is a large plastic producer worldwide and generated a net revenue of RM30.98 billion in 2018. Almost 48% of plastic production is utilised in packaging applications (Lee, 2019).

Over and above, Malaysia secures the third-largest sago producer after Indonesia and New Guinea. Naim et al. (2016) spotlight that sago production is the highest in Sarawak. In 2021, with a net worth of RM78.3 million, Sarawak exported 37,884 tonnes of sago starch worldwide (Wong, 2022). A considerable amount of sago waste is dumped into the river after starch extraction. Nearly 60 tonnes of sago scrapping are directed into the Sarawak's River daily (Zakaria, 2022). Research by Rosli et al. (2020) denotes that major river in the district of Sarawak, like Mukah, Lawas, and Kuching, are under poor water quality margin due to their high pH and ammonia concentration, biochemical oxygen demand, and trace metals. Hence, to surmount this problem, this study investigates biocomposite production using sago waste fiber (SWF) and mycelium of the fungus *Pleurotus ostreatus* as the binding agent. This study also characterizes the physical properties of biocomposite suitable for packaging application.

MATERIALS AND METHODS

Previous research by Joshi et al. (2019), Elsacker et al. (2020), and Fairus et al. (2021) referred to make a general protocol for making mycelium biocomposite from Sago Waste Fiber (SWF). The mycelium of the fungus *Pleurotus ostreatus* was inoculated in the Potato Dextrose Agar (PDA) and incubated for 14 days under room temperature. The SWF was sieved through a 5 mm soil sieve and placed in distilled water for 24 hours at room temperature, followed by drying in a hot oven at 60^0C for 3 hours. Next, the dried SWF was autoclaved at 121^0C for 20 minutes. The sterilized SWF and pieces of fully-grown mycelium of *Pleurotus ostreatus* were mixed into mushroom cultivation bags, followed by incubation at room temperature for 14 days. Then, the mixture was transferred into a plastic mould and incubated for 30 days to obtain the desired shape for the water absorption test adhering to the ASTM D1037-12.

For the WA test, five samples (R_1, R_2, R_3, R_4, R_5) sized 20 x 20 x 20 mm^3 each were prepared and measured. The weight of the dry mycelium biocomposite denotes the initial dry weight (W_i). Then, the samples were immersed in water for one week at room temperature, measuring the final weight (W_f) every day. The same method was employed in Styrofoam for comparability. The percentage of water absorption was calculated by Eq. (1)

$$\text{water absorption (\%)} = \left(\frac{W_f - W_i}{W_i} \right) \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

There are concerns about the water-absorbing behaviour of natural fibre-based biocomposites due to their hydrophilic nature. Hence, the water absorption test is essential in analysing the degree of hydrophilicity that suits packaging applications. Figure 1 depicts results of the water absorption rate (WA) of mycelium biocomposite (MB) and Styrofoam for a period of seven days. Generally, all samples of MB show a drastic increase in the WA rate from Day 1 to Day 3 and a gradual increment from Day 4 to Day 7.

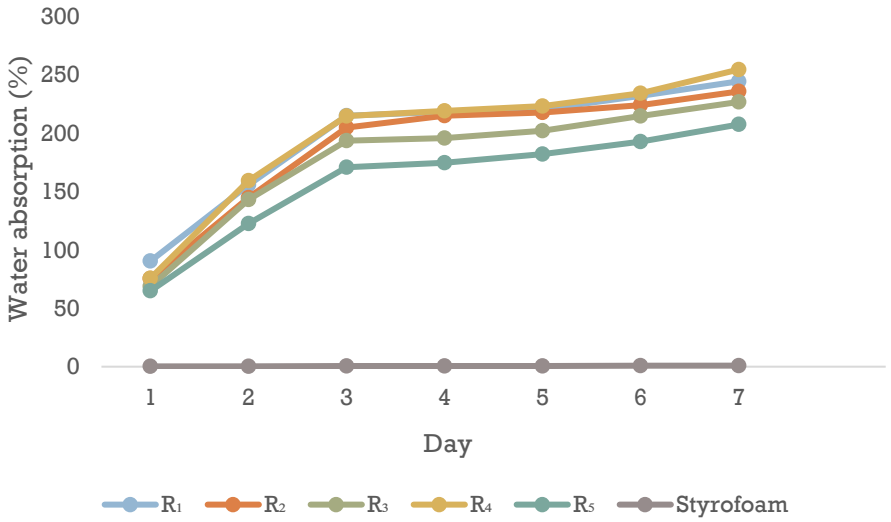


Figure 1. Water absorption behaviour of mycelium biocomposite and styrofoam.

For the sample R4, the WA rate on Day 1 was 75.63%, Day 3 was 214.34%, and Day 7 was 254.12%. On average, the WA rate of MB was 230% though previous research claimed 300% (Attias et al., 2019). The complex interaction between the hydrophobic nature of mycelium, the strong hydrophilic characteristics of cellulosic material, and the high porosity of the mycelium may have changed with water, accounting for the rigorous WA rate of MB. In contrast, the WA rate of Styrofoam was only about 0.9%, owing to the presence of hydrocarbons attached to the benzene ring (Thormann et al., 2008). Therefore, further fortification is needed to downscale the WA rate of MB before it can be applied in packaging.

CONCLUSION

The mycelium of the fungus *Pleurotus ostreatus* can degrade and colonize Sago Waste Fiber (SWF) to form a biocomposite. However, the water absorption rate was about 230%, which requires further research to reduce it, either by increasing the mycelium colonizing rate into the fibers, employing a hot/cold press when fabricating the biocomposite and researching other types of fungal mycelium that have greater strength and density.

ACKNOWLEDGEMENT

My gratitude to the Department of Crop Science, Universiti Putra Malaysia Bintulu Sarawak Campus, for the laboratory facilities.

REFERENCES

Attias, N., Danai, O., Abitbol, T., Tarazi, E., Ezov, N., Pereman, I. and Grobman, Y.J. 2020. Mycelium bio-composites in industrial design and architecture: Comparative review and experimental analysis. *Journal of Cleaner Production*, *246*: 119037.

Bulk Bag Reclamation. 2022. What Is Green Packaging? Available at: https://bulkbagreclamation.com/what-is-green-packaging/ (Accessed on 10th May 2022)

Elsacker, E., Vandelook, S., Van Wylick, A., Ruytinx, J., De Laet, L. and Peeters, E. 2020. A comprehensive framework for the production of mycelium-based lignocellulosic composites. *Science of The Total Environment*, *725*: 138431.

Fairus, M.J.M., Bahrin, E.K., Natasha, E., Arbaain, N. and Ramli, N. 2022. Mycelium-based composite: A way forward for renewable material. *Journal of Sustainability Science and Management*, *17*(1): 271-280.

Joshi, K., Meher, M.K. and Poluri, K.M. 2020. Fabrication and characterization of bioblocks from agricultural waste using fungal mycelium for renewable and sustainable applications. *ACS Applied Bio Materials*, *3*(4): 1884-1892.

Lee, E. 2019. The State of the Nation: Single-use plastics ban stirs concern among the manufacturers. *The Edge Markets*.

Naim, H.M., Yaakub, A.N. and Hamdan, D.A.A. 2016. Commercialization of sago through estate plantation scheme in Sarawak: The way forward. *International Journal of Agronomy*, *2*: 1-6.

Rosli, N., Gandaseca, S., Gerusu, G.J., Heng, R.K.J., Ahmed, O.H., Idris, M.H. and Mohamad Pazi, A.M. 2020. Quality of tropical river water in different catchments of canopy cover. *Malaysian For*, *83*(1): 128-148.

Thormann, E., Simonsen, A.C., Hansen, P.L. and Mouritsen, O.G. 2008. Interactions between a polystyrene particle and hydrophilic and hydrophobic surfaces in aqueous solutions. *Langmuir*, *24*(14): 7278-7284.

Wong, J. 2022. *Sarawak pushing for sago cultivation*. Available at: https://www.thestar.com.my/business/business-news/2022/02/21/sarawak-pushing-for-sago-cultivation (Accessed on 16 June 2022)

World Wildlife Fund. 2020. WWF Releases Report Proposing Effective Solution to Mitigate Plastic Pollution in Malaysia: *World Wildlife Fund, Inc*. (Accessed on 14 July 2022)

Zakaria. A. 2022. *Sago waste too good to waste*. Available at: https://upm.edu.my/content/sago_waste_too_good_to_waste-25176 (Accessed on 16 June 2022).

Development and Characterisation of Noodles Fortified with Dabai Fruit Powder (*Canarium odontophyllum* Miq.)

Farah Syahirah Abdul Shukri[1,2], Norhashila Hashim[1,*], Mohd Nazren Radzuan[1], Mohd Zahid Abidin[2], Mohd Sabri Pak Dek[3]

[1]Department of Biological & Agricultural Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[2]Centre for Research of Innovation and Sustainable Development (CRISD), University of Technology Sarawak, 96000 Sibu, Sarawak, Malaysia

[3]Department of Food Science, Faculty of Food Science & Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

**Corresponding author's email: norhashila@upm.edu.my*

INTRODUCTION

Wheat flour has an average of 20-50% usage in Asian noodles making. Asian noodles are popular among Asian people and come second after rice (Niu and Hou, 2020). Wheat flour is mainly composed of carbohydrates (72%), followed by protein (13.2%), fibre (10.7%), and fat (2.5%) (Fari et al., 2011). Nowadays, more people are concerned about their health when making the choice of buying and eating food. Many researchers spend their time and effort in enhancing the quality of food where it should be nutritious, able to satisfy consumer expectations, and affordable (Li et al., 2017). Enrichment of noodles can be made by adding wheat, apricot, or soybean to increase the nutrient content or specifically increase the protein and minerals content of noodles since milling of wheat causes nutrient loss.

Dabai is an underutilised fruit that is popular among the people in Sarawak. Large amounts of dabai can be found in the market during May until June and December until January. Due to its high perishable properties, utilising dabai in any other product is challenging. However, its high nutritional value increases the effort of researchers to study it. Based on studies, dabai provides a significant amount of oleic acid and linoleic acid, which is good for lowering blood lipid levels (Tan and Azlan, 2016). The objective of this paper is to observe the feasibility of developing noodles fortified with dabai fruit powder, to characterize its physicochemical and textural properties and to evaluate the sensory properties of noodles incorporated with dabai fruit powder.

MATERIALS AND METHODS

Dabai fruit was obtained from the Sarikei Market in Sarawak. dabai flesh and skin were peeled and dried using an oven at 60ºC for 6 hours. Dried dabai flesh and skin were ground and sieved. A total of 900 g of salt and 5 kg of wheat flour were purchased from the Doremon Supermarket in Sarikei, Sarawak. The chemicals and reagents used were food-grade. All analyses were conducted following the AOAC standards. The formulation of dabai noodles are presented in Table 1.

Table 1. Formulation of dabai noodles.

No.	Ingredients (g)	Samples			
		Control	F_1	F_2	F_3
1.	Wheat flour	100	85	70	55
2.	Dabai powder	0	15	30	45
3.	Water (mL)	35	35	35	35
4.	Salt	1	1	1	1

Wheat flour content decreased from 100 g to 55 g from control to formulation 3. Meanwhile, dabai powder content increased from 0 g to 45 g from control to formulation 3. Water (35 mL) and salt (1 g) remained the same for all samples. Dabai powder and wheat flour were mixed with water and salt, and the dough was allowed to rest for 30 minutes. The dough was rolled using a rolling pin and extruded using a noodle extruder to obtain long and thick noodles. Dabai noodles were steamed at 100°C for 1 hour and 15 minutes. After steaming, dabai noodles were dried at 60°C for 30 minutes in a drying oven before vacuum packaging into polyethylene bags and stored under a refrigerated temperature (4°C) for further analysis (Adegunwa et al., 2012). Figure 1 shows the preparation of dabai noodles.

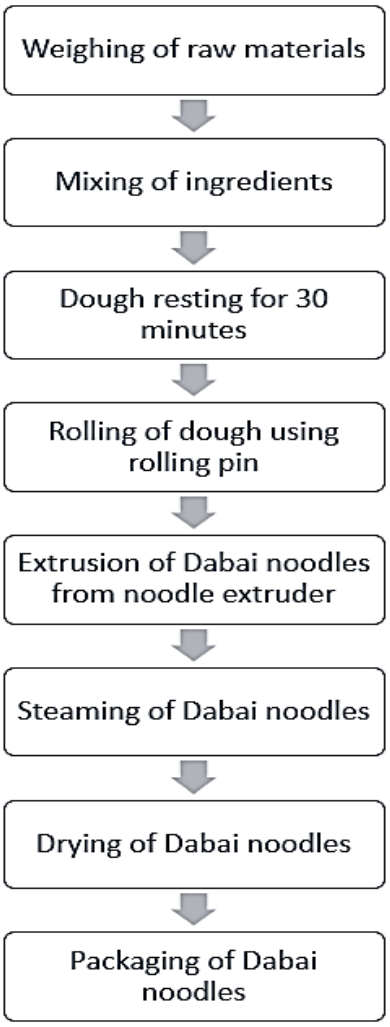


Figure 1. Preparation of dabai noodles.

RESULTS AND DISCUSSION

Colour and appearance of dabai noodles

All formulations showed a darker appearance compared to the control due to the original dark purple colour originating from the dabai fruit itself (Figure 2). The appearance does not affect the sensory evaluation results as panellists found these noodles are unique.

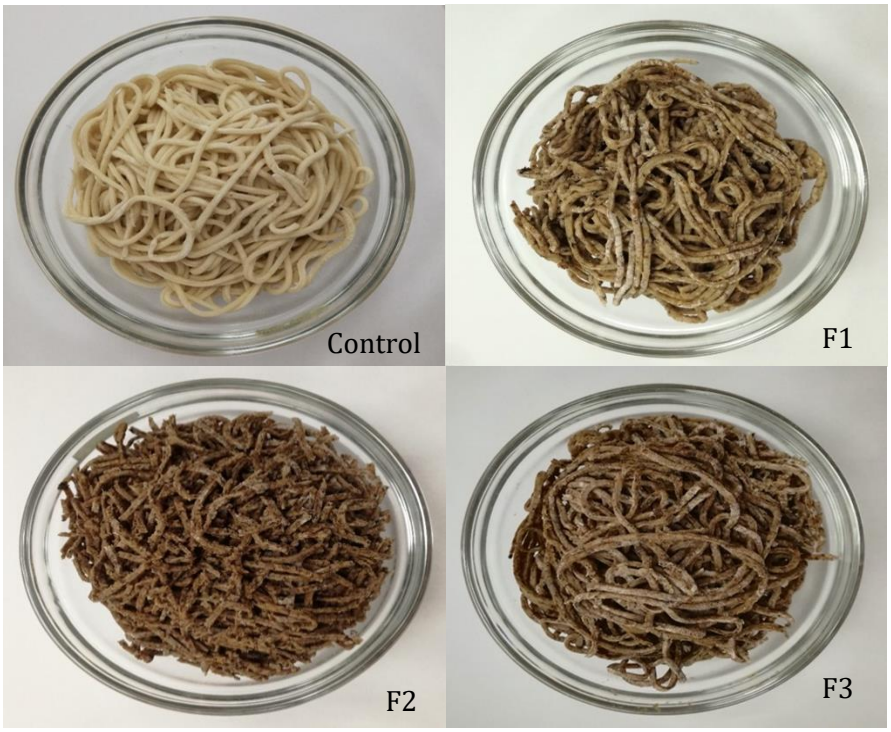


Figure 2. Colour and appearance of dabai noodles

Proximate analysis

The proximate analysis of dabai noodles is shown in Table 2. By incorporating noodles with dabai powder, the amount of protein increased significantly for F3 (23.33%) compared to control (18.23%) while the carbohydrate content decreased in F1 to F3 (67.12% and 63.52% respectively). The percentage of protein and carbohydrates are mostly contributed by wheat flour itself, while dabai contributes to the increase in fat and ash. The result obtained for dabai can be related to the study by Pakhare et al. (2016), as dabai fruit used in this research has quite a similar amount of fat and protein content. The ash and moisture content in this research was slightly higher, while carbohydrates were lower than in other studies (Pakhare et al., 2016). However, the amount of carbohydrate content was comparable to dabai Sarikei or Song, as stated by Tan and Azlan (2016). The nutritional composition of dabai fruits can be affected by climate, growing region, cultivar, maturity, and cultural practice.

Table 2. Proximate analysis of dabai noodles.

Formulation	**Control**	**F_1**	**F_2**	**F_3**
	Percentage, %			
Protein	18.23±1.34^a	25.67±1.34^c	23.92± .34^c	23.33±2.20^c
Fat	0.01±0.01^a	0.93±0.72^b	1.91±0.40^b	3.92±0.40^c
Ash	0.99±0.31^a	1.37±0.12^b	1.73±0.15^b	2.05±0.20^c
Moisture	3.20±0.20^b	2.37±0.30^a	2.87±0.06^a	2.43±0.11^a
Carbohydrates	70.25^b	67.12^a	65.55^a	63.52^a

Mean values in the same row with different alphabets (a>b>c) are significantly different at $p<0.05$ (ANOVA, Tukey' test). Values are given in means±standard error, and values in bracket are the range.

Sensory evaluation of dabai noodles

Figure 3 shows the radar chart of the sensory evaluation for dabai noodles. The radar chart was plotted based on data tabulated from the sensory evaluation sheet. For colour, the Mauchly's Test of Sphericity assumed a value of $p>0.05$. No significant difference among the samples was detected for colour where p>0.05 for within- subjects' effects. Based on Figure 3, F3 has a mean of 8.12 followed by F2 with a mean of 7.70. Most of the panelists like the colour of F3 over the control formulation. Control has the whitest colour and the colour of dabai noodles gets darker from F1 to F3. The panelists have higher acceptance towards the darker-coloured noodles. Most of the panelists liked the appearance of the control noodle over F1, F2, and F3. The appearance of the control noodle is smooth, while the appearance of Dabai noodles gets rougher from F1 to F3. Acceptance towards smoother noodles was higher among the panelists.

For aroma, Mauchly's Test of Sphericity was not assumed ($p<0.05$), and Greenhouse-Geisser was used as a correction for sphericity not assumed. There was no significant difference among the results for aroma as Tests of within-subjects effects have $p>0.05$). Referring to Figure 3, Formulation 1 has a mean of 6.50 followed by Formulation 3 with 6.23. About 80% of the panelists like the aroma of F2 over the control, F3, and F2. F2 dabai noodles were incorporated with 30% dabai powder. Thus, panelists familiar with dabai fruits could accept the aroma of F2. Feedback from the panelists indicated F2 has a higher score because dabai contributed some flavour to the bland noodles, while they dislike F1 and F3 as they have a stronger taste and more acidic.

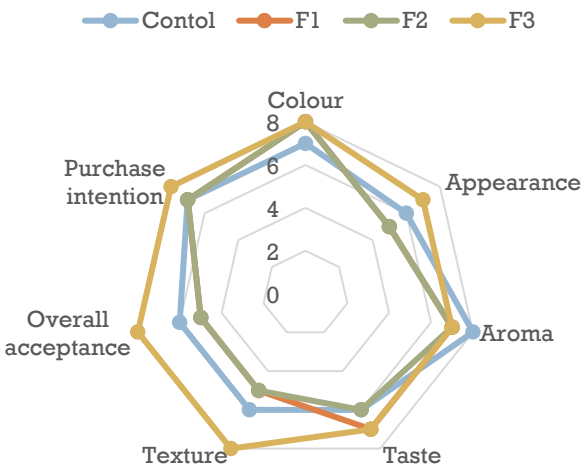


Figure 3. Sensory evaluation of dabai noodles.

CONCLUSION

The objectives of the study were achieved. The most recommended dabai noodles formulation is F2, where 30% of dabai powder was incorporated into the noodles. Dabai has high-fat content and has a unique aroma, which can be used as an exclusive ingredient in the making of wheat flour noodles. This will further help promote indigenous dabai fruit as a new food ingredient while contributing to national food security.

ACKNOWLEDGEMENT

Thanks to Universiti Putra Malaysia and University of Technology Sarawak for the support and assistance throughout the study. The authors declare that there is no conflict of interest.

REFERENCES

Adegunwa, M.O., Bakare, H.A. and Akinola, O.F. 2012. Enrichment of noodles with soy flour and carrot powder. *Nigerian Food Journal, 30*(1): 74-81.

Fari, M.J.M., Rajapaksa, D. and Ranaweera, K.K.D.S. 2011. Quality characteristics of noodles made from selected varieties of Sri Lankan rice with different physicochemical characteristics. *Journal of the National Science Foundation of Sri Lanka*, *39*: 53-60.

Li, M., Dhital, S. and Wei, Y. 2017. Multilevel structure of wheat starch and its relationship to noodle eating qualities. *Comprehensive Reviews in Food Science and Food Safety, 16*(5): 1042- 1055.

Niu, M. and Hou, G.G. 2020. Whole grain noodles. *Asian Noodle Manufacturing*, 95-123.

Pakhare, K.N., Dagadkhair, A.C., Udachan, I.S. and Andhale, R.A. 2016. Studies on preparation and quality of nutritious noodles by incorporation of defatted rice bran and soy flour. *Journal of Food Processing & Technology*, *7*(10): 1-4.

Tan, C.X. and Azlan, A. 2016. Nutritional, phytochemical and pharmacological properties of *Canarium odontophyllum* Miq. (Dabai) fruit.

Quality of Dried Midin Fern (*Stenochlaena palustris*) After Rehydration

Rajmah Muzli Razili*, Mei Hua Ng and Rahimah Sallehuddin
Agriculture Research Centre Semongok, Department of Agriculture Sarawak, KM 20, Borneo Heights Road, 93250 Kuching, Sarawak

**Corresponding author's email: rajmahmr@sarawak.gov.my*

INTRODUCTION

Midin (*Stenochlaena palustris*) is a crunchy-succulent fern that is endemic to Sarawak. It grows widely and wildly in disturbed forests, secondary forests, rubber gardens, oil palm plantations, riverbanks, and roadsides. The fronds of this local fern are pinnate, light green or red when young, and green when mature. The young fronds are curled up like fiddleheads and are usually picked to be utilized as vegetables by rural communities because it is most succulent at this stage (Chai, 2016). The fern naturally tastes neutral. Hence, it can be cooked deliciously with other ingredients such as small-dried shrimps, anchovies with slices of shallot, garlic and chili. Midin can also be developed into value-added food products, Miding Kimchi (Razili, 2010). Furthermore, Midin is used in traditional medicine to treat fever, skin diseases, ulcers, diarrhoea and stomachache as it is reported to contain phytochemicals such as alkaloids, flavonoids, hydroquinone phenols, steroids, and saponins (Sumathy et al., 2010; Chai et al., 2012; Saragih et al., 2017).

Sarawak has exported tilapia fish, laksa paste, kelulut honey, bird's nest, and black pepper directly to Singapore. Midin has also shown great potential to be exported to Singapore as 200 kg of Midin are sold out within just 18 hours at a substantial price of RM36 per kilogram after the news of the shipment was made public on Sarawak Trade and Tourism Office Singapore (STATOS) social media page on Oct 29, 2020 (Lian, 2020). Nevertheless, the shelf life of Midin is short; it will begin to turn black after one day of harvesting, even if kept in a refrigerator. This limits the commercialization of Midin. Hence, modern processing innovation that can diversify its usage and prolong its shelf life is needed. This study is carried out to determine the quality of dried Midin fern after rehydration.

MATERIALS AND METHODS

Thirty-five (35) kg of fresh Midin was purchased from Carus Flora Midin Farm in Betong, Sarawak. After reaching the Postharvest Technology Centre, Agriculture Research Centre Semongok, good quality Midin with no defect was selected and cut into the standard length of 12 to 14 cm. Then, 0.3 kg of fresh Midin was weighed and analysed for colour, texture and water activity. About 1 kg of midin was sent to Analytical Chemistry Laboratory, Agriculture Research Centre Semongok for the proximate composition analyses, which include moisture, crude protein, crude fat, crude fibre, ash, and carbohydrate contents.

The remaining Midin was blanched in hot boiling water for 1 minute and taken out. It was then soaked in ice water for 1 minute and tossed properly before being oven dried at 60°C for 16 hours. The next day, dried Midin was taken out and weighed. Later, 0.3 kg of dried Midin was sampled randomly for colour, texture, and water activity analyses. The other dried Midin was rehydrated by immersing in hot water for 1 minute. Colour, texture, water activity, and proximate composition analyses were also conducted on rehydrated Midin.

The colour, which was expressed in L* (lightness), a* (redness), and b* (yellowness) were measured using a Chroma meter (CR-400, Minolta, Japan). Texture and water activity were determined using Texture Analyzer (TA.XT Express, Stable Micro Systems, UK) and Water Activity Analyzer (Pre Aqua Lab, Decagon, USA), respectively. Every analysis was done in 10 replicates.

RESULTS AND DISCUSSION

Colour changed significantly in fresh, dried, and rehydrated Midin. As shown in the decreasing L* (lightness), -a* (green shade) and +b* (yellow shade), dried Midin turned darker, less green and yellow colour as compared to fresh Midin. After rehydration, the colour of dried Midin was restored to a brighter, greener and yellower with increasing L*, -a* and +b* values.

Table 1. Colour of fresh, dried, and rehydrated midin.

Midin Sample	Colour		
	L*	a*	b*
Fresh	34.61±4.10^{b}	-15.20±2.02^{a}	30.38±3.41^{c}
Dried	20.04±2.44^{a}	-2.10±0.79^{c}	9.59±1.43^{a}
Rehydrated	40.81±6.39^{c}	-8.99±1.22^{b}	24.53±3.99^{b}

Values are means±standard deviations. Values within column followed by the same superscript letter are not significantly different according to Tukey's test at α=0.05.

Table 2. Texture and water activity of fresh, dried and rehydrated midin.

Midin Sample	Texture		Water activity
	PPF*	PA*	
Fresh	1298.466±727.295^{a}	1108.549±385.578^{a}	0.972±0.007^{c}
Dried	747.632±372.999^{a}	1079.613±576.586^{a}	0.532±0.006^{a}
Rehydrated	2026.045±338.685^{b}	2282.921±767.234^{b}	0.956±0.005^{b}

Values are means ± standard deviations. Values within column followed by the same superscript letter are not significantly different according to Tukey's test at α=0.05.

Water activity in fresh Midin was reduced greatly after oven drying, so it can last longer. As Saragih et al. (2017) reported, the low water content in food products will slow down their deterioration rate, whether microbiological or chemical. The transportation cost of dried Midin can also be reduced as it weighed lighter. Dried Midin regained its turgidity and firmness after rehydration. In the evaluation of sensory quality conducted by Hetty et al. (2012), all sensory characteristics of cooked rehydrated Midin were comparable with the cooked fresh Midin. There were significant differences in moisture, protein, fat, ash, and carbohydrate contents between fresh and rehydrated Midin. Meanwhile, fibre content showed no significant difference. Short blanching and rehydration time is vital in reducing nutrient loss of Midin.

Table 3. Comparison in proximate compositions of fresh and rehydrated midin.

Percentage (%)	Midin samples	
	Fresh	Rehydrated
Moisture	91.70±0.27^{b}	82.41±1.11^{a}
Protein	33.85±1.63^{a}	38.25±1.07^{b}
Fat	0.86±0.32^{a}	1.31±0.34^{b}
Fiber	16.88±1.58^{a}	16.19±0.67^{a}
Ash	9.09±0.34^{b}	7.10±0.59^{a}
Carbohydrate	56.20±1.83^{b}	53.35±1.39^{a}

Values are means ± standard deviations. Values within each line followed by the same superscript letter are not significantly different according to T-test at α=0.05.

CONCLUSION

Drying can extend the lifespan of Midin and make it possible to be a delicacy of wider markets.

ACKNOWLEDGEMENT

Thanks to staff of the Postharvest and Product Development Section for their assistance in accomplishing this project. We are also grateful to the Analytical Chemistry Laboratory for their great work in doing various analyses. This work was funded by the Department of Agriculture, Sarawak.

REFERENCES

Chai, T.T., Panirchellvum, E., Ong, H.C. and Wong, F.C. 2012. Phenolic contents and antioxidant properties of *Stenochlaena palustris*, an edible medicinal fern. *Botanical Studies*, *53*(4): 439-446.

Chai, P.P. 2016. Midin (*Stenochlaena palustris*), the popular wild vegetable of Sarawak. *UTAR Agriculture Science Journal (UASJ)*, *2*(2).

Hetty, S.A., Lau, C.Y. and Sallehuddin, R. 2012. *Annual Report: Effect of Blanching, Drying, Rehydration Time and Temperature on Quality of Midin Fern (Stenochlaena palustris).* Sarawak: Research Division, Department of Agriculture.

Lian, C. 2020. Statos: Midin selling at a princely RM36 per kg in Singapore. *Dayakdaily*. Retrieved from https://dayakdaily.com/statos-midin-selling- at-a-princely-rm36-per-kg-in-spore/

Saragih, B., Prakoso, H.T., Rahmadi, A., Emmawati, A. and Kurniadinata, O.F. 2017. Phytochemicals, quality and glycemic response fern red herbal (*Stenochlaena palustris*). In *ASEAN/Asian Academic Society International Conference Proceeding Series*.

Razili, R.M. 2010. *Annual Report: Product Development of 'Miding Kimchi'.* Sarawak: Research Division, Department of Agriculture.

Sumathy, V., Lachumy, S.J., Zuraini, Z. and Sasidharan, S. 2010. Effects of *Stenochlaena palustris* leaf extract on growth and morphogenesis of food borne pathogen, *Aspergillus niger*. *Malaysian Journal of Nutrition*, *16*(3).

Seaweed (*Caulerpa lentillifera*): A Potential Sustainable Food Source

Mohd Fakhrulddin Ismail[1], Shiamala Devi Ramaiya[2], and Mohd Azrie Awang[3]
[1]International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, 71050 Port Dickson, Negeri Sembilan, Malaysia
[2]Department of Crop Science, Faculty of Agriculture Sciences and Forestry, Universiti Putra Malaysia Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia
[3]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu Sabah, Malaysia

**Corresponding author's email: m_fakhrulddin@upm.edu.my*

INTRODUCTION

Seaweeds are an important marine renewable resource that is high in minerals and natural bioactive compounds, making them ideal for alternative food (Domettila et al., 2014). The diversity in the biochemical composition of seaweed provides an opportunity for further exploration of this species (Holdt and Kraan, 2011). *Caulerpa lentillifera*, often known as "seagrape/latok," is a green seaweed that could be found and consumed in practically all Malaysian coastal areas, especially in North Borneo (Zawawi et al., 2014). Locals, particularly in Sabah, ate these species raw as salads, but *Caulerpa lentillifera* was preferred because it has a more delectable flavour and keeps longer after harvest (Nagappan & Vairappan, 2014; Zawawi et al., 2014). They were offered for sale in Sabah's local market alongside other seaweed varieties like *Eucheuma* and *Kappayphycus*, but no monitoring information is available, making it impossible to access data on biomass production and gathering (Phang et al., 2019). Despite its increasing popularity as a unique functional food among Malaysians, limited cultivation and cultural research on the plant has been undertaken, limited or non-existent in West Malaysia (Ahmad et al., 2012).

In contrast to neighbouring countries, the majority of the plant in the market was collected from their native habitat, resulting in species deterioration (Ratana-Aporn and Chirapart, 2006). *Caulerpa* is also a valuable commodity with a significant economic impact on the local market and exchanges in Southeast Asian countries (Dumilag, 2019). *Caulerpa lentillifera* has traditionally been cultured in the gravel bottoms of fishponds in the Philippines (Tanduyan et al., 2013), but this is not the case in Malaysia, particularly West Malaysia. Understanding plant behaviour under various salinity and light intensity circumstances are thus crucial for providing basic knowledge on plant growing in Malaysia environments. The primary goal of this study was to see how salinity and light intensity affected the growth rate, frond length, and ramuli diameter of *C. lentillifera* in the laboratory. The study's findings will be used to help Malaysia build a successful *C. lentillifera* production system.

MATERIALS AND METHODS

Culture under different salinity and light variation

Caulerpa lentillifera were collected and cleaned at I-AQUAS, UPM beach (2°27'53.4"N 101°50'52.8"E). The experimental unit consists of a glass tank (45 cm in height, 120 cm in length, and 30 cm in water level) and four baskets (9 cm height, 9 cm wide, and 33 cm in length) (Figure 1). Each basket receives approximately 500 g of *C. lentillifera* as replication. Each experimental unit was exposed to varying salinity levels of 10, 15, 20, 25, and 30 psu for four weeks. There was no water change to minimize nutritional intervention in growth rate, and the culture was kept under the same light conditions (1032.6 - 152.54 mol) as a constant factor. The specific growth rate (SGR) for each week was calculated using the formula below.:

$$\frac{(ln\ Final\ weight - ln\ Initial\ weight)}{days/weeks} x\ 100 = Specific\ Growth\ Rate\ (SGR)\%$$

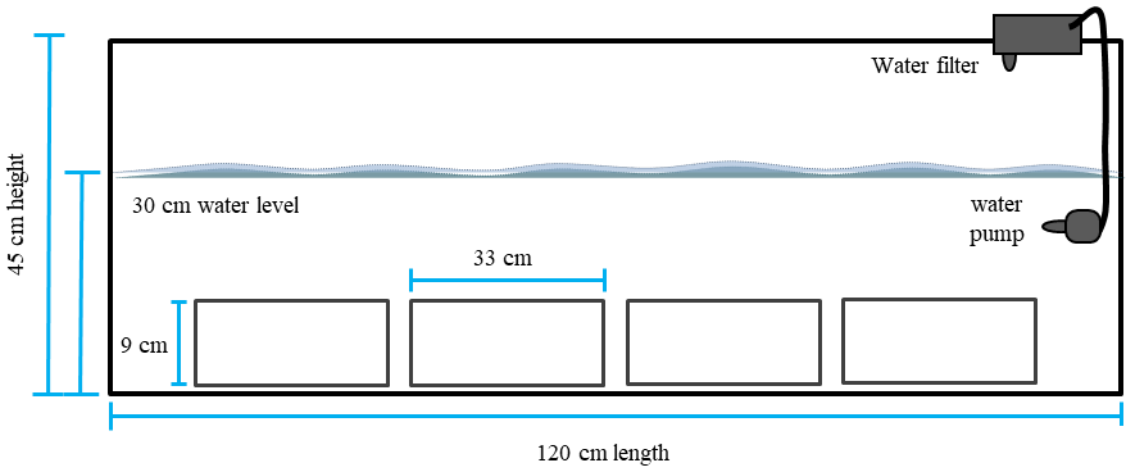


Figure 1. The experimental unit consist of water pump for water circulation.

The plant was examined under various light intensities, including an open region receiving full sun light - light 1 (1032.6 - 152.54 mol), a shaded area receiving undirect sun light - light 2 (220.8 - 400.21 mol), and artificial light from an LED diode - light 3 (80.55 - 81.33 mol). The constant salinity of the water was fixed at 25 psu as standard salinity, as *Caulerpa* reported growth between 20 – 45 psu. The morphology was measured using Mitutoyo Vernier calliper and documented using specified parameters such as frond length and ramuli diameter (Figure 2).

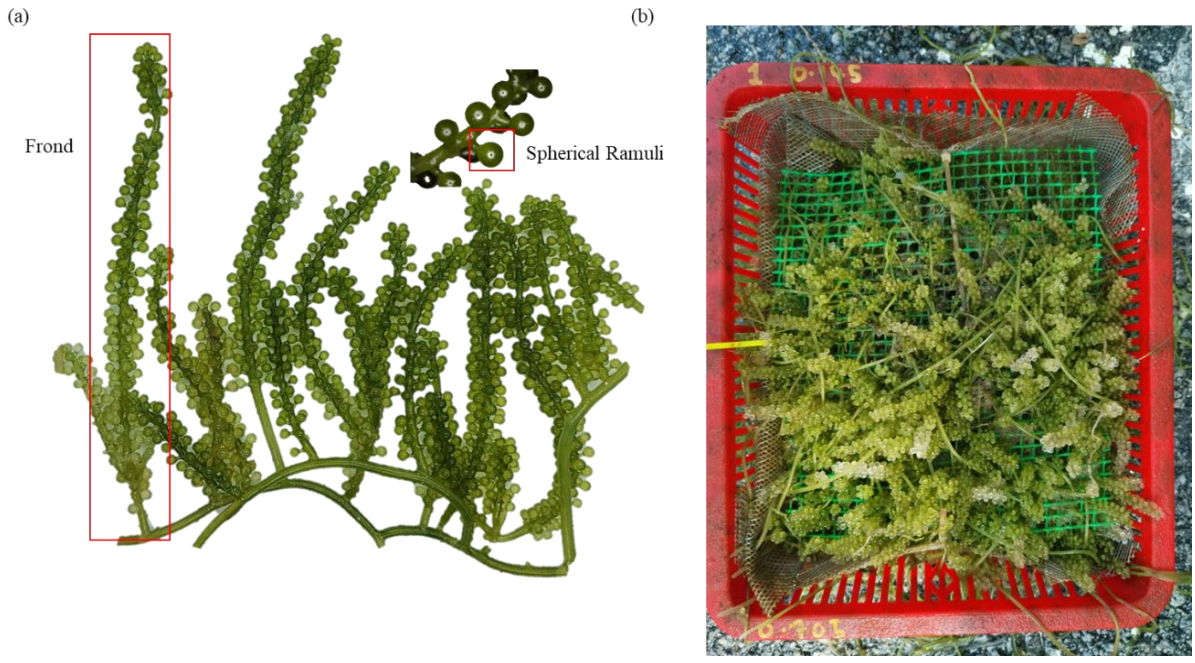


Figure 2. (a) Measured morphology of *Caulerpa lentillifera*, frond length (mm), ramuli diameter (mm), (b) *Caulerpa lentillifera* culture inside basket.

RESULTS AND DISCUSSION

As depleted minerals in water were utilised for growth, the weekly growth rate fell over time (Figure 3a) (Hui et al., 2015). At week 3, the plant grown at 15 psu died, and Rabia (2016) observed a similar phenomenon in *Caulerpa* sp. grown below salinity 30 psu. Both plants cultivated in 25 and 30 psu exhibited no significant weekly growth rate. When comparing the first and last weeks, the SGR for plants under 30 psu (2.42 g percent wk-1) was just slightly higher than 25 psu (2.21 g percent wk-1), indicating that plants favour salinity range at 25 and 30 psu. Furthermore, the study found that plants grown in light 1 (1032.6 - 152.54 mol) developed much longer fronds (95.91 mm) than plants grown in light 2 (220.8 - 400.21 mol) and the shortest under light 3 (80.55 - 81.33 mol) (Figure 3b). Estrada et al. (2020) observed that as water depth decreased (light intensity increased), frond length increased, which is similar to the observation in this study where high light (natural light) contributes to longer frond lengths as the plant produced longer frond to optimize photosynthesis.

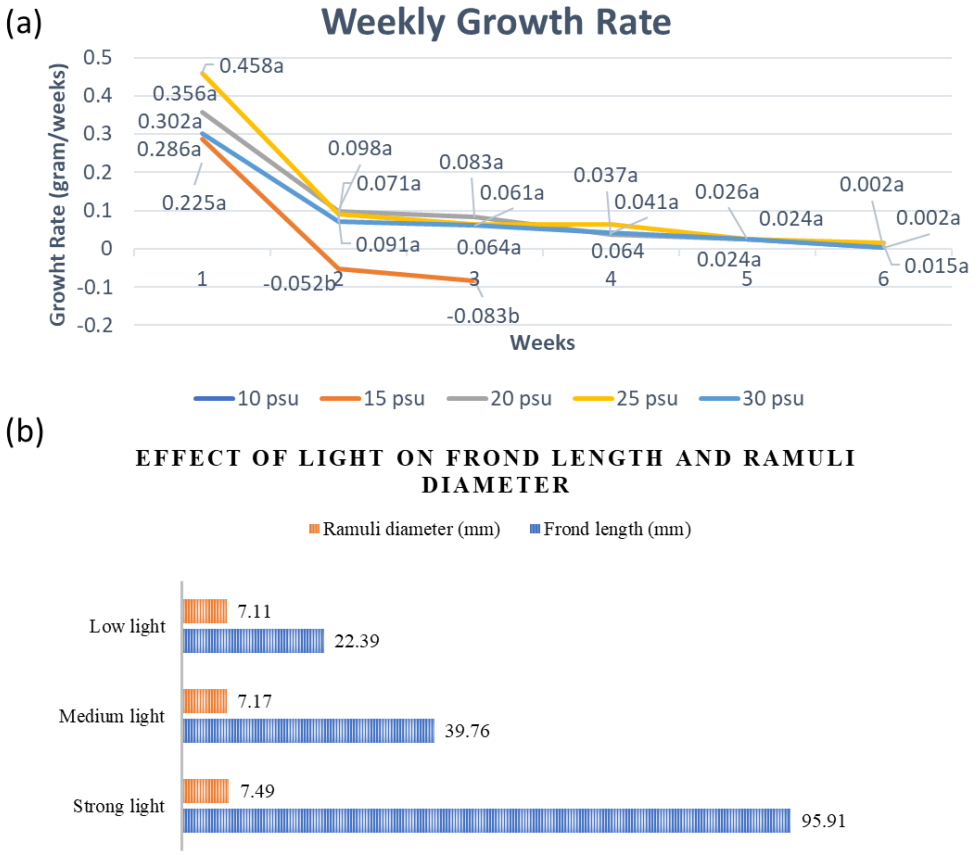


Figure 3. (a) The weekly growth rate under different salinity, (b) the frond length and ramuli size under different light intensity. Mean±SD with the same alphabet on same weeks/light conditions is not significantly different (DNMRT, p>0.05).

CONCLUSION

Caulerpa lentillifera cultured at salinity 25 and 30 psu showed similar growth rate weekly or SGR. The plant showed mortality when exposed to salinity 15 psu after 3 weeks. *Caulerpa lentillifera* respond to different light condition by growing longer frond when exposed to high light. As the fundamental plant requirements were met, *Caulerpa lentillifera* culture can be done indoors. As a result, it should be possible to cultivate this species on a large scale in the future.

ACKNOWLEDGEMENT

Special thanks to Universiti Putra Malaysia (UPM) for Funding the grant entitled as *Caulerpa lentillifera* Morphology Under Selected Laboratory Culture "VOT:9655400".

REFERENCES

Ahmad, F., Sulaiman, M.R., Saimon, W., Yee, C.F. and Matanjun, P. 2012. Proximate composition and total phenolic content of selected edible seaweed from Semporna, Sabah, Malaysia. *Borneo Sciences,* 85-96.

Domettila C., Brintha, T.S.S., Sukumaran, S. and Jeeva, S. 2013. Diversity and distribution of seaweeds in the Muttom coastal waters, south-west coast of India. *Biodiversity Journal. 4*(1): 105-110.

Dumilag, R.V., 2019. Edible seaweeds sold in the local public markets in Tawi-Tawi, Phil_pines. *Science*, *148*(4): 803–811.

Estrada, J.**,** Bautista**,** N.S. and Diaonisio-Sese, M.L. 2020. Morphological variation of two common sea grapes (*Caulerpa lentillifera* and *Caulerpa racemosa*) from selected regions in the Philippines. *Biodiversitas, 21*(5): 1823-1832.

Holdt, S.L. and Kraan, S. 2011. Bioactive compounds in seaweed: functional food applications and segislation. *Journal of Applied Phycology, 23*(3): 543-597.

Hui**,** G., Yao, J.**,** Sun**,** Z. and Duan**,** D. 2015. Effect of salinity and nutrients on the growth and chlorophyll fluorescence of *Caulerpa lentillifera*. *Chinese Journal of Oceanology and Limnology, 33*(2): 140-148.

Nagappan, T. and Vairappan, C.S. 2014. Nutritional and bioactive properties of three edible species of green algae, genus *Caulerpa* (Caulerpaceae). *Journal of Applied Phycology*, *26*(2): 1019–1027.

Phang, S.M., Yeong, H.Y. and Lim, P.E. 2019. The seaweed resources of Malaysia. *Botanica Marina*, *62*(3): 265-273.

Rabia, M.D.S. 2016. Cultivation of *Caulerpa lentillifera* using tray and sowing method in brackish water pond. *Environment Science, 4*(1): 23-29.

Ratana-arporn, P. and Chirapart, A. 2006. Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva retilculata*. *Kasetsart Journal* (*National Science*), *40*: 75-83.

Tanduyan. S.N., Ricardo B. Gonzaga, and Virginia D.B. 2013. Off bottom culture of *Caulerpa lentillifera*. *Galaxea, Journal of Coral Reef Studies*, 123–132.

Zawawi, M.H., Idris, M.H., Kamal, A.H.M. and King, W.S. 2015. Taxonomic assessment of seaweed community from the coastal areas of Bintulu, Sarawak, Malaysia. *Songklanakarin Journal of Science and Technoloy, 37*(2): 147-153.

Wild Tuber Food Resources Among The Bateq and Semoq Beri Tribes in The East Coast of Peninsular Malaysia

Jamilah Mohd Salim[1,2], Tengku Rozaina Tengku Mohamad[1,3], Siti Nursyadiq Anuar[1], Dome Nikong[2], Khatijah Omar[1,4] and Nur Azura Sanusi[4]

[1]Institute of Tropical and Sustainability and Development (Bio-D Tropika), Universiti Malaysia Terengganu (UMT), Mengabang Telipot, 21030 Kuala Nerus, Terengganu, Malaysia

[2]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu (UMT), Mengabang Telipot, 21030 Kuala Nerus, Terengganu, Malaysia

[3]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[4]Faculty of Business, Economy and Social Development, Universiti Malaysia Terengganu (UMT), Mengabang Telipot, 21030 Kuala Nerus, Terengganu, Malaysia

**Corresponding author's email: jamilah@umt.edu.my*

INTRODUCTION

Primary forest in the backbone of Peninsular Malaysia supports at least three tribes of indigenous people or Orang Asli (in Malay), specifically the tribes of Senoi, Proto Malay, and Negrito. The tribe of Semang or Negrito is known to be the earliest and the smallest group among indigenous tribes in Malaysia, known to be isolated and scattered but mainly distributed in the Northern and middle part of Peninsular Malaysia, concentrated in the highlands of Kelantan and Terengganu. Bateq is a sub-ethnic of Negrito, inhabiting a remote area North of Pahang, West of Terengganu, and South of Kelantan. Another sub-ethnic of Peninsular Malaysia Orang Asli is originated from Senoi ethnic group, known as Semoq Beri, living at the edge boundary of Pahang and Terengganu. Some of them live in a nomadic style and forage for their foods in the nearby forest and forested area and still highly depend on the forest for their livelihood and sustenance (Jamilah et al., 2021). These sub-tribes consume several species of 'Ubi' or plant tubers and seeds as their food. Wild tubers as classified as Ubi or Akar (in Malay) are uncommonly known to be edible or expected to be edible to those who are not familiar (Burkhill, 1935). Many of the 'Ubi' are unknown to the larger community due to a lack of documentation on scientific and systematic information.

Documentation on the utilization and management of wild plant parts by the indigenous tribes, particularly tubers and seeds, is still lacking compared to the plant parts that are utilized as medicine or for health purposes. Thus, the study was conducted to document those Ubi or wild tubers edible and eaten by sub-tribes of Bateq and Semoq Beri in the east coast of Peninsular Malaysia. Nutritional value in the selected edible wild tuber could add up to provisional values under the ecosystem services of tropical forest and option value when those resources potentially provide the food for the future. The outcomes of this research are important to scientifically identify and document new food sources from the forest for future food security. This paper present part of the findings from larger research conducted on traditional ecological knowledge (TEK) on the wild tuber and seeds resources utilized as food by the Bateq and Semoq Beri sub-tribes on the coast of Peninsular Malaysia.

MATERIALS AND METHODS

The documentation was mostly based in Kuala Koh, Kelantan, and Kenyir, Terengganu. Field visits and sampling were conducted opportunistically by following the Bateq foraging activities. Wild tuber plant species were identified based on the Bateq and Semoq Beri informant using the name of the wild tuber in their dialect. The tuber was dug, and photography images were captured to include the tuber, leaf, and habit of that particular plant. Voucher specimens were prepared for

identification purposes. However, many of the species of morphospecies of tubers are climber with no access visibility to their leaf. Botanical identification was confirmed with Forest Research Institute of Malaysia (FRIM), Kepong Herbarium. Raw tubers were also planted in a polybag with soil medium as part of an effort to get a visible look at the leaf, the habit of the plant or possibly the bulbil or reproductive structure of flowers and fruits.

RESULTS AND DISCUSSION

To date, a total of 11 morphospecies of wild tuber eaten by the Bateq and Semoq Beri subtribes were sampled from the forest nearby their settlement (Table 1). From those 11 morphospecies sampled, nine are confined to *Dioscorea* genus except for two species (*Amorphophallus campanulatus* and *Gnetum tenuifolium*) which are from Araceae dan Gnetaceae, respectively. Our findings in general is parallel with previous reports where Dioscoreaceae or Yam family that comprises of about 650 species, is being recognized as the fourth most important tuber crop after potatoes, cassava, and sweet potatoes and contributes about 10% of the total root and tubers production globally (Viruel et al., 2016). The Genus of *Dioscorea* is being the most important genus in the family comprising of about 600 species (Pandhan and Panda, 2020). Only three from 10 species of those wild tubers sampled are confidently confirmed to their identification to their lowest taxa which are *D. prainiana* (Ubi Rem), *D. orbiculata* (Ubi Takob) and *D. piscatorum* (Ubi Ciak). Lack of a reasonably good herbarium voucher specimen from the field has caused difficulty in botanical identification which is due to lack of visible access to leaf for the climbing species at accessible height and unavailability of reproductive organ of flower and/or fruit or bulbil during the sampling time. The identification based on tuber's morphological is far from adequate. Thus, we foresee that the botanical identification of those wild tubers will be the main challenge to deal with. At the current stage of our research, the identification used for that collected tuber morphospecies is based on vernacular names given by the sub-tribes.

The moisture of freshly harvested wild tubers is in the range of 56% to 82%. Tubers with lower moisture can potentially be kept longer in storage until future used or for industrial processing. Low moisture could help in extending the storage of the tubers. Further analysis of ash content and proximate composition analysis for all sampled wild tubers are due to be conducted as the research advances. Proximate composition analysis is highly important to highlight wild tubers food quality and includes moisture, ash, crude fat, crude protein, crude fiber, and carbohydrate (Pandhan and Panda, 2020). The outcomes of this research also contributed to the scientific identification and documentation of potential new food sources from the forest for future food security.

CONCLUSION

In this paper, the documentation of wild tubers eaten by the Bateq and Semoq beri sub-tribes are mostly confined to the Dioscorea genus, in line with the previous report of Dioscorea being the main tubers used worldwide. Identification of the lowest taxa could be the biggest challenge of this study.

ACKNOWLEDGEMENT

This research is conducted with a financial support provided under Fundamental Research Grant Scheme (FRGS), FRGS/1/2019/WAB13/UMT/02/1 from the Ministry of Higher Education (MOHE) of Malaysia. The authors also acknowledge the permission granted by the Jabatan Kemajuan Orang Asli (JAKOA) Malaysia to conduct the research with Orang Asli Bateq and Semoq Beri sub ethnics in Terengganu, Pahang and Kelantan.

Table 1. Wild tubers resources utilized by the Bateq and Semoq Beri subtribes in Terengganu and Kelantan.

No.	Vernacular/Local Name	Scientific Name	FRIM ID Ref	FAMILY	Fresh Moisture Content	Cooking method
1.	Ubi Qasek	*Dioscorea* sp.	NA	Dioscoreaceae	NA	Roast
2.	Ubi Rem	*Dioscorea prainiana*	PID 100422-07	Dioscoreaceae	NA	Boil
3.	Ubi Woh	*Dioscorea* sp.	PID 090422-07	Dioscoreaceae	NA	Roast/Boil
4.	Ubi Takop	*Dioscorea orbiculata*	NA	Dioscoreaceae	NA	Roast/Boil
5.	Ubi Cengel (Ubi Pasir)	*Dioscorea alata*?	PID 080422-07	Dioscoreaceae	NA	Roast/Boil
6.	Ubi Hau	*Dioscorea* sp.	PID 120422-07	Dioscoreaceae	68.75±0.30	Roast/Boil
7.	Ubi Ciak	*Dioscorea piscatorum*	PID 130422-07	Dioscoreaceae	77.06±0.17	Boil
8.	Ubi Makel	*Dioscorea* sp.	NA	Dioscoreaceae	56.36±0.31	Roast/Boil
9.	Ubi Sang	*Dioscorea* sp.	NA	Dioscoreaceae	NA	Boil
10.	Ubi Hakal (Lekir)	*Amorphophallus campanulatus*	NA	Araceae	NA	Boil
11.	Ubi Tampak	*Gnetum tenuifolium*	PID 110422-07	Gnetaceae	NA	Boil

NA = Not available yet at the time of printing

REFERENCES

Behera, K.K., Sahoo, S. and Prusti, A. 2009. Relative agronomic performance of different *Dioscorea* species found in different parts of Orissa. *Natural Science*, *7*(3): 23-35.

Burkill, I.H. 1935. A dictionary of the economic plants of the Malay Peninsula. Crown Agents for the Colonies, London.

Jamilah, M.S., Suzairi, Z., Muhamad, R.S. and Mohd, T.A. 2021. Sustainable Utilisation of Forest Plant Resources by the Bateq and Semoq Beri Sub-Tribes in Kelantan and Terengganu States of Peninsular Malaysia. In Mohd Tajuddin Abdullah, Candyrilla Vera Bartholomew and Aqilah Mohammad, eds. Resource Use and Sustainability of Orang Asli: Indigenous Communities in Peninsular Malaysia. Springer Nature, Switzerland AG. 243 pp. ISBN 978-3-030-64961-6 (eBook).

Padhan, B. and Panda, D. 2020. Potential of neglected and underutilized yams (*Dioscorea* spp.) for improving nutritional security and health benefits. *Frontier in Pharmacology*, *11*: 496.

Viruel, J., Segarra-Moragues, J.G., Raz, L., Forest, F. and Sanmartin, I. 2016. Late Cretaceous-early eocene origin of yams (Dioscorea, Dioscoreaceae) in the Laurasian Palaearctic and their subsequent Oligocene-Miocene diversification. *Journal of Biogeography*, *43*(4): 750-762.

Terap Seeds: A Promising Source of Nutrients and Oil with Functional Properties

Halifah Afiza Ismail[1], Shiamala Devi Ramaiya[1,2*], Maisarah Matali[1], and Goh Ei Ping[1]
[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia
[2]Institut Ekosains Borneo, Universiti Putra Malaysia Bintulu Campus, 97008 Sarawak, Malaysia

**Corresponding author's email: shiamala@upm.edu.my*

INTRODUCTION

Artocarpus odoratissimus or 'Terap' is primarily found in some parts of the Borneo Islands, such as Brunei, Kalimantan (Indonesia), Sabah, and Sarawak (Malaysia). However, this species has now been introduced in some other Southeast Asian countries, and its edible fruits have been exported to some other tropical countries (Bakar and Bakar, 2018). In Borneo, the young, immature fruit *A. odoratissimus* were cooked as vegetables whereas the pulp of the matured fruits was eaten raw, and the seeds became edible when boiled (Tang et al., 2013). According to Noorfarahzilah et al. (2017), local communities in Sabah would collect *A. odoratissimus* seeds from matured fruits and fry them until it reaches golden brown, whereas, in Indonesia, it is fried and added with flavour to be sold as snacks. It is known to have a slightly fatty flavour which is similar to hazelnuts. The seeds of *A. odoratissimus* are believed to have much potential to be marketed and produced into various products as they are not only tasty but also highly nutritious. Although *A. odoratissimus* seeds were used in various dishes, information on the nutritional composition and phytochemical properties is still lacking. Therefore, this study aims to evaluate the nutritional and phytochemical properties of the seeds and seed oil of *A. odoratissimus* to help in discovering their potential uses.

MATERIALS AND METHODS

The oven-dried seed samples were used for analyses of the proximate and mineral composition following the Association of Official Analytical Chemists (2000). The fatty acid composition of the seed parts was determined by using gas chromatography. The freeze-dried samples were used for the analysis of phytochemical compositions. The total phenolic content (TPC) and total flavonoid content (TFC) of the seed oils were determined spectrophotometrically following the standard protocol by Ramaiya et al. (2018). The total antioxidant activity (TAA) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined using protocols of Malacrida and Jorge (2012).

RESULTS AND DISCUSSION

The proximate composition in Table 1 shows that the seeds of *A. odoratissimus* possessed good protein content (21.89±0.01%) compared to the flesh (14.59±0.19%). As the seeds were edible, it could be an alternative source of protein for consumers, particularly from rural areas. The seeds also possessed high composition of crude fat at the value of 18.23±0.20%. The crude fat in the seed of *A. odoratissimus* were about 7 times higher than the crude fat in A*. champeden* (2.40%). Additionally, the seeds contain a relatively high amount of carbohydrates at 59.20±0.13%, similar with the composition of breadfruit (58.90%) (Akubor et al., 2000).

The mineral composition of *A. odoratissimus* is shown in Table 2. The ash content in the seeds of *A. odoratissimus* (0.62±0.29%) is relatively higher and confirms that it contains high minerals value. The trend of the mineral compositions in the seeds was K > Ca > P > Mg > Na > Cu. As in many other seeds, K is the most abundant mineral in the seeds of *A. odoratissimus*. Potassium (K)

Table 1. Proximate composition of *Artocarpus odoratissimus* and the seeds of other commercial *Artocarpus* species.

Species	Moisture	Ash	protein	Crude fat	Crude fibre	Carbohydrate	Trend	References
Artocarpus odoratissimus	49.25±0.31^{c} (48.76-49.82)	0.62±0.29^{c} (0.05-0.92)	21.89±0.00^{a} (21.89-21.89)	18.23±0.20^{a} (17.90-18.60)	0.05±0.003^{c} (0.05-0.06)	59.20±0.13^{c} (58.97-59.41)	C > M > P >Fat > A > Fi	Present study
Artocarpus champeden	52.9-72.8	3.2-5.1	9.9-11.2	0.8-2.4	3.9-7.1	2.8-3.5	M > P > Fi > A > C > F	Lim *et al*. (2011)
Artocarpus heterophyllus	61.8	0.15	11.85	1.0	-	26.2	M > C > P > Fi > F	Gupta *et al*. (2011)

Means with different letters (a>b>c) within a column were significantly different at the level $p<0.05$ (ANOVA, Tukey's test) values are expressed as mean±standard error (n=4) and values in bracket are the range.

Table 2. Mineral composition (mg 100 g^{-1}) of *Artocarpus odoratissimus* and the seeds of other commercial *Artocarpus* species.

Species	K	P	Na	Ca	Mg	Trend	References
Artocarpus odoratissimus	905.61±18.89^{d} (878.15-1220.73)	167.40±1.68^{a} (164.16-169.77)	131.86±18.22^{c} (88.80-388.64)	666.48±54.30^{c} (488.41-912.23)	165.01±7.73^{b} (162.80-279.02)	K > Ca > P > Mg > Na > Cu	Present study
Artocarpus altilis	940.0	-	90.0	290.0	96.0	K>Ca>Mg>Na>Cu	Tukura and Obliva (2015)
Artocarpus heterophyllus	769.32	128.83	5.39	40.86	102.94	K>P>Mg>Ca>Na>Cu	Sulaiman (2019)

Means with different letters (a>b>c>d) within a column were significantly different at the level $p<0.05$ (ANOVA, Tukey's test). Values are expressed as mean±standard error (n=4) and values in bracket are the range. K-potassium, P-phosphorus, Na-sodium, Ca-calcium, and Mg-magnesium.

content in the seeds of *A. odoratissimus* (905.61±18.89 mg 100 g^{-1}). The second predominant mineral content in *A. odoratissimus* seeds was the Ca at 666.48±54.30 mg 100 g^{-1}. The Mg content in *A. odoratissimus* is also relatively higher (165.01±7.73 mg 100 g^{-1}), than in *A. heterophyllus* (102.94 mg 100 g^{-1}). The seeds also possessed higher Na content where it was 14 times greater content was recorded compared to *A. altilis*.

Fatty acid composition in seed of *Artocarpus odoratissimus*

Fat content in *A. odoratissimus* reported in the present study was 17-18%. The seed oil content of *A. odoratissimus* was slightly similar to soybean oil (18-20%) (Abitogun et al., 2008) but higher than corn oil (6.63%) (Amos et al., 2013). The seed oil of palm oil (54.18%) is relatively higher than *A. odoratissimus* seed oil (Kuntom et al., 1994). The fatty acid compositions of the seed oil are presented in Figure 1. Unsaturated fatty acid (UFA) was found as the predominant fatty acid content in the seed of *A. odoratissimus*. Under the unsaturated fatty acid, 52.40% were recorded as monounsaturated fatty acid (MUFA), which predominantly consisted of nervonic acid (45.32%), oleic acid (5.99%) and cis-11-Eicosenoic acid (1.11%). There is increasing evidence that dietary supplementation with nervonic acid is healthy for babies and infants during the early stage of brain development (Amminger et al., 2012). Basically, saturated fatty acid (SFA) contains 42.9% of the seed oil *in A. odoratissimus*. The major constituents of SFA recorded in this study was palmitic acid (26.22%) followed by behenic acid (9.26%). The presented fatty acid composition is the first complete documentation of fatty acid composition in *A. odoratissimus* seeds.

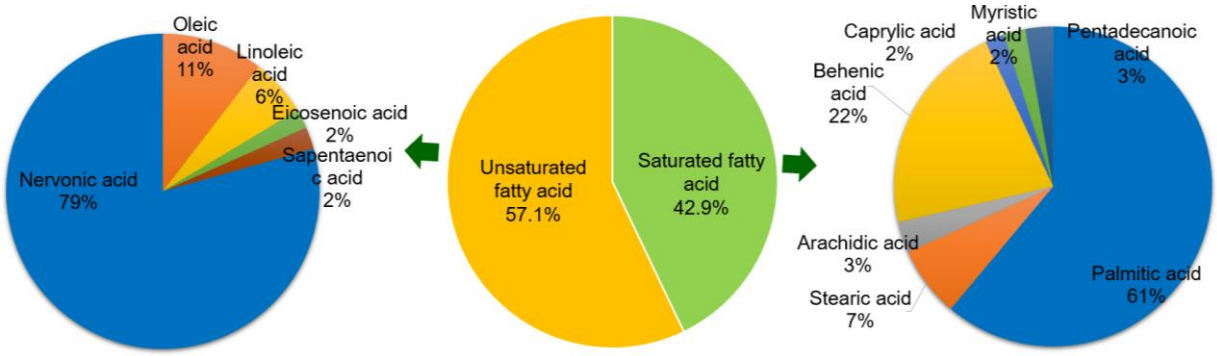


Figure 1. Fatty acid composition in seeds of *Artocarpus odoratissimus.*

Phytochemical properties of the seeds of *Artocarpus odoratissimus*

The phytochemical properties of *A. odoratissimus* seeds oil is presented in Table 3. The TPC content in the seeds of *A. odoratissimus* is the highest (18.67±0.89 mg GAE g^{-1}), compared to A. *integer* and *A. kemando* with values of 11.87 mg GAE g^{-1} and 11.67 mg GAE g^{-1}, respectively. The results supported that the *A. odoratissimus* were rich in phenolic that might contribute to high antioxidant activities (Jagtap and Bapat, 2010). As for the TFC, the seeds of *A. odoratissimus* also possessed higher concentration (12.64±0.17 mg QE g^{-1}). The presence of phenolic and flavonoid is effective against cancerous diseases, and cardioprotective agents and acts as antioxidants, antibacterial, skin protection from UV radiation and capability as the application in the pharmaceutical and medical industry (Andreu et al., 2018; Meng et al., 2018). The present results indicated that the *A. odoratissimus* seed oil possessed free radical scavenging activity. The antioxidant activity of the seed oil ranged from 9.15-10.80 mg mL^{-1}. The seed part displayed high scavenging activity compared to the flesh. This is in agreement with the previous study by Abu

Bakar et al. (2009), which reported that the seed of *A. odoratissimus* displayed higher antioxidant activity and has higher phytochemical contents than the flesh part of the fruit.

Table 3. Phytochemical properties of the seeds of *Artocarpus odoratissimus.*

Species	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	FRAP (µm TE g^{-1})	DPPH (mg mL^{-1})	References
Artocarpus odoratissimus	18.67±0.89 (16.90-19.74)	12.64±0.17 (12.32-12.88)	68.26±0.22 (68.00-68.69)	10.25±0.55 (9.15-10.80)	Present study
Artocarpus integer	11.87	10.18	-	76.58	Abu Bakar *et al.* (2015)
Artocarpus kemando	11.67	8.98	-	61.45	Abu Bakar *et al.* (2015)

Values are expressed as mean ± standard error, and values in brackets are the range.

CONCLUSION

The finding revealed new and additional information on the nutritional and phytochemical properties of *A. odoratissimus* fruits that support the ethnobotanical uses by the local people. From a broad perspective, the information gathered in this study could support the local uses and gain visibility in food and health promoters and the by-products, mainly the seeds to be utilized due to their nutritional and phytochemical attributes.

ACKNOWLEDGEMENT

This research was granted with funding support by Sarawak Research Development Council (SRDC) under RDCRG/RIF/2019/15.

REFERENCES

Abu Bakar, M.F., Abdul Karim, F. and Perisamy, E. 2015. Comparison of phytochemicals and antioxidant properties of different fruit parts of selected *Artocarpus* species from Sabah, Malaysia. *Sains Malaysiana*, *44*(3): 355-363.

Gupta, D., Mann, S., Sood, A. and Gupta, R.K. 2011. Phytochemical, nutritional and antioxidant activity evaluation of seeds of jackfruit (*Artocarpous heterolphyllus* Lam.). *International Journal of Pharma and Bio Sciences*, *2*(4): 336-345.

Tang, Y. P., Linda, B. L. L. and Franz, L. W. 2013. Proximate Analysis of *Artocarpus odoratissimus* in Brunei Darussalam. *Food Research Journal, 20*(1): 409-415.

Total Phenolic, Flavonoid, and Antioxidant Content in Five Selected Indigenous Food Flavouring Plants

Nurul Aisyah Yusli[1], Noorasmah Saupi[1,2,*], Shiamala Devi Ramaiya[1,2], and Hanisah Kamilah Abd Razak[1]

[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia

[2]2Institut Ekosains Borneo, Universiti Putra Malaysia Bintulu Campus, 97008 Sarawak, Malaysia

**Corresponding author's email: noorasmah@upm.edu.my*

INTRODUCTION

Flavour has become the most critical factor influencing food quality and global competitiveness (Weerawatanakorn et al., 2015). In Sarawak, there were few indigenous food flavouring plants that were used by the locals like *Pangium edule* (daun kepayang), *Premna serratifolia* (daun singkil), *Pycnarrhena tumefacta* (daun tubu), *Scorodocarpus borneensis* (daun kesinduk*)*, and *Syzygium polyanthum* (daun bungkang) as flavour enhancer or modifier in their cooking. Previous studies have found that these plants are used as leafy vegetables in the daily diet of local communities in Sarawak (Saidin et al., 2016; Saupi et al., 2019; Saupi et al., 2020). Basically, food flavouring was used to make savoury dishes more appealing in terms of general palatability, richness, pleasantness, and hedonic (McCabe and Rolls, 2007). However, locals-only consumed these plants for the purpose of flavouring plants, but they are not very aware of the nutrients of the plants eaten.

Pangium edule Reinw. is a species from family Flacourtiaceae which also known as "kepayang" or "picung" in Borneo Island. This species is a tall tree that can reach until 40 m height with sparse crown spreading. Many studies recorded that the fruit and seeds of *P. edule* have been widely used as a preservative for meat, fish, and shrimps in Indonesia and Sarawak, Malaysia (Heruwati et al., 2009; Mustaffer et al., 2021). The food that has been preserved using *P. edule* usually have richer flavour and aroma. *Premna serratifolia* L. is a species from family Lamiaceae. In Malaysia, this species is known as "buas-buas", "singkil", "pecah piring", or "limau pantai". The leaves and roots have an astringent flavour and strong scent (acridly sweet). The leaves are typically prepared as vegetables or added to high-fat recipes by the natives. Many studies also have recorded that *P. serratifolia* has many medicinal properties such as anti-inflammatory, anti-bacterial, anti-hypoglyceamic, anti-coagulant, and anti-arthritics that can treat cough, constipation, headache, fever, obesity, tumour, and skin-disease.

Pycnarrhena tumefacta Miers. also known as "daun tubu", "mekai", "bekai", "daun sengkubak", or "appak" by the locals in Sarawak. This species is from the family Menispermaceae. The plant is a thin, twining shrub that regularly climbs big trees, and it is typically found in hilly locations above 500 to 1500 m a.s.l. The leaves possess umami flavour and are use as a glutamate substituted by the locals. According to Mohammed et al. (2020), *P. tumefacta* is a medicinal plant that can be used as medicine to treat fever and headache. *Scorodocarpus borneensis* Becc. usually known as "bawang hutan", "kesinduk", or "kayo kesindo" which from Olacaceae family. This plant can grow up to 40 m tall and is widely distributed around Peninsular Thailand, Peninsular Malaysia, and Borneo. This species has a strong garlic-like smell which is present in the leaves, flowers, and fruits. Locals usually add *S. borneensis* into the dishes as a seasoning as a garlic substitute. A species of the Myrtaceae family known as "Indonesian bay leaf," "daun salam," "serai kayu," or "samak" in Malaysia is *Syzygium polyanthum* (Wight) Walp. Halim and Maryani (2022) assert that this species is distinct from the Lauraceae species *Laurus nobilis*, also known as the true bay leaf. *Syzygium polyanthum* is typically used as a culinary herb or a traditional medicine since it has a

faint aroma and a mildly sour or astringent flavour (Ravindran, 2017). The phytochemical content in this flavoring plant has not been reported yet in previous studies (Saidin et al., 2016; Saupi et al., 2019; Saupi et al., 2020). Thus, this study was conducted to identify the total phenolic, total flavonoid, and antioxidant activity of these five selected indigenous food flavouring plants.

MATERIALS AND METHODS

Sample preparation

Fresh samples were collected from bushes around Bintulu, Sarawak. Then, samples were cleaned and washed using tap water, and inedible parts were discarded. Later, the samples were oven-dried at 60˚C for 24 hours and ground into a fine powder using the heavy-duty blender. The samples were stored in an air-tight container for further analysis and labeled.

Sample extraction

About 1 g of the oven-dried grounded samples were weighed and soaked in 10 mL of 70% methanol. Samples were extracted using an orbital shaker at 110 rpm for 48 hours at room temperature. The extracts were centrifuged at 1000 rpm for 10 minutes and filtered using Whatman No. 2 filter paper.

Total phenolic content

Phenolic content was determined using Folin Ciocalteu's method by Singleton et al. (1999). One (1) mL of sample extract was pipetted into the test tubes. The standard gallic acid in a concentration of 10, 20, 40, 60, 80, and 100 µg/mL also was pipetted into the test tubes. Afterward, 5 mL of distilled water was pipetted into the test tubes, followed by 0.5 mL of Folin Ciocalteu's reagent. The solution was mixed and shaken. After 5 minutes, 1.5 mL of 20% sodium carbonate was added and made up to 10 mL with distilled water. The tubes were covered with parafilm, and the mixture was incubated for two hours at room temperature for color development. The absorbance was measured at 750 nm by using UV-VIS Spectrophotometer (Lambda 25 Perkin Elmer, Germany). The TPC content in the samples was calculated by equation from the calibration curved and expressed in gallic acid equivalent (mg GAE/ 100 g) dry mass.

Total flavonoid content

Total flavonoid content was determined by aluminium chloride complex forming assay as described by Kamtekar et al. (2014). Quercetin solution with 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL prepared in methanol was used as the standard solution, whereas the blank standard was prepared using distilled water. A quantity of 1 mL aliquots and 1 mL of prepared standard quercetin was positioned into test tubes, then added with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution and left for five minutes. Afterward, a quantity of 0.3 mL of 10% aluminium chloride solution was added and allowed to stand for six minutes. Lastly, 2 mL of 1 M sodium hydroxide was added, making up the volume of up to 10 mL with distilled water. The mixture was mixed well until the solution perceived yellowish-orange color. The reading was measured at 510 nm of absorbance using UV-VIS Spectrophotometer (Lambda 25 Perkin Elmer, Germany). The calibration curve of the quercetin standard solution was used as an equation to determine the TFC expressed as mg of quercetin equivalents per 100 g quercetin equivalents (mg QE/100 g) in a dry mass basis.

DPPH assay

DPPH free radical scavenging activity was conducted according to Brand-Williams et al. (1995) with some modifications. The stock solution of DPPH was prepared with absolute methanol (100 μM). The sample extracts were diluted into a series of known concentrations using 80% methanol. Then, the assay started by adding 1.0 mL of sample extract with 3.0 mL of DPPH stock. The solutions were incubated for 30 min under dark condition, and the absorbance was taken at 517 nm using PerkinElmer Lambda 25 UV/Vis Spectrometer. The concentration of sample required to scavenge 50% of DPPH (EC_{50}) was calculated by plotting the linear equation of sample concentrations versus percentage inhibition (%). The result was expressed as mg/mL.

Statistical analysis

Data on phenolic, flavonoid, and antioxidant activity were analyzed by Statistical Analysis Software (SAS 9.3). The mean of each parameter measured was analyzed using one-way analysis of variance (ANOVA). The difference between means was then compared using Tukey's Range Test at a significant level $p \leq 0.05$.

RESULTS AND DISCUSSION

Generally, the result in Table 1 clearly indicated that all the selected ILV were the richest phenolic sources compared to flavonoid. The total phenolic content of selected indigenous food flavouring plants in the current study varied widely, ranging from 307.06-382.16 mg GAE/100 g. *Syzygium polyanthum* contained the highest amount of phenolic, and *P. serratifolia* contained the lowest amount of phenolic. However, the total flavonoid content was highest for *P. serratifolia* and lowest for *S. polyanthum*. The total flavonoid content of selected indigenous food flavouring plants ranges from 116.23-288.10 mg QE/ 100 g). For DPPH assay, *S. borneensis* (2.604 mg/mL) showed significantly low antioxidant activity compared to other species. A lower EC_{50} value suggested that the sample had higher antioxidant activity (Ramaiya et al., 2014)

Table 1. Total phenolic content, total flavonoid content, DPPH Scavenging assay for selected indigenous food flavouring plants.

Species	TPC (mg GAE/100 g)	TFC (mg QE/100 g)	DPPH (mg/mL)
Pangium edule	365.37 ± 3.04^{b}	135.59 ± 6.71^{c}	0.108 ± 0.01^{c}
Premna serratifolia	307.06 ± 3.50^{e}	288.10 ± 5.67^{a}	0.096 ± 0.01^{c}
Pycnarrhena tumefacta	351.33 ± 5.94^{c}	154.93 ± 2.50^{b}	0.345 ± 0.001^{b}
Scorodocarpus borneensis	330.12 ± 3.34^{d}	134.83 ± 5.90^{c}	2.758 ± 0.20^{a}
Syzygium polyanthum	382.16 ± 3.79^{a}	116.23 ± 5.36^{d}	0.168 ± 0.01^{bc}

Mean values in the same column with different alphabets (a>b>c) are significantly different at $p<0.05$ (ANOVA, Tukey' test). Values are given in means ± standard deviation.

CONCLUSION

All selected indigenous food flavouring plants in this study showed a significant antioxidant content. The high antioxidant content in plants can benefit human health. This also can allow a further study for these species to be developed as functional food, which can improve the utilization of the species besides being a food seasoning in local cuisine.

ACKNOWLEDGEMENT

The authors are grateful for the financial support from Universiti Putra Malaysia undergraduate Research Fellowship (GRF) programme. The authors are thankful for the use of laboratory facilities of the Faculty of Agricultural and Forestry Sciences, University Putra Malaysia Bintulu Campus. The authors also thank Sarawak Biodiversity Centre for the approval (18) JKM/SPU/608-8/2/1 Vol.3 to conduct research and collect the living sources for research purposes.

REFERENCES

Brand-Williams, W., Cuvelier, M. and Berset, C. 1995. Use of a Free Radical Method to Evaluate Antioxidant. *LWT-Food Science and Technology, 28*: 25-30.

Halim, Y. and Maryani. 2022. Functional and sensory properties of Indonesian bay leaf (*Syzygium polyanthum*) herbal tea. *Food Research, 6*(2): 25-33.

Heruwati, E., Ismaini, L. and Mangunwardoyo, W. 2009. Antibacterial test of *Pangium* (*Pangium edule* Reinw.) extract against the growth of fish spoilage bacteria. *Indonesian Fisheries Research Journal, 15*(2): 65-73.

Kamtekar, S., Keer, V. and Patil, V. 2014. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *Journal of Applied Pharmaceutical Science, 88*(10): 61-65.

McCabe, C. and Rolls, E. 2007. Umami: a delicious flavour formed by convergence of taste and olfactory pathways in the human brains. *European Journal of Neuroscience, 91*: 1855-1864.

Mohammed, N., Muhialdin, B., Masri, N., Sukor, R., Abd-El Aziem, F. and Meor Hussin, A. 2020. Chemical compositions, antioxidant and antimicrobial activities of Tubu (*Pycnarrhena longifolia*) leaves used as ingredient in traditional functional foods. *Food Research*: 1-8.

Mustaffer, N., Ramli, N., Tongkhao, K., Vangnai, K., Rosni, N. and Rukayadi, Y. 2021. Changes in physiochemical properties and antioxidant activity of fermented kepayang (*Pangium edule* Reinw.) seeds. *Food Research, 5*(4): 47-58.

Ramaiya, S., Bujang, J. and Muta Harah, Z. 2014. Assessment of total phenolic, antioxidant, and antibacterial activities of *Passiflora* species. *The Scientific World Journal, 4*: 1-10.

Ravindran, P. 2017. *The Encylopedia of Herbs and Spices.* Cambridge, United Kingdom: CABI.

Saidin, S., Saupi, N., Sarbini, S.R. and Muta Harah, Z. 2016. Mineral content of five indigenous leafy vegetables from bintulu market, Sarawak Malaysia. *Journal of Medicinal Herbs and Ethnomedicine*, *2*: 26 –35.

Saupi, N., Saidin, A.S., Zakaria, M.H., Sarbini, S.R. and Yusli, N.A. 2020. An ethnobotanical study of indigenous leafy vegetables among local communities in Bintulu, Sarawak, Malaysia. *Borneo Journal of Resource Science and Technology*, *10*(2): 155-165

Saupi, N., Saidin, A.A, Ya, M.H.Z. and Sarbini, S.R. 2019. Diversity, utilization and proximate composition of indigenous leafy vegetables consumed in Malaysia. *Journal of Agriculture Food and Development, 5*: 1-8.

Singleton, V., Orthofer, R. and Lamuela-Raventos, R. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. *Methods of Enzymology, 299*: 152-178.

Weerawatanakorn, M., Wu, J., Pan, M. and Ho, C. 2015. Reactivity and stability of selected flavour compounds. *Journal of Food and Drug Analysis, 23*: 176-190.

Utilization of Indigenous Species as Crafts Among Iban People in Bintulu, Sarawak

Nurul Aisyah Yusli[1], and Noorasmah Saupi*
Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia

**Corresponding author's email: noorasmah@upm.edu.my*

INTRODUCTION

The majority ethnic group of Sarawak is the Iban, also referred to as the Sea Dayak. Iban women are particularly adept in crafts like weaving meanwhile the men are skilled in carving (Anggo and Laja, 2018). In Malaysia, Iban is one of the ethnic groups famous for their culture and handicrafts such as pottery, mat weaving, basket weaving, "pua kumbu", and carving. The Iban ethnic group usually uses indigenous plants as a basis for the production of handicrafts. According to previous studies, rattan (*Calamus*), bamboo (*Bambusa vulgaris*), bemban (*Donax canniformis*), palm leaves, and cotton yarn are the primary indigenous raw materials used to make handicrafts (Hasan et al., 2011; Abdul Gapor et al., 2019). However, for mass production, it is preferred to use replacement materials like plastic that are more dependable or accessible. Although its purpose is the same, the new aesthetics gradually eliminate the original inspirations, aesthetic value, and authenticity of the craft that represents their ethnic identity (Hasan et al., 2011). This has led to the current generation increasingly not recognizing the indigenous plants used for craft making and the loss of traditional knowledge. Thus, the objective of this study is to record and document the indigenous species used by the Iban in the Bintulu area as craft crops.

MATERIALS AND METHODS

An ethnobotanical survey was conducted around local settlements and longhouses around Bintulu Division (Figure 1). 148 respondents participated in this field survey, which been conducted in semi-structured from October to December 2021. Information was collected based on the respondents' answers to the survey questionnaire. Questionnaires consisted of three sections; personnel information, basic information about the craft crop, and the type of craft product made from the indigenous plants. Each respondent was briefed prior to filling out the questionnaire. The species and type of crafts were identified immediately after the survey with assistance from the survey respondents via transect survey and a brief interview session. The utilization of indigenous plants as crafts was analysed using the Statistical Package for Social Science (SPSS), IBM ®V22.0 Software. Data was analysed descriptively to show frequency.

RESULTS AND DISCUSSION

In this study, 15 species from 11 different families were identified as indigenous species used by the community in Bintulu for craft making (Table 1). Arecaceae and Dipterocarpacecae is the biggest families, which are represented by three species each. Followed by Moraceae with two species. Meanwhile, Poaceae Maranthaceae, Lauraceae, Hypericaceae, Pandanaceae, Apocynaceae, and Zingiberaceae are represented by one species only. More than half of the respondents involved in this study were female 68.2% and only 31.8% were male (Figure 2). The result showed that female respondents had more knowledge of indigenous handicrafts plants than males. The age of respondents varies from 15 to above 60 years old. Respondents between the age 31 to 59 years old was the highest group involved, with 62.8%. Followed by respondents above 60 years old with 31.1% then respondents aged 15 to 30 years old with 6.1%. Generally, the selection of the age range was decided in order to prevent any missing traditional knowledge,

as the younger generation may not possess the knowledge because of a lack of practices and urbanization occurred. Many research conducted on traditional knowledge showed that traditional knowledge was found be disregarded by newer generations.

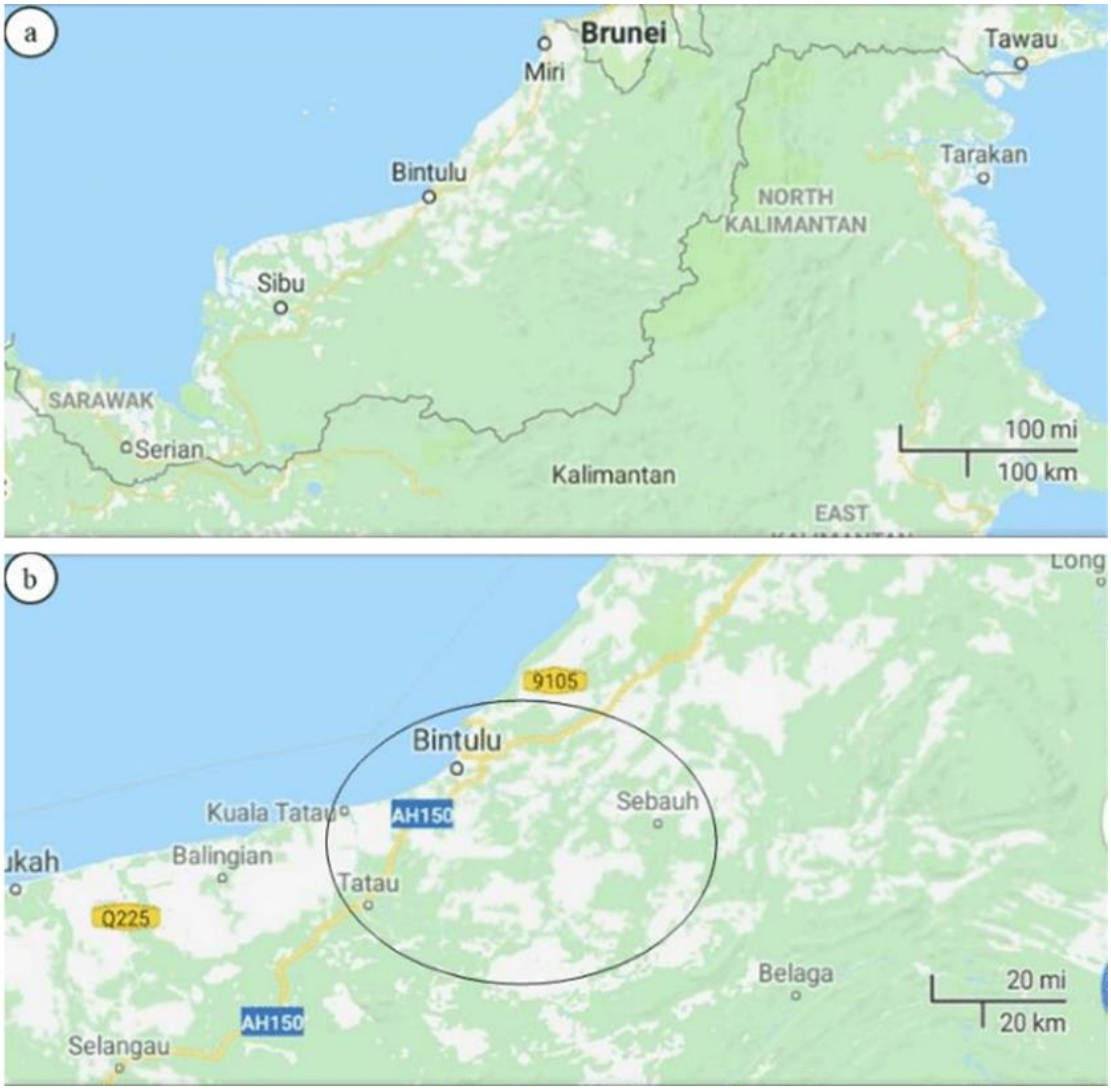


Figure 1. Location of the study: (a) Bintulu district, and (b) Tatau and Sebauh districts

CONCLUSION

Therefore, this study able to record and documented 15 species of indigenous species and how the Iban in Bintulu utilized the plant as craft. It is recommended to widen the study area in order to provide full documentation of indigenous plants used by the Iban in Sarawak, Malaysia.

REFERENCES

Anggo, F. and Laja, L. 2018. The function and role in the Iban social rank in their communities. *International Journal of Engineering & Technology*, *7*: 639-641.

Hasan, R., Sahari, F. and Lucas, T. 2011. Sarawak Craft Council: Sustaining the Indigenous Craft of Sarawak. International Conference on Creativity and Innovation for Sustainable Development., Kuala Lumpur, pp. 1-13.

Table 1. Indigenous plants used as craft by the Ibans in Bintulu, Sarawak.

No.	Scientific name	Family	Common name	Plant part use	Type of craft
1.	*Alstonia scholaris*	Apocynaceae	Pelaik, pulai	Stem	Cut and carved as base for traditional music instrument called "sapeh". Carved as "parang" casing and holder for agriculture uses and self-defence.
2.	*Artocarpus elasticus*	Moraceae	Tekalong	Stem, stem skin	Stem was cut and carved as "parang" holder. Stem skin was soaked and tapped for ladies' traditional garment.
3.	*Artocarpus integer*	Moraceae	Cempedak	Stem	Carved as "parang" casing and holder for agriculture uses and self-defence.
4.	*Calamus hispidulus*	Arecaceae	Rotan	Stem	Cut, dried, and woven as mat for house decoration purpose. Shaped and woven as frame for domestic utensils such as chairs, mat, and basket.
5.	*Cratoxylum arbonescens*	Hypericaceae	Gerunggang	Stem	Carved as "parang" casing and holder for agriculture uses and self-defence.
6.	*Dendrocalamus asper*	Poaceae	Buluh	Culm	Soaked with water, dried, and woven as mat for house decoration purpose. Shaped and woven as frame for domestic utensils such as chairs, mat, and basket.

Continued Table 1.

No.	Scientific name	Family	Common name	Plant part use	Type of craft
7.	*Donax grandis*	Marantaceae	Bemban	Leaves	Dried, cut, and woven for hat making, or ritual attire.
8.	*Dryobalanops oblongifolia*	Dipterocarpaceae	Keladan	Stem	Cut, soaked, and carved as base for traditional music instrument called "sapeh".
9.	*Eusideroxylon zwageri*	Lauraceae	Belian	Stem	Cut and carved as "parang" casing and holder for agriculture uses and self-defence.
10.	*Hornstedtia reticulata*	Zingiberaceae	Senggang	Leaves	Cut and woven as small toy for kids. Dried and woven for hat making, or ritual attire.
11.	*Licuala grandis*	Arecaceae	Palas	Leaves	Washed and woven for hat making, or ritual attire.
12.	*Metroxylon sagu*	Arecaceae	Sagu	Stem	Cut, soaked with water and dried for "parang" holder making.
13.	*Pandanus artocarpus*	Pandanaceae	Mengkuang	Leaves	Drooped, dried and woven for ladies' traditional garment.
14.	*Shorea macrophylla*	Dipterocarpaceae	Engkabang	Stem	Cut and carved for furniture making and usually used as home decoration.
15.	*Shorea* spp.	Dipterocarpaceae	Meranti	Stem	Cut and carved for furniture making and usually used as home decoration and "parang" casing and holder for agriculture uses and self-defence.

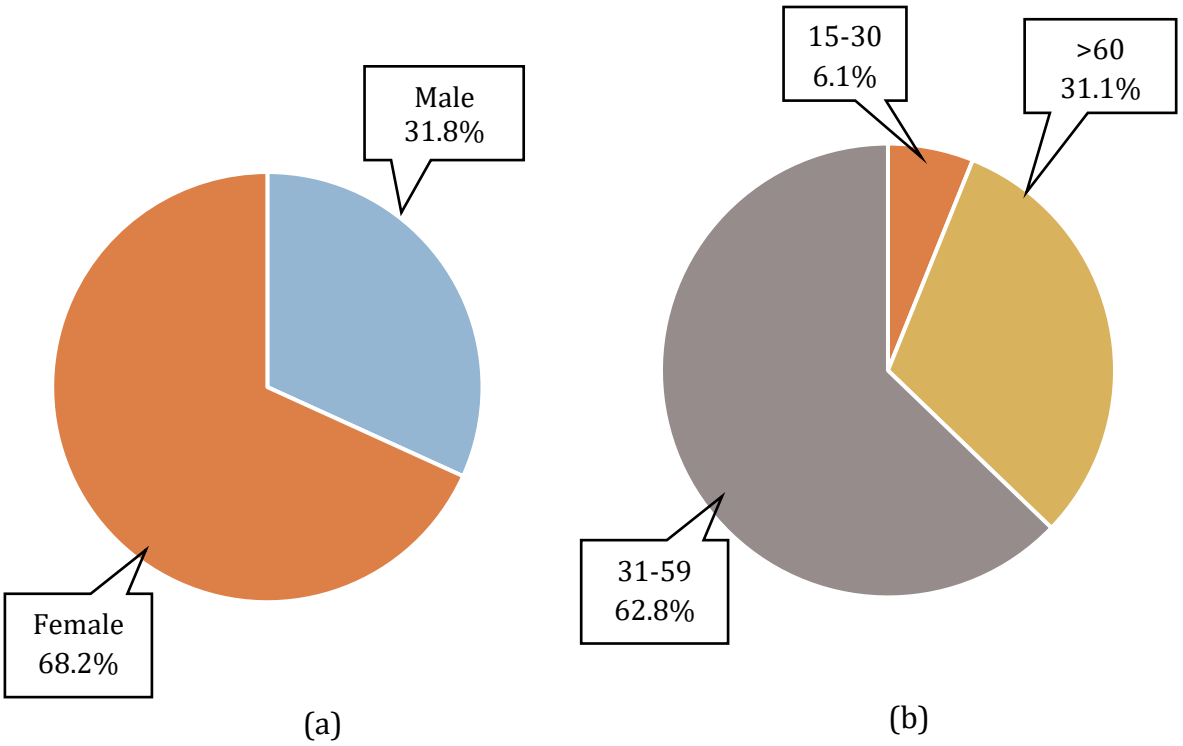


Figure 1. Demographic of Iban people in Bintulu, Sarawak: a) gender and b) age.

Flower Development of Crystal Longan (*Pometia pinnata* J.R. Forst & G. Forst)

Angeline Limok Anak Mong* and Rajmah Muzli Razili
Agriculture Research Centre Semongok, KM 20, Jalan Puncak Borneo, 93250, Kuching, Sarawak, Malaysia

**Corresponding author's email: a.limok@sarawak.gov.my*

INTRODUCTION

Crystal longan (*Pometia pinnata* J.R. Forst & G. Forst), also locally known as Kasai is a fruit species from the Sapindaceae family. It has a wide natural distribution from Sri Lanka and the Andaman Islands throughout Southeast Asia to Taiwan, Fiji, and Samoa. It is a small to fairly large tree, typically 12-20 m tall and 10-20 m in canopy diameter (Thomson and Thaman, 2006). It makes an open type of growth with long, pinnate leaves. Crystal longan tree can be distinguished by its new flushes, which are deep red wine-coloured.

Crystal longan floral inflorescence is like that of lychee, with its panicle covered with small whitish flowers. The appearance of the floral inflorescences is highly variable. The panicle with a cluster of flowers is borne at the end of the branches, conspicuously projecting beyond the foliage. Flowers of Crystal Longan are bisexual, with both male and female flowers on the same tree and within the same panicle. Female flowers may appear bisexual, but the anthers are reduced and sterile (Thomson and Thaman, 2006). Male flowers usually mature first and outnumber female flowers. Fruits mature 3-4 months after flowering. Its fruits are highly variable, with its exocarp variously coloured, which are either greenish-yellow, yellow, red, or purple. While the flesh is semi-transparent white, sweet, aromatic, and juicy. The various exocarp colouration shows considerable variation and genotypes, but information regarding its taxonomy is limited.

In Sarawak, Crystal longan is cultivated on a small-scale farm or in a backyard garden. The cultivation is scattered throughout Sarawak; up to date, not much information can be obtained on its flower and fruit morphological characteristics. Therefore, this study aims to document the flower morphology of Crystal Longan planted under local conditions.

MATERIALS AND METHODS

Plant materials

The observation and sampling were conducted at a private orchard at Kampung Jangkar, Lundu. Flowers of the Crystal longan tree (fruit with purple-coloured exocarp) were closely observed and recorded during the recent flowering season.

Observation of flower morphology

The flower panicle samples were collected from trees in the flowering stage. Flowers at different stages were collected before and during anthesis, and its morphological characteristics were examined under the Olympus SZX7 stereo microscope at Entomology Laboratory at Agriculture Research Centre, Semongok.

RESULTS AND DISCUSSION

As can be seen in Figure 1, the inflorescence of Crystal longan was formed at the terminal branch, with its panicle branches covered with clusters of small whitish flowers. Each flower was held

together by its pedicel, which was attached to a single peduncle. Each peduncle has a varying quantity of flowers within the range of 3-7 flowers on a single peduncle (Figure 2). Based on the observation, Crystal longan is a monoecious plant with both male and female flowers existing on the same tree and within the same panicle.

Figure 1. Floral inflorescence of Crystal Longan that was formed at the terminal branch.

Figure 2. Cluster of flowers (3-7 flowers) on a single peduncle.

Both male and female flowers within the same panicle mature at different time over a period of 4-6 weeks (Figure 3). As observed, male flowers open first as compared to female flowers. In certain panicle, the appearance of male flowers outnumbered the female flowers.

Figure 3. Crystal longan flower (male and female) at a different maturity stage

The flower samples were collected and investigated further under a microscope. As shown in Figure 4, the male flower has consisted of petals, pedicel, and stamen. There is no pistil observed on the male flower. Each male flower only has 5 stamens which are composed of filament and an anther. The filaments are hairy towards the base and sit separately above the base of the flower. The anthers are pinkish purple in colour and will dehiscent during anthesis to release pollen for pollination of female flowers.

Figure 4. Male flower consisted of petals, filaments, and anthers (0.8x mag.).

Figure 5. Female flower consisted of pedicel, petals, stigma, style, ovary and stamen (1.0x mag.)

As shown in Figure 5, the female flower consists of a stigma, style, ovary, and stamen. Although the female flower may appear bisexual, the anthers are indehiscent during anthesis. Just like the male flower, the female flower also consists of 5 stamens that are hairy towards the base. The ovary is 2-lobed and hairy, and the style is undivided and hairy. As can be seen in Figure 4 and Figure 5, both flowers were covered with 5 small petals, which were whitish in colour.

CONCLUSION

The present observation revealed that Crystal longan is a monoecious plant with both male and female flowers on the same tree. Although the female flowers appeared bisexual, the anthers never dehiscent during anthesis and will subsequently degenerate. The anthers in the male flower dehiscent during anthesis, and the pollens released from the male flowers will pollinate the female flowers. This study should be continued further by investigating the duration from flowering to fruiting and, subsequently its harvesting index, as the information is not well documented.

ACKNOWLEDGEMENT

The authors would like to express sincere gratitude to all those involved in this research, especially the supporting staff from Fruit Crops Unit that has assisted in the on-field data collection. Special appreciation also goes to Entomology Unit for their assistance in this research.

REFERENCES

Thomson, L.A.J. and Thaman, R.R. 2006. *Pometia pinnata* (tava), V2.1 In: Elevitch.

Whitman, W.M.F. 1971. The Olosapo, the Sunsapote and the Fijian longan. Rare Fruit Council International. pp. 323-325.

Windarsih, G. and Efendi, M. 2019. Short communication: Morphological characteristics of flower and fruit in several rambutan (*Nephelium lappaceum*) cultivars in Serang City, Banten, Indonesia. *Biodiversitas Journal of Biological Diversity, 20*(5): 1442-1449.

Synergistic Effects of Growth-Promoting Microorganisms on Seedling Production of Terap (*Artocarpus odoratissimus* Blanco)

Goh Ei Ping[1], Shiamala Devi Ramaiya[1,2,*] and Noorasmah Saupi[1,2]
[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia
[2]Institut Ekosains Borneo, Universiti Putra Malaysia Bintulu Campus, 97008 Sarawak, Malaysia

**Corresponding author's email: shiamala@upm.edu.my*

INTRODUCTION

Borneo is recognized as the land of diversity, and there are about 152 indigenous species were recorded in Sarawak. Among that, *A. odoratissimus* also known as terap belongs to the Moraceae family (Zerega et al., 2004), is one of the indigenous fruits that is popular for its odour, gaining visibility in the local fruit industry and valued for durable timber (Bolanle-Ojo et al., 2008). This fruit is identified by the Department of Agriculture (DOA) Sarawak as one of the promising indigenous species that play a significant role in nutrition, food security, and income generation for local communities. Although there has been an increasing demand and attributes of this fruit in recent years, less effort has been made to cultivate this species on a small or large scale, and lack of information available on their propagation techniques. Seed propagation techniques may greatly facilitate the domestication of the tree species by enabling the rapid multiplication of selected genotypes and the production of superior planting stocks.

When a plant is in its earliest stages of growth, substrates are the utmost important factor to be looked up. It has been shown that applying effective microorganisms (EMs) in the planting media improves plant development and tolerance to environmental and production challenges, including problems with water quality. These soil EMs develop mutualistic symbioses with over 80% of terrestrial plant species, including numerous crops. Numerous EMs have been discovered that supply host plants with mineral nutrients (particularly nitrogen and phosphorus) and water in return for plant nutrients (Smith and Read, 2008). Plant growth-promoting microorganisms such as *Trichoderma*, *Bacillus*, and mycorrhizal fungi indicate enhanced tolerance to abiotic stresses during plant growth, partly due to improved root growth and improvement in the water-holding capacity of plants (Vinale et al., 2008; Radhakrishan et al., 2017; Bzdyk et al., 2018). The present study examines the synergistic effects of the substrate composition with effective microorganisms, i.e., *Trichoderma harzianum*, *Bacillus subtilis*, and Arbuscular mycorrhizal fungi (AMF), on the growth and development of the *A. odoratissimus* seedlings.

MATERIALS AND METHODS

Treatment preparation and the experimental design

Two-week germinated seedlings were transferred into the polybag and placed under 50% shade. The planting media were prepared in the polybags (16" x 16") potting mixture with 3 volumes of topsoil: 2 volumes of sand: 1 volume of compost. The effective microorganisms, i.e., *Trichoderma harzianum*, *Bacillus subtilis,* and AMF as additivity in planting media. The pot experiment was designed following the Randomized Completely Design (RCBD) with eight replications per treatment. The following treatments were evaluated in this study: Treatment 1: Soil + Arbuscular Mycorrhizal Fungi (AMF); T2: Soil + *Trichoderma harzianum*; T3: Soil + *Bacillus subtilis*; T4: Soil + AMF + *Bacillus subtilis*; T5: Soil + *Trichoderma harzianum* + *Bacillus subtilis*; T6: Soil + AMF + *Trichoderma harzianum*; T7: Soil + AMF + *Trichoderma harzianum* + *Bacillus subtilis*; T8: Soil only (control).

Data collection and statistical analysis

Weekly observation has been made to record the plant height (cm), stem diameter (mm), the number of leaves (n), and chlorophyll index, which were collected for 12 weeks. The growth rate was also calculated based on the measurement of the plant's height per day. Following the 12 weeks of monitoring, the plant samples were harvested to record the data of root length (cm), fresh root mass (g), fresh aerial mass (g), and total fresh mass (g). The plant sample was dried in an oven at 60° C until a constant weight was achieved, followed by calculating total dry mass, dry aerial mass (g), and dry root mass (g). The quality of the seedling produced was determined using Dickson's Quality Index (DQI). Analysis of variance (ANOVA) was used to detect significant differences among treatments whereas, post hoc Tukey's test (p<0.05) was used to compare treatments mean using Statistical Analysis System version 9.4.

RESULTS AND DISCUSSION

Effect of different planting substrates on seedling growth and development

The plant height, collar diameter, and the number of leaves for *A. odoratissimus* seedlings have been recorded weekly, and the results are presented in Table 1. Among the substrates, seedlings grown in T7 (Soil + AMF + *T. harzianum* + *B. subtilis*) were recorded significantly higher (p<0.05) plant height at 47.13±10.8 cm, and this is not comparable with T3 (Soil + *B. subtilis*) at 46.83±2.63 cm and T4 (Soil + AMF + *B. subtilis*) at 40.96±0.20 cm. The least plant height was recorded in T8, soil only (control) at 34.13±0.80 cm. These findings can be explained by the effectiveness of plant-growth promoting microbes to enhance plant growth and crop yield (Rani et al., 2019). Seedlings grown in the substrate with *B. subtilis* alone (T3) or in combination with other effective microbes (T7) showed a strong effect on the growth and development of *A. odoratissimus* seedlings. According to Anand et al. (2010), the plant treated with *B. subtilis* showed maximum shoot and root length in chili. The authors have explained that *B. subtilis* managed to fix atmospheric nitrogen and promoted nodulation by other bacteria, resulting in improved colonization of native symbiotic rhizobacteria. Similar to the plant height trend, a significantly higher collar diameter was recorded in seedlings grown in T3 (Soil + *B. subtilis*) with the value of 8.29±0.53 mm and T7 (Soil + AMF + *T. harzianum* + *B. subtilis*) at 8.25±1.28 mm. This shows the application of *B. subtilis* alone or in combination with AMF and *T. harzianum* could improve the substrate structure, nutrient solubility, and plant physiology (Halpern et al., 2015). On the other hand, the number of leaves does not significantly (p>0.05) affect by the substrate treatments.

Effect of substrates on biomass composition of *A. odoratissimus* seedlings

Figure 1 presents the aerial, root, and total biomass of *A. odoratissimus* seedlings. T7 recorded significantly higher aerial and root dry mass at 25.00±0.64 g and 11.66±1.08 g, respectively. Unlike in plant height and collar diameter, the T3 showed significantly lower root biomass at 9.66±0.49 g compared to the T7. However, treatment with soil only (T8) contributed to less significant results in their fresh and dried mass than the other treatments. In terms of shoot: root ratio, there is no significant difference between the treatments. Notably, the combination of microbes in T7 improved the *A. odoratissimus* seedling growth and biomass accumulation with significant contribution by the p-solubilizing bacteria, i.e., *B. subtilis*, to improve plant root establishment, together with the *T. harzianum* and AMF that improves crop yield and resilience to abiotic stress through increasing nutrient uptake and defend plant root infection fungi (Talaat et al., 2015; Kakabouki et al., 2021). This finding is further supported by the higher DQI value obtained in T7 which was 0.72±0.03 (need to put other DQI data for other treatments for comparison in Table of Figure). This value implied the quality of *A. odoratissimus* seedlings was obtained in the substrate with the combination of effective microbes. The higher DQI value represented the promising seedling quality, which integrates biomass distribution and morphological traits for successful field planting. This is followed by T3 at 0.60±0.04 in the

substrate with single-strand inoculum *B. subtilis*, however, the less efficacious composite was found with the control (T8) treatment.

Table 1. Plant height (cm, stem diameter (mm), and number of leaves (n) for *Artocarpus odoratissimus* seedlings in different substrate composition (data per weekly or end of the observations (12 weeks) – in methods mentioned weekly observation).

Trt	Compositions	Plant height (cm)	Stem diam. (mm)	Number of leaves (n)
T1	Soil + AMF	36.43±0.50^{b}	7.14±0.56^{bc}	9.66±1.15^{a}
T2	Soil + *Trichoderma harzianum*	34.26±1.15^{b}	7.76±0.19^{b}	9.66±0.57^{a}
T3	Soil + *Bacillus subtilis*	46.83±2.63^{a}	8.29±0.33^{a}	10.33±0.33^{a}
T4	Soil + AMF + *Bacillus subtilis*	40.96±0.20^{ab}	7.46±0.30^{bc}	9.66±0.57^{a}
T5	Soil + *Trichoderma harzianum* + *Bacillus subtilis*	39.40±0.81^{b}	7.34±0.76^{bc}	9.66±0.57^{a}
T6	Soil + AMF + *Trichoderma harzianum*	36.76±0.47^{b}	6.81±0.60^{c}	10.00±1.00^{a}
T7	Soil + AMF + *Trichoderma harzianum* + *Bacillus subtilis*	47.13±10.8^{a}	8.25±1.28^{a}	9.66±0.57^{a}
T8	Soil only (control)	34.13±0.80^{b}	6.89±0.43^{c}	9.66±0.57^{a}

Mean values in the same column with different alphabets (a>b>c) in the same category are significantly different at p<0.05 (ANOVA, Tukey's test). Values are given in means±standard deviation and values in brackets are the range.

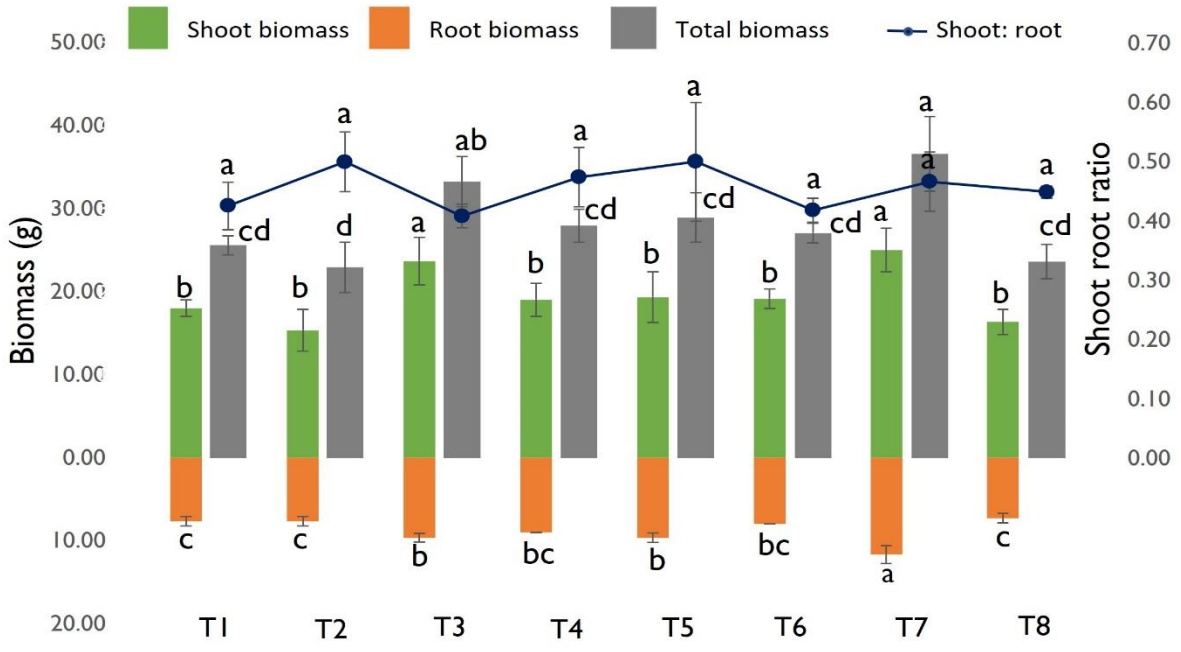


Figure 1. Biomass composition on *Artocarpus odoratissimus* seedlings. Mean values with different alphabets in the same category are significantly different at (p<0.05).

CONCLUSION

The use of effective microorganisms in the planting substrate is the essential element for successful seedling production and improved cultivation of *A. odoratissimus*. As a result, the T7 (Soil + AMF + *T. harzianum* + *B. subtilis*) improved the early stages of seedling development. This result was in line with a more significant DQI value of 0.72. It could be concluded that the combination of AMF, *T. harzianum*, and *B. subtilis* leads to interactions that may enhance the cultivation of the *A. odoratissimus* seedlings. Appropriate cultivation methods could be suggested to farmers and growers for high-quality commercial production of *A. odoratissimus* seedlings.

ACKNOWLEDGEMENT

This research was granted with funding support by Sarawak Research Development Council (SRDC) under RDCRG/RIF/2019/15.

REFERENCES

Bolanle-Ojo, O.T., Akinyele, A.O, and Aduradola, A.M. 2018. Morphological and physiological response of *Artocarpus heterophyllus* Lam. seedlings to selected environmental factors. *Journal of Agricultural Science & Food Research*, *9*: 1-7.

Kakabouki, I., Tataridas, A. Mavroeidis, A. Kousta, A. Karydogianni, S. Zisi, C. and Papastylianou, P. (2021). Effect of colonization of *Trichoderma harzianum* on growth development and CBD content of hemp (*Cannabis sativa* L.). *Microorganisms*, *9*(3): 518.

Radhakrishnan, R., Hashem, A. and Abd_Allah, E.F. 2017. *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*: 667.

Rani, A., Kumar, N. Ram, A. Dev, I. Uthappa, A.R. Shukla, A. and Parveen, S. 2019. Effect of growing media and arbuscular mycorrhiza fungi on seedling growth of *Leucaena leucocephala* (Lam.) de Wit. *Indian Journal of Agroforestry*, *21*: 22-28.

Zerega, N., Ragone, D. and Motley, T.J. 2004. Complex origin of breadfruit: Implication for human migrations in Oceania. *American Journal of Botany*, *91*, 760-766.

Traditional Ecological Knowledge of Orang Asli in Malaysia: The Utilization of Plant Tubers as Food Resources

Siti Nursyadiq Anuar[1,*], Jamilah Mohd Salim @ Halim[1,2], Dome Nikong[2], Norhayati Ab Manaf[1], Nur Azura Sanusi[3] , Khatijah Omar[1,3], Tengku Rozaina Tengku Mohamad[4]

[1]Institute of Tropical and Sustainability and Development (Bio-D Tropika), Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Nerus, Terengganu, Malaysia

[2]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Nerus, Terengganu, Malaysia

[3]Faculty of Business, Economy and Social Development, Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Nerus, Terengganu, Malaysia

[4]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

**Corresponding author's email: p4492@pps.umt.edu.my*

INTRODUCTION

Orang Asli is a term used by the Malaysian Government in referring to the aborigines in Peninsular Malaysia. Under the Orang Asli Act (Act 134) 1954, which was revised once in 1974, a person is considered Orang Asli if one of his/her parents is a member of an Orang Asli ethnic group and lives according to the laws, beliefs, and customs of that group. Orang Asli communities utilised the tropical rainforest as their main home and depends on various wild food plant for their livelihood and sustenance. They also use a variety of natural resources in their daily life by engaging in many activities such as hunting, collecting, and gathering forest resources. This community has a great traditional ecological knowledge in many aspects, which makes them a great source of information of flora and fauna within the forest. The utilization of the wild food plant was strongly guided by traditional ecological knowledge inherited from their ancestor. Therefore, there is a need to conserve the existing knowledge that is significance to the Orang Asli community.

Traditional ecological knowledge is defined stated as a network of knowledge, beliefs, spirituals, and traditions aimed to maintain and connect indigenous relationships with heritage, culture and place, which are transferred to their traditions, ritual practices, and other activities among kin communities directly and indirectly (Berkes, 1999). Meanwhile, Harun and Othman (2011) elaborated that traditional ecological knowledge was also known as indigenous knowledge used by the local community to make a living in a particular environment. It is believed that traditional indigenous knowledge is considered as knowledge, spirituals, and beliefs which are inherited from generation to generation by heritage and cultural transmission of livelihood with one another and their environment.

A study was conducted to investigate the traditional ecological knowledge of plant tubers as a food resource utilized by the Orang Asli, specifically Bateq tribes in Kelantan and Terengganu in Peninsular Malaysia. This also provides a general description of the tribes towards the practices of the plant resources in maintaining the plant tubers for their survival and well-being. The outcome of this research is important to scientifically identify and document new food sources from the forest that could contribute to those tribe' livelihood and future food security.

MATERIALS AND METHODS

The study was conducted in May 2022 in a village known as Perkampungan Orang Asli Kuala Koh and lies between 4.8735°N to 4.8757°N latitude and 102.4512°E to 102.4634°E longitude. The population of Orang Asli, mostly the Bateq sub-tribes live inside the National Park, situated in the

middle part of Peninsular Malaysia covers an area which includes three ~~3~~ States which are Pahang, Kelantan, and Terengganu.

Official permission was obtained from the Orang Asli Development Department (JAKOA), followed by their village head or Tok Batin to interview the villagers. The data was collected through semi-structured questionnaires, field visits, group discussions, and key informant interviews in settlement of the tribes. The semi-structured questionnaire that was used to gather information on traditional ecological knowledge regarding the utilization, including the plant part used, mode of preparation, method of processing the plant, and the practice of harvesting and consuming the plant. Interviews were conducted through verbal communication in Bahasa Melayu language with the help of an interpreter.

The 45 interviewed informants were made up of 15 males and 30 females. The informants were selected using the snowball sampling method, identified by community members as knowledgeable on the utilization of the plant tubers. The plant mentioned by the informants were collected with the help of the research assistant during the field visit. The plant was photographed, and, if possible, the plant species were identified on-site. Thereafter, the voucher specimen was prepared and identified by the Forest Research Institute of Malaysia (FRIM) and other taxonomists and then deposited in the Herbarium.

RESULTS AND DISCUSSION

The study revealed that Orang Asli consumed at least 11 species of plant tubers, among others known locally as Ubi Takop, Ubi Rem, Ubi Kebak, Ubi Pasir (Ubi Cengel), Ubi Woh, Ubi Hakay, Ubi Kaserk, Ubi Pam, and Ubi Haw as their food (Table 1).

The plant tubers were reported to be gathered from diverse localities, from moderately slopping ground to hilly environments, flat areas, and at the banks of the main rivers. The diversity and availability of the plant are available year-round, although the rates of return were highest in May and October. The harvesting activity for plant tubers also shows a seasonal pattern because Orang Asli will not dig for plant tubers during the season of fruits. This report is supported by the study of Karen Endicotts in 1975-1976. The tool needed for the digging procedure is very simple and easily portable. Orang Asli harvested the plant tubers by using a wooden digging stick tipped with chisel-like metal blades fashioned from an old bush knife or small metal shovel blades. They wrapped the plant tubers they collected in a large leaf and tied the bundles securely with vines. They used a basket or cloth slings to carry the tubers back to the camp. Most of the time, they will share the plant tubers with the rest of the community in the camp.

Most of young Orang Asli learn to harvest plant tubers through practice and experience. Based on this context, a skilled plant tubers harvester may identify the plant's growth cycle through visual, touch, taste, and smell for some notable species. Young Orang Asli is not given direct spoken instruction; rather, they learn to recognize the growth and form of plant tubers via intimate engagement in their parents' lives and by replicating what their parents do. By the time they reach the age of seven, young Orang Asli may have participated in a number of digging activities, and by the time they reach the age of ten, all children have watched their parents dig countless times and are able to identify the most crucial component of the tubers.

Digging up tubers needed a significant amount of knowledge. First, they had to have a general idea of where to dig. The Orang Asli can choose the most suitable location for digging by noticing the presence of climbing woody stems, leaves, and flowers in the forest. This helps them locate the best spot. The intertwining of the climbing pieces is one of the most significant indications that can be found there. Sometimes they kept these locations in mind for a year or two and went to them only when they thought the tubers would be ready to harvest. Orang Asli would also look for dry, dead leaves on the trees and then examine the ground for a portion of the vine that would point them in the direction of the tubers. They would search the region thoroughly until they

found what it is that they are looking for if they did not have any prior knowledge of locations where tubers were growing.

Table 1. List of plant tubers utilized by orang asli for food resources.

Local Name	**Scientific Name**	**Family**	**Remark**
Ubi Takop	*Dioscorea orbiculata*	Discoreaceae	A dependable famine food, long and thin, good flavour, roasted or boiled, the largest source of tuber, usually grow on slopes away from streams.
Ubi Rem	*Dioscorea prainiana*	Dioscoreaceae	Enormous root grows on one spot near to surface and does not spread in every direction.
Ubi Gadong	*Dioscorea hispida*	Dioscoreaceae	Bitter, contain toxic alkaloid dioscorine, must be leach out before its edible, processing required at least one full day and usually two, tasty when properly prepared. Useful food resource.
Ubi Kebark	Undetermine	Undetermine	Grow in the hilly area, gives an intoxicating effect to the person who eats it.
Ubi Pasir / Ubi Cengel	*Dioscorea alata*	Dioscoreacea	Preferred the sandy of rivers away from streams.
Ubi Woh	*Dioscorea*	Dioscoreacea	Hard to dig for, produce many flowers that drop close together, thus the vine tend to be found in cluster, thorny steam.
Ubi Hakay	*Amorphophallus campanulatus*	Aracea	Elephant-foot yam, grow near Malay dwellings, the flesh was cooked in bamboo.
Ubi Kaserk	*Dioscorea pentaphylla*	Dioscoreacea	Grow near the banks of the river.
Ubi Hau	*Dioscorea* sp.	Dioscoreacea	Grow in the lowland and hilly area.
Ubi Ciyak	*Dioscorea piscatorum*	Dioscoreacea	Grow in the lowland area near the river, Thorny, Fish poison can be extracted from the tuber.
Ubi Tampak	*Gnetum tenuifolium*	Gnetaceae	Woody tuber grows close to surface in a small bunch coming off the vine. Growing in flat, moderately sloping ground.

The survey reveals that the choice of utilizing the plant tubers is motivated by deliciously tasted, nutritious, easy to cook, and unpolluted natural food. Even when there is rice on the camp, Orang Asli may still go digging for plant tuber, but the plant tuber is no longer an absolute dietary need. This is because digging for plant tubers is a significant cultural symbol for the Orang Asli. Before the advent of the cultivated plant, plant tubers were typically thought to have been the primary sources of carbohydrates (Endicott and Bellwood (1991). Digging still carries high cultural value, but the dietary significance of plant tuber has been replaced with rice. The cooking method of the plant tubers was basically very simple. The tubers usually were roasted directly over the fire or boiled in a cooking pot.

Orang Asli frequently replants the tuber head. Tuber replanting is done quite casually. All that is needed is some gentle digging, the tuber head thrown back into the soil, and a few digs and scrapes of the digging stick to bury it. This is a case of substantial knowledge that can be expressed simply. There are no spells, rituals, incantations, or other mystical words to spice up the conversation. To avoid injuring the plant's stalk, they never cut the stems and never excavate the spot that is close to the base of the stem. After a period, they gradually develop a habit of returning to the spot where they had dug in before. This urge comes from the fact that they want to check on the progress of the tuber that they had planted in the past.

CONCLUSION

Understanding what lies behind traditional ecological knowledge and the practice of using plant resources, those indigenous tribes could possibly contribute to the future food security and preservation of the natural heritage of plant biodiversity.

ACKNOWLEDGEMENT

This research is conducted with a financial support provided under Fundamental Research Grant Scheme (FRGS), FRGS/1/2019/WAB13/UMT/02/1 from the Ministry of Higher Education (MOHE) of Malaysia.

REFERENCES

Berkes, F. 1999. Sacred Ecology: Traditional ecological knowledge and source management. Philadelphia: Taylor and Francis.

Endicott, K.M. and K. L. Endicott. 2008. The headman was a woman: The gender egalitarian Batek of Malaysia. Long Grove, IL: Waveland Press.

Endicott, K. 201. The hunting methods of the batek negritos of Malaysia. *The Asia Pacific Journal of Anthropology*, *2*: 7–22.

Lye Tuck-Po, 2005. Changing Pathways Forest degradation and the Batek of Pahang, Malaysia United State: Lexington Books.

Effect of Storage Period and Temperature on External Quality of Durian Nyekak (*Durio kutejensis* (Hassk.) Becc.)

Pearlycia Brooke* and Rajmah Muzli Razili
Agriculture Research Centre Semongok, Department of Agriculture Sarawak, Borneo Height Road, 93250 Kuching, Sarawak, Malaysia

**Corresponding author's email: pearlycb@sarawak.gov.my*

INTRODUCTION

Durio kutejensis (Hassk.) Becc. or commonly known as durian nyekak is one of the six commonly edible durian species. It is an endemic species in Borneo and can be found growing in virgin and secondary forests with sandy clay soil (Priyanti et al., 2013). In Sarawak, durian nyekak is popular in the rural markets and is highly appreciated by the locals. The trees can be found in the central and northern regions of Sarawak and are planted in home gardens. They received little or no horticultural attention but were allowed to grow and bear fruits naturally.

Agriculture Research Centre (ARC) Semongok, as the research division of the Department of Agriculture Sarawak, has studied the different varieties of durian nyekak since the 1990s. A number of superior clones were screened and successfully selected after many years of field evaluation and quality testing. These clones, namely DK 5, DK 6, and DK 8 were selected due to their good eating quality and size. In Indonesia, particularly in eastern Kalimantan, durian nyekak is commercially cultivated on a large scale with wide superior varieties like 'Rencong', 'Kayan', 'Nangka' and 'Batuah' were released and made available to the consumers (Santoso, 2010). Although durian nyekak is not as popular as the common durian *(D. zibethinus)*, there are several characteristics which may render this species suitable for commercial exploitation. The bright yellow to orange aril gives durian nyekak an attractive appearance. This appealing colour and its firm pulp texture make the fruits suitable for minimal processing. Besides colour, its mild aroma is undoubtedly a characteristic appreciated by those who find the smell of durian offensive and overpowering. The soft odour provides the opportunity for the fruits to be exported to the wider overseas market and has huge market potential in catering to the needs of consumers who prefer less pungent durians, especially those from western countries.

Similarly, to other durian species, durian nyekak is normally allowed to ripen naturally and fall from the tree before they are being harvested. However, several fruit traders in the local markets have pointed out that tree-picked durian nyekak has longer shelf life than the mature dropped fruits and they can be stored for several days if kept under ideal storage condition. This is an added advantage for the traders as the fruits take a longer time to ripen and can be transported to the distant markets. In view of the reasons, this study was undertaken to assess the external quality changes of durian nyekak when subjected to different storage period and temperature.

MATERIALS AND METHODS

Fruit preparation and storage

Durian nyekak fruits (DK 3) were harvested in ARC Semengok's farm at a maturity stage where there was an overall change in the husk colour of the fruits, from light yellow to golden yellow. The fruits were transported to ARC Semengok's Post Harvest Centre the same day and used immediately for experiment. Subsequently, the fruits were sorted to eliminate obvious defects and randomly divided into three treatments where they were stored at (1) ambient temperature (28±3°C and 70 RH% for 6 days), consistent with the practice of local traders, (2) 15°C for 6 days and (3) 15°C for 12 days. Three replications of four fruits were used for each treatment and were assessed on Day 0, 2, 4, 6, 8 and 12 during the whole storage duration.

Fruit external quality assessment

Post-harvest exocarp browning was monitored visually by recording the number of fruits with browning symptoms and converting it to percentage. The fruits were also monitored daily for splitting which occurred along the suture lines to determine the onset of husk dehiscence. The percentage of weight loss was obtained by measuring the difference in individual fruit weight before and after storage using a top-loading balance. Presence of fungal infection was based on the observation of characteristic symptoms, *e.g.*, the appearance of a white mycelial growth and discoloration on the husk.

RESULTS AND DISCUSSION

Browning

In this study, the decline in the visual quality of fruits was mainly due to browning. This greatly affects marketability as durians with brownish husk are not regarded as fresh. Fruits kept in both 15°C and ambient temperature (28°C) showed symptoms of exocarp browning starting on Day 4 with browning percentage of 53% and 15%, respectively. Although the incidence of browning was higher in fruits kept in 15°C on the aforementioned day, no increase in percentage of brown fruits was observed until Day 6. However, when the storage period was extended to 12 days, it was observed that 100% of the fruits turned brown on Day 8. In contrast, all fruits kept in ambient temperature turned brown on Day 6 of the storage period. From our study, it can be concluded that storing durian nyekak fruits in both temperatures caused browning however the rate of browning was greatly accelerated at ambient temperature. This is in agreement with Wills et al. (1981), who reported that the recommended optimum temperature range for ripening fruit in general is 18-21°C. Temperature outside this range, as in this study, can cause poor colour development and encourage mould growth.

Dehiscence

According to Sriyook (1994), water loss and ethylene production were the two major reasons that caused ripened durian fruit to dehisce. Water losses caused the pericarp to shrink and pull the carpels from each other along the suture at the middle of each locule. Ethylene, on the other hand, weakened the cells in the dehiscence region which consists of parenchyma cells without chlorophyll. In this study, husk dehiscence was first observed on both storage temperatures on Day 6 with the highest percentage of dehisced fruits (100%) recorded in fruits stored at ambient temperature. Fruits stored in 15°C, on the other hand, had delayed onset with only 53% of the fruits dehisced on Day 6. Subsequently, all fruits kept in this temperature dehisced completely on Day 12 when the storage period was extended. The high rate of dehiscence in ambient temperature could be due to high endogenous ethylene and carbon dioxide (CO2) production in the husk of fruits stored under normal air (Booncherm and Siripanich, 1991; Wongs-Aree and Noichinda, 2014). In addition, dehiscence is greater under low humidity conditions as it caused an increase in internal ethylene concentration (Ketsa and Pangkool, 1994). The durian fruit, therefore, should be stored under relative humidity of 85 to 90% to delay fruit dehiscence.

Weight loss

Durian fruits have high rates of weight loss after harvest (Ketsa and Pangkool, 1994). On Day 6, durian nyekak fruits stored at ambient temperature had lost about 38.2% of their initial fresh weight, with an average daily loss of 6.3% as compared to fruits stored at 15°C (29.0%). In comparison, a study by Tirtosoekotjo (1990) reported a weight loss of 15.3% (average daily loss of 5.1%) in *D. zibethinus* during a 3-day holding at ambient conditions. On Day 8 and 12, the initial weight of fruits stored at 15°C was reduced by 37.8% and 48.21%, respectively (Figure 1). The

high weight loss was probably due to dehiscence of some fruits (53%) which was already observed on Day 6 of the storage period. In addition, the presence of numerous spines in durian fruits which increases the surface area for transpiration, resulted in making the fruits more prone to water loss (Añabesa et al., 2006). At this weight loss, browning and dehiscence of the fruits became objectionable, and dryness, rendered the fruits unacceptable for sale.

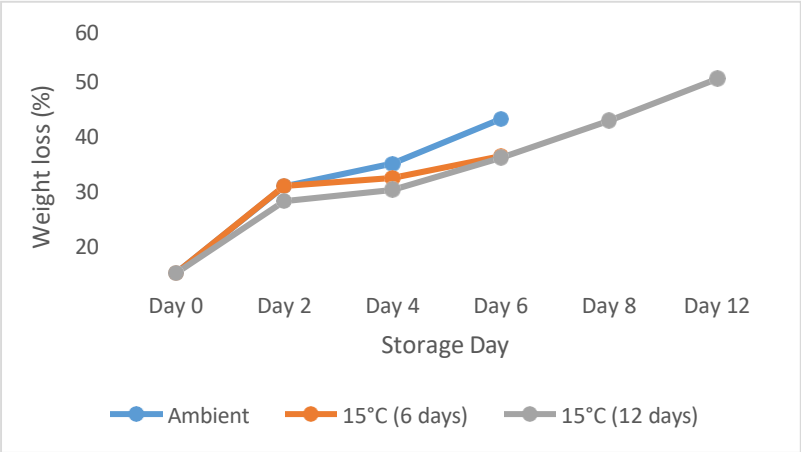


Figure 1. Weight loss at two different storage temperatures, *i.e.* (1) ambient (28±3°C), (2) 15°C at 6 days and (3) 15°C at 12 days.

Fungal infection

Fruits stored at ambient temperature started to show symptoms of fungal infection on Day 4 of storage with 18% fruits infected. At the end of the storage period (Day 6), the percentage of infected fruits had increased significantly to 55.6%. The high incidence of fungal infection in the fruits was probably due to the storage temperature (28°C), which falls in the optimum growth temperature range for most fungi that is between 25°C and 30°C (Ibrahim et al., 2011).

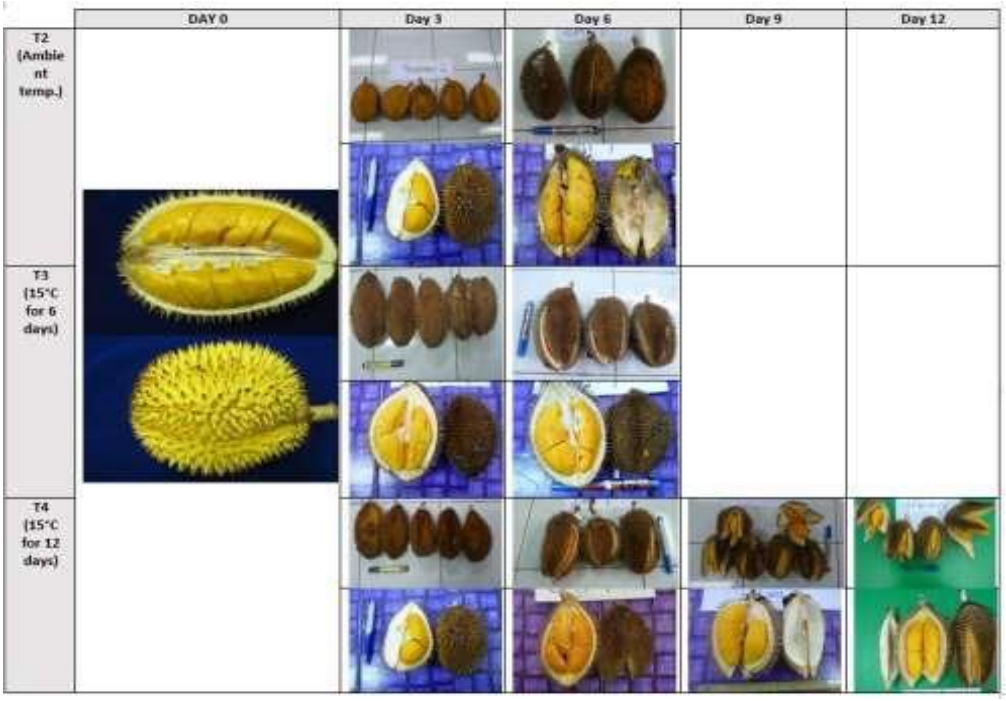


Figure 2. Exocarp browning, husk dehiscence and incidence of fungal growth in fruit samples during increasing storage time.

Although the fungus in this study was not isolated and identified, a study conducted by Sivapalan et al. (1998) had consistently isolated *Colletotrichum gloeosporioides, Cylindrocladium scoparium, Geotrichum candidum, Gliocephalotrichum bulbilium* and *Lasiodiplodia theobromae (Botryodiplodia theobromae)* from affected fruits of *D. kutejensis.* No fungal infection was observed on fruits kept at 15°C during the whole storage period (Figure 2). This in accordance with a study by Nur Azlin et al. (2020) who demonstrated that storage of *D. zibethinus* cv. Musang King in low temperature helped in retarding fungal growth.

CONCLUSION

Based on fruits external quality evaluation in this study, it can be suggested that durian nyekak stored under 15°C can be kept for up to 8 days as compared to only 4 days for ambient temperature stored fruits. Fruits stored at 15°C exhibited tremendous quality deterioration at the end of the storage period when compared to their initial quality at harvest. The observations made in the present study serve as an impetus for a more thorough investigation on prolonging the shelf-life of tree-picked durian nyekak using other postharvest techniques. The optimum storage temperature should also be investigated further to determine the best condition to maintain its postharvest quality.

ACKNOWLEDGEMENT

The authors wish to acknowledge their sincere gratitude to Mr. Jeffery Chemagat and Ms Josephine Finia Doam of Fruit Crops Section and also Mr. Drahman Sapong of Postharvest Technology Section (ARC Semengok) for their work in data collection and documentation.

REFERENCES

Añabesa, M.S., Oria, D.V., Esguerra, E.B. and Sarcos, M.A. 2006. Postharvest behavior & storage life of 3 durian cultivars with varying maturity, waxing & temperature. *Philippine Journal of Crop Science, 31*(1): 29-46.

Brooncherm, P. and Siriphanich, J. 1991. Postharvest physiology of durian pulp and husk. *Kasetsart Journal Natural Science, 25*: 119-125.

Ibrahim, M., Rabah, A.B., Liman, B. and Ibrahim, N.T. 2011. Effect of temperature and relative humidity on the growth of *Helminthosporium fulfum*. *Nigerian Journal of Basic and Applied Science, 19*(1): 127-129.

Ketsa, S. and Pangkool, S. 1994. The effects of humidity on ripening of durian. *Postharvest Biology and Technology, 4*: 159-165.

Santoso, P. J. 2010. Lai, durian berwarna daging atraktif: potensi ekspor. Balai Penelitian Buah Tropika, Solok, Indonesia.

Sivapalan, A., Metussin, R., Hamdan, F. and Zain, R.M. 1998. Fungi associated with postharvest fruit rots of *Durio graveolens* and *D. kutejensis* in Brunei Darussalam. *Australasian Plant Technology, 27*(4): 274-277.

Sriyook, S., Siriatiwat, S. and Siriphanich, J. 1994. Durian fruit dehiscence-Water status and ethylene. *HortScience*, *29*(10): 1195-1198.

Tirtosoekotjo, R. 1990. Postharvest physico-chemical characteristics of durian fruit (*Durio zibethinus* Murr.) *Penelitian Hortikultura, 4*(1): 42-45.

Wills, R.H.H., Lee, T.H., Graham, D., McGlasson, W.B. and Hall, E.G. 1981. *Postharvest: An Introduction to the Physiology and Handling of Fruit and Vegetables,* New South Wales, New South Wales University Press, 161 pp.

Wongs-Aree, C. and Noichinda, S. 2014. Postharvest physiology and quality maintenance of tropical fruits. In: Postharvest Handling: A System Approach.

Molecular Identification of Bacteria with Quorum Sensing Inhibition Properties from Matang Mangrove Forest Reserve

Fathima Zahraa Ozeer, and Kamariah Hasan*
Faculty of Applied Sciences, Quest International University, Jalan Raja Permaisuri Bainun, 30250 Ipoh, Malaysia

**Corresponding author's email: Kamariah.hasan@qiu.edu.my*

INTRODUCTION

Quorum Sensing (QS) process is used in bacteria to regulate gene expression as a group when the population density achieved certain threshold. In pathogenic bacteria, QS has always been associated with the ability to cause harm to its host. The signaling molecules known as autoinducers (AI) is the key player in triggering various gene expressions involved in bioluminescence, pathogenesis while evading host immune responses, and biofilm formation among a few (Jiang et al., 2019). Bacteria with quorum sensing ability has exhibited higher pathogenicity compared to those without this mechanism. However, QS pathways can be interrupted by certain molecules known as anti-Quorum Sensing (anti-QS) compounds, which can be found in plants, fungi and abundantly in bacterial species (Ab Ghani, 2017). Although several plants have exhibited the ability to produce anti-QS molecules, the largest repository of anti-QS molecules can be found in bacterial species as they are utilized for regulatory and competitive purposes in nature. Matang Mangroves Forest reserve, located near Kuala Sepetang, Taiping offers a rich reservoir of microorganisms and is the site of choice to isolate bacteria with potential secondary metabolites production. The objective of this study is to isolate bacteria that can produce anti-QS molecules and prevent interaction especially between pathogenic bacteria. A strain of *Chromobacterium violaceum* isolated from river water in Meru, Ipoh was used as the anti-QS indicator strain via the inhibited production of the purple pigment violacein. Potential isolates were studied and molecularly identified using 16s rRNA gene sequencing analysis. The unique behaviors and appearances of the isolated bacterial species may suggest undiscovered potential for anti-QS properties and provide preliminary data for further analysis of their secondary metabolites.

MATERIALS AND METHODS

Isolation of *C. violaceum* from the environment

Indicator strain, *C. violaceum* strain was isolated from sediments, sludge and water from a river outlet in Meru, Ipoh, Perak. The water sample (10 µL) was spread on NA and left to incubate at room temperature until colonies formed. Suspected *C. violaceum* grown as violet colonies were chosen and streaked on NA plates to obtain pure colonies. 16S rRNA gene amplification was performed and the PCR fragment was sent for sequencing to confirm the strain.

Isolation of Anti-QS bacteria from soil samples

Soil sediments were collected at an adequate depth from 3 sampling sites in Matang Mangrove Forest Reserve, Kuala Sepetang, Perak, Malaysia. The sediments were serially diluted with 0.9% saline water up to 10^{-3} dilution and 100 µl of each dilution were spread on nutrient agar plates. The plates were incubated at ambient temperature until colonies can be seen. Unique colonies were isolated and incubated at ambient temperature to get pure isolates.

Screening for anti-QS properties

The mangrove isolates and *C. violaceum* were grown in a shaker incubator, and the screening procedure was performed as detailed by Ma et al. (2018). *C. violaceum* culture was mixed with agar and poured into Petri dishes. Once solidify, discs containing unique isolates were placed on the agar and the plates were kept at room temperature for several days. Isolates that formed haloes of clear zones or had inhibited growth of *C. violaceum* were considered as positive isolates.

Extraction of genomic DNA and amplification of 16s rRNA gene

The genomic DNA of *C. violaceum* was extracted using 'Bio Basic Inc. EZ-10 Spin Column Genomic DNA Minipreps Kit' as instructed by the kit. DNA extraction for the bacterial isolates were performed based on EtNa method described by Vingataramin and Frost (2015). The 16s rRNA gene amplification was performed using the forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer 1494R (5'-CTACGGCTACCTTGTTACGA-3') for all isolates. After the positive isolates were identified, they were grown in LB broth and stored in 20 % glycerol stock at -80°C for future use.

DNA sequencing and identification

The 16s rRNA gene PCR products from the isolates were sent to Apical Scientific Sdn. Bhd. for DNA sequencing. The DNA sequences were trimmed to remove ambiguous reads and BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed with 16s rRNA gene sequences of other species. After *C. violaceum* was sequenced and identified, the forward and reverse sequences were used to make a consensus sequence for submission to Genbank.

Statistical analysis

The violacein zone of inhibition diameters obtained from the triplicates of each potential anti-QS isolate and were analyzed using IBM SPSS Statistics software version 25. The means for zone of inhibition diameters within and between triplicates of isolates were analyzed using one-way ANOVA and Tukey's *post hoc* tests. A statistical significance level of $p<0.05$ was established.

RESULTS AND DISCUSSION

Morphology and molecular identification of *C. violaceum*

The isolated *C. violaceum* was confirmed to be Gram-negative coccobacilli and the 16s rRNA gene sequence was submitted to the GenBank database as *C. violaceum* strain QIU-Z14 (Accession number: MZ540364).

Screening of anti-QS isolates

A total of 8 potential bacteria that exhibited anti-QS activity against *C. violaceum* QIU-Z14 were isolated bases on the zone of inhibition diameter as shown in Table 1. The statistical differences in the mean diameter of inhibition of isolates between and within the triplicate diameters from the one-way ANOVA test was shown to be significantly different ($p<0.05$). After conducting the *post-hoc* Tukey's test, the isolates were statistically grouped into three homogenous subsets.

Molecular identification of the anti-QS isolates

Gram-negative isolates were dominant among the results. 16S rRNA gene sequence showed that the isolated bacteria have high similarity with *Serratia marcescens*, *Pseudomonas aeruginosa, Vibrio fluvialis, Vibrio parahaemolyticus, Bacillus cereus, Staphylococcus epidermidis, Vibrio sinaloensis and Pseudomonas* spp. *Staphylococcus epidermidis* has not been previously reported to have anti-QS properties.

Table 1. The zone of inhibition diameter and identification of the anti-QS positive isolates.

Isolates	Mean Inhibition (cm)	Suggested species	% Similarity
RED	2.40±0.00^{c}	*S. marcescens*	99.02%
N2	2.30±0.00^{c}	*P. aeruginosa*	98.89%
S4	0.90±0.12^{a}	*V. fluvialis*	99.04%
S10	0.90±0.00^{a}	*V. parahaemolyticus*	98.27%
N9	1.77±0.25^{b}	*B. cereus*	99.19%
N6	1.60±0.30^{b}	*S. epidermidis*	99.28%
N10	0.97±0.12^{a}	*V. sinaloensis*	97.86%
S3	2.00±0.00^{bc}	*Pseudomonas* spp.	98.21%

Means that do not share the same letter are significantly different (p<0.05) based on Tukey's Test results.

Characterization of anti-QS isolates

Serratia marcescens

The RED isolate, with high similarity to *Serratia marcescens*, exhibited the largest diameter of overpowering growth with a clear zone surrounding it. The AHL-dependent system in *S. marcescens* is responsible for several characteristics including carbapenem antibiotics, as well as the red pigment, prodigiosin. Further photometric analysis is required to confirm if the obtained result is due to anti-QS or antibacterial properties.

***Pseudomonas* spp**.

The N2 and S3 isolates showed high similarity to *Pseudomonas aeruginosa (98.89%) and Pseudomonas* spp. (98.21%), respectively. These isolates produced clear zone and reduced the violacein pigment without preventing growth of *C. violaceum*, suggesting potential anti-QS capabilities. N2 produced green pigment, pyocyanin, widely utilized as antibacterial agents in various industries. The S3 isolate, was found to lack pyocyanin at first, but still had a hollow zone showing anti-QS capabilities.

***Vibrio* spp.**

The S4, S10 and N10 isolates were found to have high identity similarities to *Vibrio* species, producing various AIs which can negatively regulate the AHL QS system. Although the *Vibrio* species had not shown a hollow zone of inhibition, the production of violacein by *C. violaceum* QIU-Z14 was prevented within the area of growth for all three *Vibrio* isolates. This may suggest the possible presence of anti-QS molecules.

Gram-positive bacteria

The isolate N9 has high similarity with Gram positive *Bacillus cereus* (99.19%). *Bacillus cereus* was found to degrade AHLs like C_6-HSL used by *C. violaceum,* supporting our results (Raafat et al., 2019). *Staphylococcus epidermidis* (N6) possess degradative exoenzymes controlled by the Agr system, which may explain the discoloration of the violacein produced. In contrast, the newly discovered pigment and uncommon morphology on *S. epidermidis* may contribute to probable anti-QS molecules or antibacterial properties.

CONCLUSION

Anti-Quorum Sensing molecules may be an alternative approach to reduce pathogenicity of bacteria. The 16s rRNA gene identification is a preliminary method and further analyses are

required for identification at the species level. Further studies could include metagenomic analysis to discover more species, evaluation of anti-QS bacteria extracts on clinically important pathogens and characterization of extracellular signals involved in *C. violaceum* QIU-Z14 and the potential anti-QA producers.

ACKNOWLEDGMENT

The authors would like to thank lab staff for their assistance and Quest International University (QIU) for the opportunity to perform this experiment.

REFERENCES

Ab Ghani, N. 2017. Characterisation of quorum quenching actinobacteria isolated from mangrove swamps exhibiting lactonase activity. *University of Malaya*, 1-86.

Jiang, Q., Chen, J., Yang, C., Yin, Y. and Yao, K. 2019. Quorum Sensing: a prospective therapeutic target for bacterial diseases. *BioMed Research International*, *2019*: 1–15.

Ma, Z.-P., Song, Y., Cai, Z.-H., Lin, Z.-J., Lin, G.-H., Wang, Y. and Zhou, J. 2018. Anti-quorum sensing activities of selected coral symbiotic bacterial extracts from the South China Sea. *Frontiers in Cellular and Infection Microbiology*, *8*: 144.

Raafat, M., Ali-Tammam, M. and Ali, A. 2019. Quorum quenching activity of *Bacillus cereus* isolate 30b confers antipathogenic effects in *Pseudomonas aeruginosa*. *Infection and Drug Resistance*, *12*: 1583-1596.

Saleem, H., Mazhar, S., Syed, Q., Javed, M.Q. and Adnan, A. 2021. Bio-characterization of food grade pyocyanin bio-pigment extracted from chromogenic *Pseudomonas* species found in Pakistani native flora. *Arabian Journal of Chemistry*, *14*(3): 103005.

Vingataramin, L. and Frost, E.H. 2015. A single protocol for extraction of gDNA from bacteria and yeast. *BioTechniques*, *58*(3): 120-125.

Investigation of Local Palm Hearts (Umbut) as Potential Prebiotic Ingredients Using *In Vitro* Colon Model Experimentation

Chai Lee-Ling[1], Tan Hui Yan[1], Noorasmash Saupi[1], and Shahrul Razid Sarbini[1,2]*
[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia
[2]Halal Products Research Institute, Universiti Putra Malaysia, 43400 Serdang, Malaysia

**Corresponding author's email: s.r.sarbini@gmail.com*

INTRODUCTION

In Malaysia, the growth of commodity palm industry has brought a significant impact on economic development. Palm trees from *Arecaceae f*amily, are not only cultivated for their fruits, but also for their importance resource (Sundram et al., 2003). The edible palm hearts (EPH) or known as palmito, chonta or swamp cabbage in America countries, or "*umbut*" in Malaysia is a type of vegetable harvested from palm tree species (Donoghue, 2005). These edible cores from the palm tree stems appeared firm and smooth and is described to have a flavour resembling the artichoke. However, only nutritional analysis of EPH from apong (*Nipa fruticans*) and nibong (*Oncosperma tigillaria*) palm from Sarawak, Malaysia has been reported (Hoe and Siong, 1999). To date, many type of researches revealed the presence of prebiotic carbohydrates in some food crops specifically vegetables, tuber crops and some fruits crops (Dwivedi et al., 2014; Fonteles and Rodrigues, 2018). Realizing the diverse pharmacology properties of locally available vegetables and fruits, it brings us to the research question of EPH, whereby information on the prebiotic potential of EPH and their effect towards the human health is still lacking. To fill this gap, this study can provide information on the proximate compositions of EPH cultivated in Sarawak, notably the oil palm (*Elaeis guineensis*), sago palm (*Metroxylon sagu*) and coconut (*Cocos nucifera*). Subsequently, the stability of palm hearts in the simulated saliva, gastric and intestinal conditions, as well as the effect of EPH towards the major colonic bacterial population using *in vitro* colonic fermentation study. Therefore, this study may contribute to exploit the available source of prebiotic plants.

MATERIALS AND METHODS

Preparation of plant materials

Hearts of oil palm (*Elaeis guineensis*), sago palm (*Metroxylon sagu*) and coconut (*Cocos nucifera*) were obtained from local markets in Sarawak, Malaysia. Samples were blended and freeze-dried for further analysis.

Proximate analysis

Crude protein content, fat content, ash content, fibre content and carbohydrate content were determined according to AOAC method by Horwitz & Latimer (2005). All analyses were carried out in triplicate, and values were expressed as percentages on a dry basis (% DW).

In vitro gastrointestinal digestion

In vitro gastrointestinal digestion was performed as described in the simulation study using α-amylase for oral phase; pepsin for gastric phase; pancreatin and bile salt for intestinal phase digestion (Lee-Ling et al., 2022).

In vitro fermentation

In vitro fermentation was conducted as described by (Rawi et al., 2021) using basal nutrient media, human faecal slurry (10%), and test substrates (1%) under anaerobic condition at 37°C. Sampling was carried out at 0, 6, 12 and 24 hours. Inulin was used as a positive control.

Microbial analysis

Enumeration of bacterial cells (*Bifidobacterium spp.*, *Lactobacillus/Enterococcus* and *Clostridium histolyticum* group) were performed by using 16S rRNA targeted oligonucleotide probes labelled with the fluorescent Cy3 dye (Bajury et al., 2017).

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) version 9.4. Tukey's Multiple Range Test ($p \leq 0.05$) was used to perform multiple comparisons between the means.

RESULTS AND DISCUSSION

Proximate composition analysis

The proximate compositions of EPH in Figure 1 showed significant differences in their nutritional contents. It is significantly revealed that all EPH contains abundant amount of carbohydrate. This is justified in studies that reported most of the palm trees are considered as carbohydrates-producing crops. Thus, carbohydrates are the most abundant organic compounds found in plants of *Arecaceae* family (Brou et al., 2018). Among all the EPH, the sago palm hearts has showed the highest carbohydrates content at 66.81%. This finding is plausible with the technological purposes of indigenous sago tree in the process of sago starch extraction (Tabora et al., 1993).

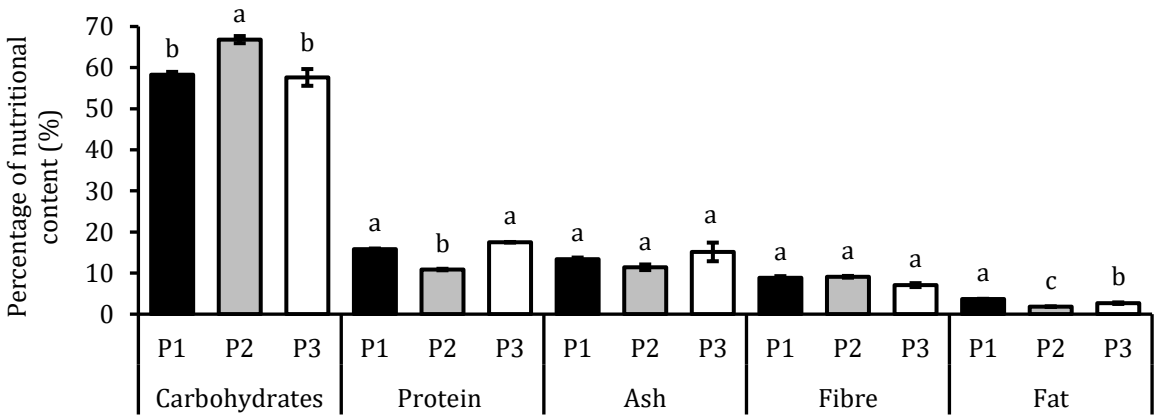


Figure 1. Proximate composition of edible palm hearts (P1 = oil palm heart, P2 = sago palm heart, and P3 = coconut palm heart).

Microbial analysis

Figure 2 showed the population changes in *Bifidobacteria, Lactobacilli,* and *Clostrodium histolyticum group* during fermentation of EPH from oil palm, sago and coconut. Results showed a significant increase of *Bifidobacterium spp.* and *Lactobacillus-Enterococcus* group population after 6 h fermentation in all samples, except for the negative control. This is due to the presence of fermentable carbohydrates, such as dietary fibres or non-digestible sugars in EPH and inulin (positive control) that supports the growth and metabolic activity (Hogg, 2013).

Surprisingly, a significant decrease of *Clostridium histolyticum* group was observed as well. This may be related to the increasing population of the probiotic that suppress the growth of

Clostridium spp. Also, the contribution of anti-microbial metabolites (i.e. SCFA) produced from the fermentation by probiotic, which inhibit the growth of certain pathogen like *Clostridium histolyticum* group (Rawi et al., 2020).

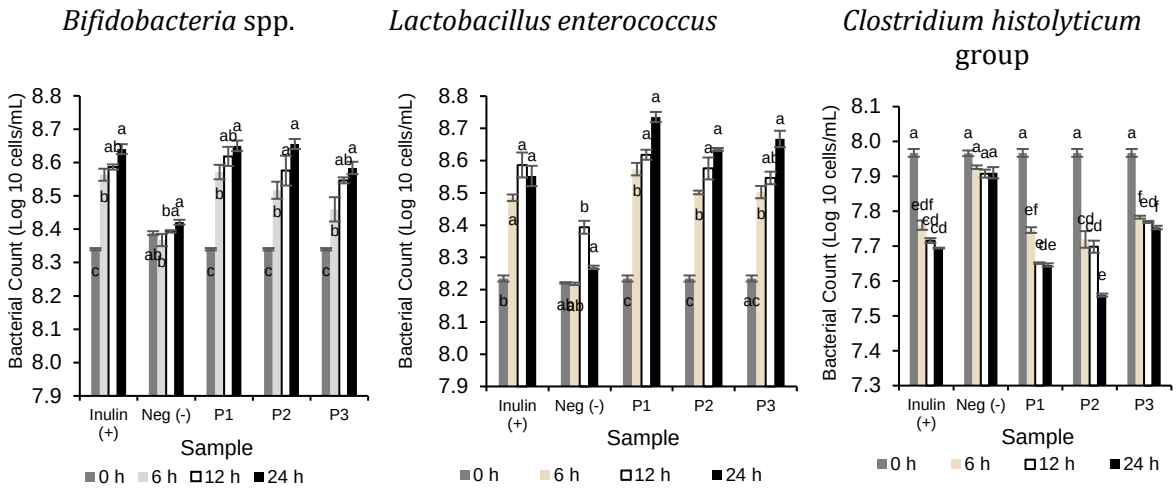


Figure 2. Population changes of *Bifidobacteria, Lactobacilli,* and *Clostrodium histolyticum* group during fermentation of EPH from oil palm, sago and coconut. Superscript letters shows significantly higher/lower population (log10 cells/mL) in comparing among samples in the same fermentation period (t) at a confidence level of 95%. * Means that shows significant difference when compared with 0 hour within the same sample at a confidence level of 95%. (P1 = oil palm heart, P2 = sago palm heart, P3 = coconut palm heart, and Neg = negative control).

CONCLUSION

Edible palm hearts (EPH) from oil palm, sago, and coconut revealed a positive prebiotic response. Particularly, by promoting probiotic growth which may contribute to the production of SCFA during colonic fermentation.

ACKNOWLEDGEMENT

The authors would like to thank the support from Fundamental Research Grant Scheme (FRGS/1/2019/WAB01/UPM/2/19) funded by the Malaysia Ministry of Education and Department of Veterinary Services Malaysia (ST-2016-013).

REFERENCES

Bajury, D.M., Rawi, M.H., Sazali, I.H., Abdullah, A. and Sarbini, S.R. 2017. Prebiotic evaluation of red seaweed (*Kappaphycus alvarezii*) using *in vitro* colon model. *International Journal of Food Science and Nutrition, 68*: 821–828.

Brou, R.M., Ekissi, G.S.E., Faulet, B.M., Fagbohoun, B.J. and Kouamé, P.L. 2018. Impacts of boiling times on physicochemical and nutritive composition from heart of oil palm tree (*Elaeis guineensis* Jacq.) *Advance Research,* 1-16.

Donoghue, M.J. 2005. Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. *Paleobiology*, *31*: 77–93.

Dwivedi, S., Sahrawat, K., Puppala, N. and Ortiz, R. 2014. Plant prebiotics and human health: Biotechnology to breed prebiotic-rich nutritious food crops. *Electronic Journal of Biotechnology*, *17*: 238–245.

Fonteles, T.V. and Rodrigues, S. 2018. Prebiotic in fruit juice: processing challenges, advances, and perspectives. *Current Opinion in Food Science, 22:* 55–61.

Hoe, V.B. and Siong, K.H. 1999. The nutritional value of indigenous fruits and vegetables in Sarawak. *Asia Pacific Journal of Clinical Nutrition, 8:* 24–31.

Lee-Ling, C., Hui Yan, T., Saupi, N., Saari, N. and Sarbini, S.R., 2022. An in vitro study: Prebiotic Effects of Edible Palm Hearts in Batch Human Fecal Fermentation System. *Journal of the Science of Food and Agriculture*, [Online].

Rawi, M.H., Abdullah, A., Ismail, A. and Sarbini, S.R.2021. Manipulation of gut microbiota using *Acacia* gum polysaccharide. *ACS Omega*, *6:* 17782–17797.

Rawi, M.H., Zaman, S.A., Pa'ee, K.F., Leong, S.S. and Sarbini, S.R. 2020. Prebiotics metabolism by gut-isolated probiotics. *Journal of Food Science and Technology, 57*: 2786–2799.

Sundram, K., Sambanthamurthi, R. and Tan, Y.A. 2003. Palm fruit chemistry and nutrition. *Asia Pacific Journal of Clinical Nutrition, 12.*

The Checklists of Insects Associated with The Sarawak Indigenous Eggplant, Terung Asam (*Solanum lasiocarpum* Dunal.)

Muhamad Azmi Mohammed[1]*, Ameyra Aman-Zuki[1], Nurul Imani Che Pa[1], Abang Abdul Rahim Ossen[1], and Salmah Yaakop[2]

[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia

[2]Centre for Insect Systematics, Department of Biological Science and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

**Corresponding author's email: mm_azmi@upm.edu.my*

INTRODUCTION

Solanum lasiocarpum L. (synonym *Solanum ferox*) or Terung Asam, Terung Dayak (Dayak Brinjal), Indian Nightshade, is a species in the Solanaceae family. This specie is known to be origin from Southeast Asia and South Asia (Voon and Kueh, 1999) and was registered under Geographical Indications (GI) certification in 2011 as Terung Asam Sarawak (GI No. GI2010-00002). A Terung Asam plant can produce fresh fruit of 2.6 kg/plant with a yield of 10.4 fruits/plant, and a hectare of Terung Asam can yield 16-26 mt/ha (Umar, 2013). However, the lack of study on insects associated with *S. lasiocarpum* carries a gap between insect pests and beneficial insects for this crop. Not knowing the identity of the insect pests can bring wrong assessment in the pest management for *S. lasiocarpum*. Therefore, this study aims to determine the associated insects with *S. lasiocarpum* or Terung Asam. Secondly, to identify insect pests and beneficial insects for this economically significant indigenous species.

MATERIALS AND METHODS

The sampling was done at an *S. lasiocarpum* planting site at Universiti Putra Malaysia (UPM) Bintulu Sarawak Campus (20 plants in a row with 1 m and 1.5 m spacing within and between rows). Two methods of insect sampling were utilized, which were active and passive sampling. The active sampling was conducted using a sweep net and manually hand-picked at five randomly different spots with a 1 m distance. Passive sampling was conducted by using yellow pan traps and a Malaise trap. All sampling efforts were replicated three times, starting from transplanting the *S. lasiocarpum* until the fruiting phase. The collected insect samples were stored in 70% ethanol before being brought to the laboratory for further analysis. Collected insect samples were sorted into morphospecies before the identification process. The identification process was aided using Stereo Microscopes Leica EZ4 and Leica Zoom 2000. Samples identification approach was followed as conducted by Choate (2011) and Triplehorn and Johnson (2005). The identified specimens were photographed by a digital camera and later stored in Entomology Laboratory for repository and future reference.

RESULTS AND DISCUSSION

Species abundance and composition

A total of 242 individuals of insect samples with 30 morphospecies and 22 families were collected at the studied *S. lasiocarpum* cultivation. The identified specimens with the number of individuals are listed in Figure 1. Seven insect orders were collected, i.e., Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea and Orthoptera. Order Diptera has the highest number of individuals collected (76 individuals= 31.4%), while Hymenoptera has the highest number of morphospecies collected (10 morphospecies= 33.3%). *Henosepilachna kaszabi* Bielawski and Fürsch (1960) holds the highest number of individuals collected (60 individuals = 24.7%).

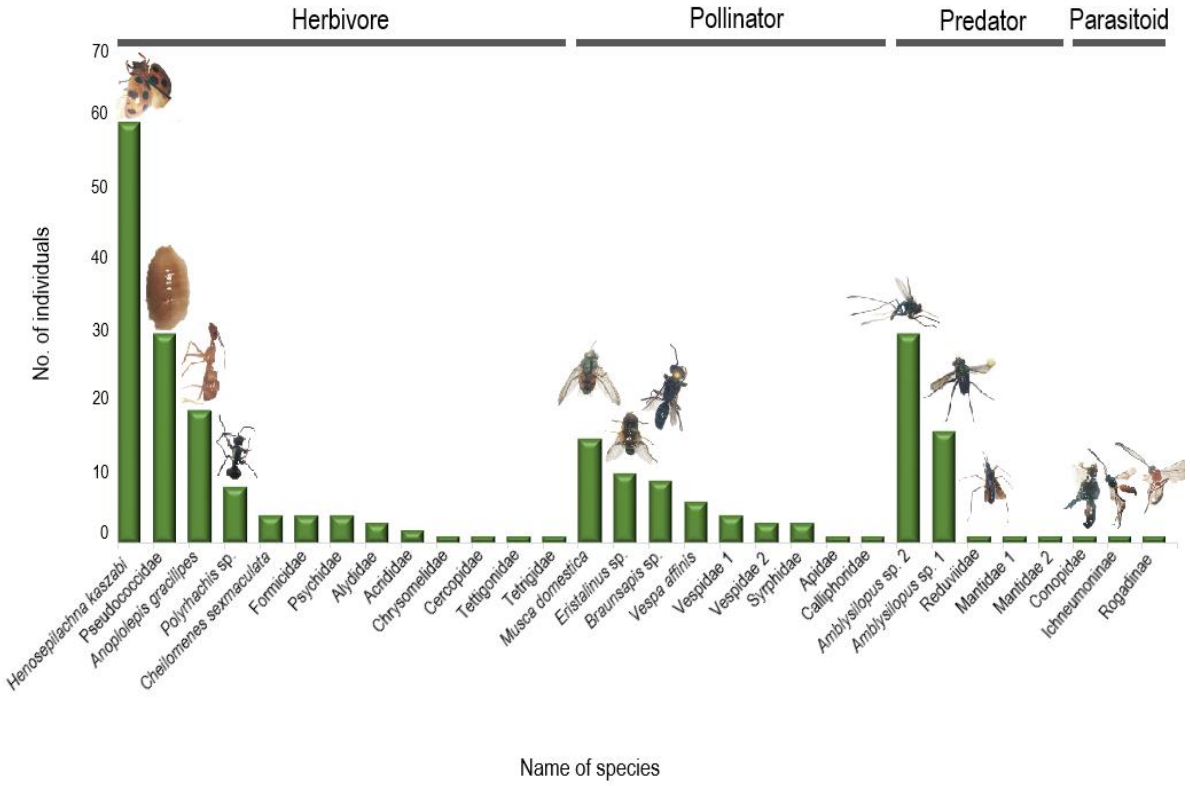


Figure 1. Number of individuals insects collected with the status of each individual.

Insect species with the various growth phase in *Solanum lasiocarpum* cultivation

Three growth phases of *S. lasiocarpum* are the vegetative, flowering and fruiting (Table 1). The list of insects collected from each growth phase is different, and this result may show the function of each insect gathered. Table 1 shows the insect species collected during three phases of the *S. lasiocarpum* plantation. Twenty-six (87%) and 17 morphospecies (57%) were recorded during the flowering and fruiting phases of *S. lasiocarpum*, respectively. *Amblysilopus* sp. 1 and sp. 2 were recorded in all *S. lasiocarpum* three growth phases.

Status of insect at *Solanum lasiocarpum* cultivation

The collected insects were divided into four bio-ecological functions: herbivore, predator, pollinator and parasitoid, as shown in Figure 1. Based on the results, we assumed two herbivore insects in this study are primary pests of *S. lasiocarpum*, i.e., ladybug *Henosepilachna kaszabi* and mealybug Pseudococcidae. Both insect species were found to infest *S. lasiocarpum* during the flowering and fruiting phases of the crop (Table 1). The larvae and adults of *H. kaszabi* infest *S. lasiocarpum* leaves from the softer part into the midrib (Figure 2). The feeding of *H. kaszabi* causes consequential damage to the leaves and affects the productivity of the crop. Larvae of *H. kaszabi* were also found boring and causing holes at the root and stem of the plant. Halim et al. (2017) found that *H. kaszabi* was infested on Solanaceae crops such as eggplants and sponge gourds.

Table 1. List of insects associated with three growing phases of *Solanum lasiocarpum.*

Order	**Family**	***S. lasiocarpum* Growing Phase**		
		Vegetative	Flowering	Fruiting
Coleoptera	*Henosepilachna kaszabi*		√	√
	Cheilomenes sexmaculata		√	√
	Chrysomelidae		√	
Diptera	Calliphoridae	√		
	Conopidae		√	
	Amblypsilopus sp. 1	√	√	√
	Amblypsilopus sp. 2	√	√	√
	Musca domestica		√	√
	Eristalinus sp.		√	√
	Syrphidae		√	
Hemiptera	Alydidae			√
	Cercopidae		√	
	Pseudococcidae		√	√
	Reduviidae			√
Hymenoptera	Apidae		√	
	Braunsapis sp.		√	
	Polyrhachis sp.	√	√	
	Anoplolepis gracilipes	√	√	√
	Formicidae	√	√	
	Ichneumoninae		√	
	Rogadinae		√	
	Vespa affinis		√	√
	Vespidae 1		√	√
	Vespidae 2		√	√
Lepidoptera	Psychidae		√	
Mantodea	Mantidae 1		√	√
	Mantidae 2		√	√
Orthoptera	Acrididae		√	√
	Tettigoniidae		√	√
	Tetrigidae	√		

Figure 2. Larva (a) and adult (b) of *Henosepilachna kaszabi* infesting the leaves of *Solanum lasiocarpum*.

CONCLUSION

Despite *S. lasiocarpum* or Terung Asam as a renowned crop in Sarawak, studies on this indigenous crop are still limited and lacking. This study draws the list of associated insects and their possible function or niche towards the plant. Through this study, we can conclude that *Henosepilachna kaszabi* and mealybugs are significant pests for *S. lasiocarpum*. Other insect collected species can be classified as pollinators, beneficial predators, and visiting insects at the cultivations. In the future, more studies should be conducted to confirm the insect's function and provide data on the insect pests and beneficial insects towards the crop for efficiently controlling the insect pests of *S. lasiocarpum*.

ACKNOWLEDGEMENT

This research was funded by research grant GPIPM/2019/9681200.

REFERENCES

Voon, B.H. and Kueh, H.S. 1999. The nutritional value of indigenous fruits and vegetables in Sarawak. *Asia Pacific Journal of Clinical Nutrition, 8*: 24-31.

Umar, S. 2013. *Terung Asam Sarawak, a geographical indications (GI)-Registered Product of Sarawak.* Agriculture Research Centre, Semongok. Sarawak Department of Agriculture.

Choate, P.M. 2011. *Introduction To The Identification of Insects And Related Arthropods*. Florida University, Florida, USA.

Triplehorn, C.A. and Johnson, N.F., 2005. *Borror and DeLong's Introduction to the Study of Insects'*. Thompson Brooks: Belmont, CA, USA.

Halim, M., Aman-Zuki, A., Mohammed, M.A. and Yaakop, S. 2017. DNA barcoding and relationships of eight ladybugs species (Coleoptera: Coccinellidae) that infesting several crops from Peninsular Malaysia. *Journal of Asia-Pacific Entomology*, *20*(3): 814-820.

Development of Micropropagation Protocol in Borneo Sour Eggplant, *Solanum lasiocarpum* Dunal. for Multiplication

Eldred Anak Embu[1], Jessica Jeyanthi James Antony[1,2,*], Noorasmah Saupi[1,2], Uma Rani Sinniah[3]

[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, P.O. Box 396, 97008 Bintulu, Sarawak, Malaysia
[2]Institut of Ekosains Borneo, Universiti Putra Malaysia Bintulu Sarawak Campus, P.O. Box 396, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia
[3]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor, Malaysia

**Corresponding author's email: jessica@upm.edu.my*

INTRODUCTION

Solanum lasiocarpum Dunal is the Solanaceae family's largest and most varied genus. There are over 1,400 species in the genus, with nearly a third belonging to the subgenus *Leptostemonum*. This subgenus includes economically important species and is usually known as the spiny solanums (Cadavid et al., 2022). Plant tissue culture techniques are well known for their application in maintaining or growing plant cells, tissues, or organs under sterile circumstances (Naik and Ravali, 2016). Tissue culture allows for the rapid growth of a high-quality, disease-free stock of planting materials (Suman, 2017).Furthermore, plant tissue culture has a significant impact on agriculture and industry by providing crops to meet the world's ever-increasing need. These have recently made substantial contributions to the improvement of agricultural science, and they are now an indispensable tool in modern agriculture (Hussain et al., 2012). Hence, this study was carried out to determine optimal clorox concentration, nodal segment size, sucrose and media strength for the development of multiple plantlets in *Solanum lasiocarpum* Dunal.

MATERIALS AND METHODS

Plant material

Solanum lasiocarpum Dunal was obtained at the yellow ripe stage from the Pasar Tamu, Bintulu, Sarawak, Malaysia. The seeds were extracted from the fruits and were placed in beaker and washed properly using tap water, followed by washing using Dettol and Tween 20 for 20 min and rinsed thoroughly using tap water thereafter. Seeds were germinated in vitro for the selection of uniform and diseases free seedlings and contamination percentage was taken in 3 weeks. The optimal Clorox concentration was used in following experiment.

Sterilization using different concentration of Clorox

The seeds were sterilized with different concentrations of Clorox (50, 60, 70, 80, 90 and 100%) for 20 min to prevent contamination (Foo et al., 2018). Next, the seeds were rinsed three times with sterile distilled water. Then, the seeds were cultured in ¼ strength Murashige and Skoog medium supplemented with 20 g/L sucrose, 1 mg/L 6-benzylaminopurine (BAP) and 0.2 mg/L of indole-3-acetic acid (IAA). The cultures were kept in light for 16 hours at a brightness of 1000 lux. The culture room temperature was kept at 25±2 °C for 3 weeks using white cool fluorescent lamps. The experiment included 6 replicates per treatment, each with 10 explant samples. The observations were recorded depending on the percentage of contamination within 3 weeks.

Optimization of explant sizes

Shoot buds were obtained respectively from 3-week-old seedlings. Shoot bud explants with sizes of (2 mm and 5 mm) were excised using sterile scalpel on sterile filter paper. Then, explants were cultured on ¼ strength MS medium supplemented with 20 g/L sucrose, 1 mg/L BAP and 0.02 mg/L of IAA. Within 3 weeks, observations for the percentage of shoot formation, the number of shoots, the size of shoots, and the number of leaves were evaluated as well as the next experiment.

Optimization of sucrose concentration

Nodule segments from germinated seedlings were cultured on ¼ MS media supplemented with 0, 10, 20, 30, 40, 50 g/L sucrose and 1mg/L BAP + 0.2 mg/L IAA and will be incubated for 3 weeks at 25±1ºC under 16 hours photoperiod.

Optimization of the media strength types

Sterilized shoot buds (obtained from germinated seeds) were cultured on MS medium (Murashige and Skoog, 1962) with 5 types of salt concentrations ranging from ¼, ½, ¾, 1 and 2 MS strength in each of the media supplemented with 20 g/L sucrose.

Statistical analysis

All the data was analysed by one-way variance analysis (ANOVA). Significant differences ($p<0.05$) between treatment methods were tested using the Duncan comparison test at a 5% probability test and statistical analysis was performed using the Statistical Analysis System (SAS) computer package through the Analysis of Variance (ANOVA) procedure.

RESULTS AND DISCUSSION

Effect of explant size

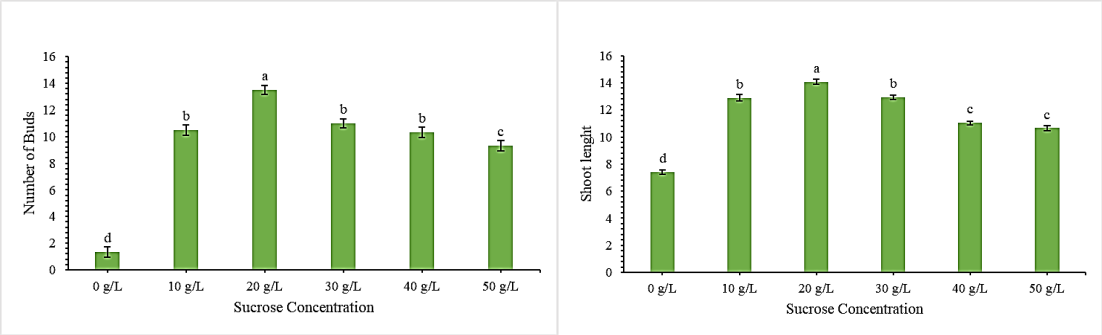
The explant with the size of 5 mm resulted in the highest number of buds (10.66±0.56), number of leaves (19.83±0.89) and shoot length (15.07±0.57 mm) compared to the 2 mm explants size tested after 3 weeks of incubation in the culture room (Table 1). The efficiency of in vitro *Solanum* sp. plantlet production does not only depend on the size of explants used but can also be affected by the combinations of plant growth regulators used (Foo et al., 2018). Therefore, these findings suggested the essential roles of plant growth regulators. Different explant sizes and combinations of hormones would result in significant differences in regeneration processes.

Table 1. Number of buds, leaves, and bud size of *Solanum lasiocarpum* Dunal plantlet in relation to different explant sizes (2 mm and 5 mm) after 3 weeks of culturing on ¼ MS medium strength with 1 mg/L BAP + 0.2 mg/L IAA.

Explant size	Number of buds	Number of leaves	Shoot length (mm)
2 mm	6.33±1.50[b]	10.33±3.29[b]	9.60±1.47[b]
5 mm	10.66±0.56[a]	18.50±1.43[a]	15.07±0.57[a]

Effect of sucrose concentration

The MS medium supplemented with 20 g/L sucrose produced significant highest number of buds (13.50±0.31), number of leaves (14.17±0.37) and shoot length (14.07±0.33 mm) compared to other treatment tested (Figure 1). Sucrose is a very important component in nutrient medium used in plant cell, tissue, and organ culture. The sucrose acts as carbon source and its addition are vital for ensuring optimal development of explant (Zahara et al., 2017). In this study, the MS medium supplemented with 20 g/L sucrose obtained optimal shoot formation in *Solanum*.

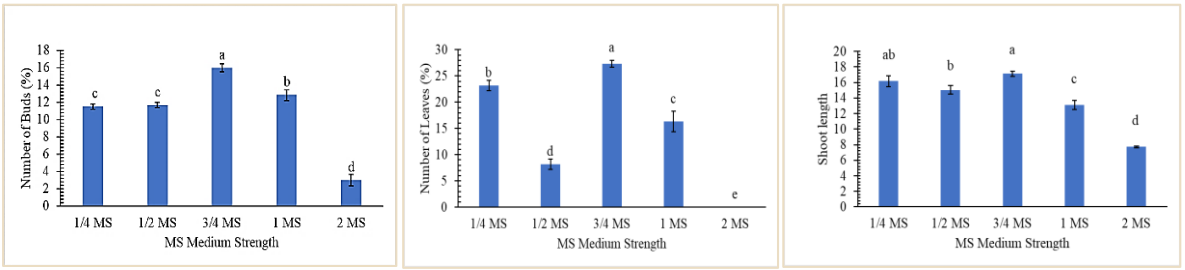


(a) (b)

Figure 1. The effects of difference sucrose concentration on regeneration of *Solanum lasiocarpum* Dunal after 3 weeks of incubation, (a) The number of buds percentage and (b) shoot length.

Effects of MS media strength

Optimization of media strength revealed a significant number of buds (16.00±0.47), number of leaves (27.33±0.33) and shoot length (10.02±0.15 mm), were achieved when nodal segment (optimized explant size: 5 mm) was cultured on ¾ MS media compared to all other treatments tested following 3 weeks of incubation in the culture room (Figure 2). The findings are consistent with those of Yesmin et al. (2018) who reported that using ½ and 1MS strength resulted in the highest number of shoots in *Solanum melongena* L. In this study, nodal segments cultured on ¾ MS media strength obtained optimal shoot formation in *Solanum lasiocarpum* Dunal.



(a) (b) (c)

Figure 2. The effects of difference MS medium strength on regeneration of *Solanum lasiocarpum* Dunal after 3 weeks of incubation, (a) The number of buds percentage (b) number of leaves and (c) Shoot length.

CONCLUSION

The results concluded that the optimal size of 5 mm nodal segments produced highest number of plantlets for in vitro plantlets in *Solanum lasiocarpum* Dunal. The most effective MS medium components for enhancing the development and growth of nodal segment explants were ¾ MS medium and the effective sucrose concentration were supplemented with 20 g/L in MS medium.

ACKNOWLEDGMENT

I would like to acknowledge the IPM Research Grant (UPM) for the Science Fund project of "Establishment of Tissue Culture Protocol for Borneo Sour Eggplant, *Solanum Lasiocarpum* Dunal. Through Optimization of Plant Growth Regulator for Multiplication Rooting and Hardening". We would like to thank the Institut of Ekosains Borneo (IEB) and Universiti Putra Malaysia (UPM) for their support.

REFERENCES

Cadavid, I.C., Giraldo, C.E., Balbinott, N., González, M.A., Uribe, S. I. and de Boer, H.J. 2022. Molecular identification of the economically important *Solanum* subgenus *Leptostemonum* (Solanaceae) using DNA barcodes. *Journal of Plant Biochemistry and Biotechnology*, 1-15.

Foo, P.C., Lee, Z.H., Ching, C.K., Subramaniam, S. and Chew, B.L. 2018. Shoot Induction in White Eggplant (*Solanum melongena* L. Cv. Bulat putih) using 6- Benzylaminopurine and Kinetin, *Tropical Life Sciences Research, 29*(2): 119-129.

Naik, M.L. and Ravali, B. 2016. Plant Regeneration in Eggplant (*Solanum melongena* L.). *International Journal of Plant, Animal and Environmental Sciences*, *6*: 121-126.

Suman, S. 2017. Plant tissue culture: A promising tool of quality material production with special reference to micropropagation of banana. *Biochemical and Cellular Archives*, *17*(1): 1-26.

Yesmin, S., Khatun, M.M., Tanny, T., Protity, A.T., salimullah, I.A. and Alam, I. 2018. Regular article in vitro regeneration of two high-yielding eggplant (*Solanum melongena* L.) varieties of bangladesh. *Current Botany, 9*: 8-12.

Zahara, M., Datta, A., Boonkorkaew, P. and Mishra, A. 2017. The Effects of Different Media, Sucrose Concentrations and Natural Additives on Plantlet Growth of Phalaenopsis Hybrid "Pink". Brazilian Archives of Biology and Technology*, 60.*

Evaluation of Plant-Based Food Wrappers by Communities in Bintulu, Sarawak

Noorasmah Saupi*, Samantha Natalie Dandi, Nurul Aisyah Yusli and Shiamala Devi Ramaiya
Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia

**Corresponding author: noorasmah@upm.edu.my*

INTRODUCTION

Food packaging was developed from natural ingredients around 10,000 years ago, and it was made using animal skins, tree stems, and leaves (Mhd Nor et al., 2018). Hence, there has been minimal need for packing of products, either for transportation or storage. Mustafa et al. (2012) state that food wrappers were required because of social developments and human transporting things in big quantities and often in further locations. Present, demand for food wrappers has come as the need for storage, sampling, selling, transportation, and food preservation. Wrapping or packaging can be defined as an object which protects, secures, and provides a specific image of the product contained within it (Mudra, 2004). Food wrappers are usually used to preserve, maintain the nutrients, and prolong the shelf life of the food (Ojekale *et al.* 2007). For instance, banana leaves can reduce food leakage and spoilage by forming closures (Luna and Recote 2021). Sari et al. (2019) state that food wrappers are used to extend the shelf life of food and provide mechanical protection from any chemical or biology contaminants. Astuti (2009) reported that the utilization of natural materials such as leaves as a food wrapper provide positive impacts to the environment and consumers because the material does not contain any hazardous ingredients or toxic chemicals, and it is also easy to be find, easy to be fold and enhance aroma to the food. The older generation utilized various sorts of leaves as a traditional method of food packaging (Ismail et al., 2021). Dosumu et al. (2014) stated that wrapping techniques vary depending on geographical areas, culture, industry level, urbanization, status of economic, manufacturer's desired impact, and the end user's choice. By comparing Sarawak and Peninsular Malaysia, it is found that there are similarities in food wrapping . However, there are slight changes that can be found in terms of their materials and methods used to process the food wrappers (Sahari et al., 2017). Subet and Hassan (2014) noted that the relevance of the material, culture is different, depending on the area or division of Sarawak. For instance, the Saribas Malay uses "nipah" leaves to make kelupis while the Northern part of Sarawak uses "daun nyirik" to make kelupis (Sahari et al., 2017).

The difficulties to obtain the plant materials were also considered as the constrain for the locals to use them as food wrappers. Also, there are limited information about the usage of the plant-based food wrappers in other parts of Sarawak. It is known that different part of Sarawak had different types of plant that used as wrapper for the same dish. Furthermore, there are little commercialized plant-based food wrappers reported in Sarawak due to the preferences to modern packaging such as the polystyrene box and paper wrappers. Hence, more information is needed to be exposed on the benefits of the plant-based food wrappers to the communities. Thus, the objectives of this study are to records the plant species that is used as food wrappers by the communities in Bintulu, Sarawak and to evaluates the application technique of the plant based-food wrappers.

MATERIALS AND METHODS

The study was conducted using the questionnaire research approach and the interview by using the semi-structure method. This survey was conducted in Bintulu that can be separated by three

districts which is Bintulu town, Tatau and Sebauh starting from December 2021 until April 2022. The sample size was 383 respondents based on Krejcie and Morgan (1970) formula.

The questionnaire is made into four sections where it focused on the respondent personal information, basic information and details about food wrapper, and the potential of the plants as food wrapper. In the section A, it focused on the respondent personal information such as the gender, location, race, age, and education level (Saupi et al., 2020). In section B, it focused on the basic information about food wrapper including about the plant that is used and the details of the plants such as the texture, cycle, and the habitat of the plants, the ability to enhance flavor, method to prepare the plant before using it as food wrappers, the folding shape of the wrappers and the consistency of the food that is going to be wrapped. Meanwhile, section C consists of the potential of the plant as food wrapper. The questions were mostly about the ability of the plants to be commercialize, to determine whether the plants are an indigenous Sarawak plant, the availability of the plants, and the suggestion from the respondents on how to enhance the usage of the plants among the community. The data were computed while the frequencies and descriptive analysis were obtained using Statistical Package for the Social Sciences version 23.0. The descriptive analysis was used to determine the mean score, median and the standard deviation of the respondents.

RESULTS AND DISCUSSION

This study had been participated by various ethnicities where the Iban ethnic has the highest percentage of 54.4%. The study was majorly participated by females (72.7%). The respondents with age group of mostly in the range of 45-54 years old constitute of 29.7%. In terms of the living area of the populations, there are 47.1% respondents are from the sub city. The occupation of the respondents is mostly sellers which is 54%. There are many respondents from the sub city due to the availability and purchasing rate of the plants species in the market (Saupi et al., 2020). According to previous study by Bhattarai et al. (2015) many wild plants were marketed commercially which is of benefit to both the local economy

In this study, 21 species of plants from 11 plant families were documented as food wrapper by the communities in Bintulu, Sarawak as shown in Table1. The plant species for food wrapper mostly come from the family of Arecaceae. Sari et al. (2017) mentioned that Arecaceae was regarded as one of the most essential plant families for human existence. The plant emits aroma of sweet, bland, pandan-like, umami, fresh, and vanilla-like aromas, and flavors. Nine species emit bland flavors, six transmit sweet flavors, three emit fresh flavors, and one emits pandan, vanilla, and umami flavors respectively. Based on previous study done by Rini et al. (2017), food that is wrapped with leaves usually will emit a pleasant aroma and will have a specific taste. Lascurain et al. (2018) studied that depending on the plant species, certain leaves have a different flavor. In terms of food consistency, all the plant species are capable to wrap solid food while only a few species which are *Musa spp., N. fruticans*, *P. amaryllifolius* and *S. brachycladum* that can wrap semi solid food and liquid food. As for the type of the food, various type of food can be wrapped with food wrappers such as the “kelupis”, “ketupat”, “bak chang”, “nasi lemak” and “tapai”. The shelf lives of the food that used the plant-based wrapper ranges from day 1 to 1 week.

There are three types of wrapping surfaces which are smooth, rough, and hairy. Only one species of plant has a rough and hairy surface respectively, compared to 20 plants with smooth surfaces. For certain species, the plant parts need to be discarded prior to the wrapping process to facilitate the folding purpose. As for example, the tip and end portion of *L. grandis* need to be discarded, whereas *A. catechu* that has an extremely tough outer layer should be removed before been used. Each plant has its different usable stage to be used as wrappers. 20 species were used during its matured stage while only species were preferred to be used when it is old.

When a plant is harvested, it must go through several methods before it can be used as a wrapper for any of the food. There are seven preparation methods that have been recorded drying, soaking then washing, soaking then wiping, washing, wiping, wiping then oiling or preheating, and wiping then preheating. Fourteen plant species only required wiping before using while two species need to be soaked before wiping. For the other preparation methods, there are only one specie for each method respectively. In some food wrapping, additional materials were required to ensure that the food would not slip out easily from the wrapping material. Based on the survey, there are various types of additional materials used for wrapping such as bamboo skewer, banana leaves, rope, and stapler. However, 14 plant species did not require any additional materials to wrap the food. There were various folding shapes in wrapping food that been recorded in this study. Fourteen plant species used the closed wrap method while two species did not require any type of folding method and one species respectively for the other folding shapes such as the elongated cone, flat wrap, roll or woven, woven, and woven or closed wrapped. 18 plant species were used to wrap the food before cooking while, two for half cooked food and one act for storage purpose only.

There are various plant species have been recorded from the respondents as plant-based food wrappers and all this plant had great potential as food wrapper in Sarawak. 71.4% of the respondents stated that the plant that been used for wrapping food were not the indigenous plant of Sarawak. While 8.9% agreed that the plant species are indigenous. More than half of the respondents agreed that the plant species for food wrapper are available around Sarawak. In addition, 37.8% of respondents claimed that the plant species has the potential to become iconic plant-based food wrapper for Sarawak. 74.7% of respondents agreed that the plant-based food wrapper should be introduced to non-Sarawakian. Several type of the plant can be grown in a garden, but most of it must be harvested from the wild. 83.3% of respondents said that the plant can be cultivated.

The plant-based food wrapper had the ability to be a good economic prospect to the communities. The utilization of the plant-based food wrapper was successful in satisfying 98.70 % of respondents. More than 90% respondents agree that the usage of the plant-based food wrapper can help the local economy. More than half of the respondents said that the plant-based food wrappers need to be processed into ready-made items. Therefore, when it comes to the production of ready-made products, 97.7 % claimed that it will have a positive response from the communities. Besides that, 90.9% respondents agree that the usage of plant-based food wrapper should be widely commercialized while 9.1% of respondents are unsure whether the food wrapper should be widely commercialized or not. Some of plant-based food wrappers are currently in the market for purchase. There are ten species of the plants that are utilized for commercial purposes, nine for personal use, and two for which it is unsure about the usage. Besides that, nine of the plant species are available in market while ten are unavailable. Lin *et al.* (2019) stated that the usage of plant-based food wrappers is beneficial as they are inexpensive, of high quality, and conveniently accessible. The usage of the plant-based food wrapper will also help the local economy. According to Ayodeji *et al.* (2016), the food wrappers *Thaumatococcus daniellii* have contribute in terms of economy especially for the rural people at the Southern Nigeria.

CONCLUSION

In this present study, a total of 21 plant species of plant-based food wrapper had been recorded in Bintulu, Sarawak. Besides that, various application technique which is the stages to handle the process of plant-based food wrapper had been evaluated. The findings for this evaluation can be a reference for future suggestions that are interested to study this plant-based food wrapper. Additionally, the nutrient content for the plant species should be considered for future research in order to know the exact physicochemical differences.

Table 1. The description of the plant species for food wrapper.

Species	Family	Common name	Habit	Availabilit	Plant part	Aroma produced	Food consistency	Type of the food	Shelf-life
Areca catechu L.	Arecaceae	Upih Pinang	Tree	Wild	Sheath	Bland	Solid	Glutinous rice	1 week
Cocos nucifera L.	Arecaceae	Daun Kelapa	Tree	Cultivated	Leaves	Bland	Solid	Tapai	2 days
Colocasia esculenta (L.) Schott	Araceae	Daun Keladi	Herb	Cultivated	Leaves	Bland	Solid	Grilled fish	1 day
Curcuma longa L.	Zingiberaceae	Daun Kunyit	Herb	Cultivated	Leaves	Bland	Solid	Ketupat	1 week
Dillenia suffruticosa (Griff.) Martelli	Dilleniaceae	Daun Simpur	Shrub	Wild	Leaves	Bland	Solid	Rice	1 day
Donax grandis (Miq.) Ridl.	Marantaceae	Daun Bemban	Tree	Wild	Leaves	Fresh	Solid	Rice	3 days
Elaeis guineensis Jacq.	Arecaceae	Daun Sawit	Tree	Cultivated	Leaves	Bland	Solid	Vegetables	3 days
Indocalamus tessellatus (Munro) Keng *f.*	Poaceae	Daun Buluh	Grass	Cultivated	Leaves	Umami	Solid	Bak Chang	1 week
Licuala bintuluensis Becc.	Arecaceae	Daun Silat	Tree	Wild	Leaves	Bland	Solid	Glutinous rice	3 days
Licuala grandis H.Wendl.	Arecaceae	Daun Palas	Tree	Wild	Leaves	Bland	Solid	Kelupis	3 days
Licuala petiolulata Becc.	Arecaceae	Daun Biru	Tree	Wild	Leaves	Sweet	Solid	Ketupat, kelupis	3 days
Licuala spp.	Arecaceae	Daun Iseng	Tree	Wild	Leaves	Fresh	Solid	Glutinous rice	1 week
Macaranga bancana (Miq.) Müll.Arg.	Euphorbiaceae	Daun Wonihan	Tree	Wild	Leaves	Sweet	Solid	Rice	3 days
Musa spp.	Musaceae	Daun Pisang	Herb	Cultivated	Leaves	Sweet	Solid and semi solid	Nasi lemak, grilled fish, pulut panggang, tepung pelita	1 day
Nepenthes ampullaria Jack	Nepenthaceae	Periuk Kera	Climber	Wild	Leaves	Sweet	Solid	Glutinous rice	2 days
Nypa fruticans Wurmb	Arecaceae	Daun Apong	Tree	Wild	Leaves	Fresh	Solid and semi solid	Celorot, rice, ketupat	1 day
Pandanus amaryllifolius Roxb.	Pandanaceae	Daun Pandan	Tree	Cultivated	Leaves	Bland	Solid	Kelupis	1 week
Phacelophrynium maximum K.Schum	Marantaceae	Daun Long	Herb	Wild	Leaves	Sweet	Solid and semi solid	Tepung pelita, ketupat, kuih tako	1 day
Phrynium pubinerve Blume	Marantaceae	Daun Lerek	Herb	Cultivated	Leaves	Sweet	Solid	Rice	1 week
Schizostachyum brachycladum (Kurz ex Munro)	Poaceae	Buluh	Grass	Cultivated	Diaphragm	Sweet	Solid and liquid	Lemang, pansuh	2 days
Terminalia catappa L.	Combretaceae	Daun Ketapang	Tree	Cultivated	Leaves	Sweet	Solid	Rice	7 days

REFERENCES

Astuti, N.P. 2009. Sifat Organoleptik Tempe Kedelai yang Dibungkus Plastik, Daun Pisang dan Daun Jati (*Doctoral dissertation, Universitas Muhammadiyah Surakarta*).

Ayodeji, O.I., Adeleye, O., Dada, O., Adeyemi, O. and Anyasor, G.N. 2016. Phytochemical constituent and antioxidant activity of *Thaumatococcus daniellii* Benn (Benth.) leaves (food wrapper). *International Journal of Pharmacology, Phytochemical and Ethnomedicine, 2*: 55-61.

Bhattarai, S., Chaudhary, R.P. and Taylor, R.S. 2009. Wild edible plants used by the people of Manang district, central Nepal. *Ecology of Food and Nutrition*, *48*(1): 1-20.

Dosumu, O.O. and Akinnuoye, G.A. 2014. Effect of steaming of beans pudding on the phytochemical composition of *Thaumatococcus Daniellii* Wrapper. *Nigerian Food Journal*, *32*(1): 110-116.

Ismail, N., Che Ishak, F.A., Arsyad, M.M., Karnjamapratum, S. and Sirison, J. 2021. The Malay's traditional sweet, dodol: a review of the Malaysia's heritage delicacy alongside with the rendition of neighbouring countries. *Journal of Ethnic Foods*, *8*(1): 1-13.

Lascurain, M., López-Binnqüist, C., Avendaño, S. and Covarrubias, M. 2017. The Plants Leaves Used to Wrap Tamales in the Mexican State of Veracruz. *Economic Botany*, *71*(4): 374-379.

Lin, F., Luo, B., Long, B. and Long, C. 2019. Plant leaves for wrapping zongzi in China: an ethnobotanical study. *Journal of Ethnobiology and Ethnomedicine*, *15*(1): 1-16.

Luna, M.B.Z. and Recote, J.M.Q. 2021. Knowledge and practices in the utilization of banana *(Musa* sp.) leaf as food contact material in the Metropolitan Manila foodservice industry. *Philippine Journal of Science*, *150*(5): 861-874.

Subet, M.F dan Hassan, S. 2014. Rahsia Kebahasaan Pantun Melayu Sarawak. *In Awang Azman Awang Pawi, Wajah Melayu Sarawak: Rampaian ilmu budaya* (pp 187-210).

Mhd Nor, O., Anwar Fikri, A. and Muhamad, A.G. 2018. Sustainable housing landscape concept as a source of wrapping materials for preserving culture activities in malay traditional food. *IDEALOGY*, *3*(2): 263-274.

Mustafa, M., Nagalingam, S., Tye, J., Shafii, A.H. and Dolah, J. 2012. Looking back to the past: revival of traditional food packaging. *In 2nd Regional Conference on Local Knowledge (KEARIFAN TEMPATAN). Jerejak Island, 1:* 1-17.

Ojekale, A.B., Makinde, S.C.O. and Osileye, O. 2007. Phytochemistry and anti-microbial evaluation of *Thaumatococcus danielli*, Benn.(Benth.) leaves.

Rini, R., Fakhrurrozi, Y. and Akbarini, D. 2017. pemanfaatan daun sebagai pembungkus makanan tradisional oleh masyarakat Bangka (studi kasus di Kecamatan Merawang). *EKOTONIA: Jurnal Penelitian Biologi, Botani, Zoologi dan Mikrobiologi*, *2*(1): 20-32.

Sahari, F., Hasan, R., Durin, A., Adruce, S A.Z. and Rahman, S.A. 2018. Traditional food wrapping and packaging: culture and identity of the Saribas Malay, Sarawak. *International Journal of Innovative Des Creativity Soc Sci*, *2*(1): 7.

Sari, Y., Afriyansyah, B. and Juairiah, L. 2019. Pemanfaatan Daun sebagai Bahan Pembungkus Makanan di Kabupaten Bangka Tengah. *EKOTONIA: Jurnal Penelitian Biologi, Botani, Zoologi dan Mikrobiologi*, *4*(2): 48-56.

Saupi, N., Saidin, A.A., Zakaria, M.H., Sarbini, S.R. and Yusli, N.A. 2020. An ethnobotanical study of indigenous leafy vegetables among local communities in Bintulu, Sarawak, Malaysia. *Borneo Journal of Resource Science and Technology*, *10*(2): 155-165.

Yusli, N.A., Saupi, N., Ramaiya, S.D. and Lirong, Y.A. 2021. An ethnobotanical study on indigenous food flavourings and aromatic enhancing plants used by the native communities of the central region of Sarawak. *Malaysian Applied Biology*, *50*(3): 105-115.

Exploration of Plant Growth-Promoting Endophytic Bacteria from The Roots of Native Plant *Uncaria borneensis*

Fauziah Abu Bakar[1,2], Muhammad Harith Iskandar Pakasa[1], Farah Eziana Sharaman[1], Wong Sie Chuong[3], and Philip Lepun[4]

[1]Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia

[2]Institut Ekosains Borneo (IEB), Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia

[3]Department of Science and Technology, Faculty of Humanities, Management and Science, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia

[4]Department of Forestry Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia

**Corresponding author: ab_fauziah@upm.edu.my*

INTRODUCTION

Endophytes are widely recognised inoculants for enhancing plant growth, and significant study on numerous plants from diverse parts, including stems, roots, leaves, and bark, has been undertaken (Dudeja et al., 2012). The endophyte bacterial community of native forest trees has gotten less attention than the rhizospheric bacterial community (Dutta and Thakur, 2017) and endophytic fungal community (Win et al., 2018).

Uncaria borneensis is a forestal tree native to Borneo, Malaya, Sumatera, and Thailand across tropical Asia's woods. The genus *Uncaria* has garnered considerable attention from researchers due to the enormous number of species thought to have therapeutic properties particularly the highly examined *Uncaria gambir* (Musdja et al., 2018 and Yunarto et al., 2021). Although the function of *Uncaria borneensis* is yet unclear, its wood is known to be utilised in the construction of medium and heavy structures, floors and musical instruments. Thus, the current study focuses on isolating and characterising the endophytic bacteria from *Uncaria Borneensis* roots and assesses their potential as plant growth-promoting bacteria.

MATERIALS AND METHODS

Isolation of endophytic bacteria

The healthy roots of *Uncaria borneensis* were collected in sterile sampling bags and brought to the laboratory. Each sample was washed and surface sterilised with ethanol and sodium hypochlorite. Surface sterile samples were marched in sterile distilled water and plated on nutrient agar. After 48 hours of incubation, colonies were chosen and streaked on Nutrient Agar to get pure culture.

Molecular identification of endophytic bacteria

The boiling method was used to extract the DNA from selected isolates. The 16S RNA gene and universal primers 27F and 1492R were used to determine the bacteria's identity.

Phosphate solubilisation ability

The isolates were tested for phosphate solubilisation using Pikovskaya medium and bromophenol blue as an indicator, as described by Jasim et al. (2013). Positive strains were those that developed a clear zone around the colonies after 5-7 days of incubation at 35°C.

Effect of bacterial isolates on root germination of tomato seedlings

Five bacteria capable of solubilizing phosphate and producing IAA were chosen for the pot experiment. Each of the bacteria chosen produced a different concentration of IAA. Maize seeds were surface sterilised for 3 days before being inoculated with 10·8 cells mL^{-1} of selected bacteria for 2 hours. The seedlings were then placed in polybags with autoclaved soil. Plants were grown in a nursery for six weeks before being measured for fresh and dry weights, root and shoot lengths, and other variables.

RESULTS AND DISCUSSION

We isolated and characterised culturable endophytic bacteria from *Uncaria borneensis* roots as a preliminary step toward understanding their potential role as biofertilizers in enhancing sustainable agriculture. This study indicated that *U. borneensis* trees exhibit a large proportion of culturable endophytic bacteria. A total of 61 endophytic bacteria were identified from the roots. The control plates that showed the absence of bacterial colonies confirmed that the isolates obtained were endophytes. All isolates were molecular identified (Figure 1) and evaluated for various important parameters.

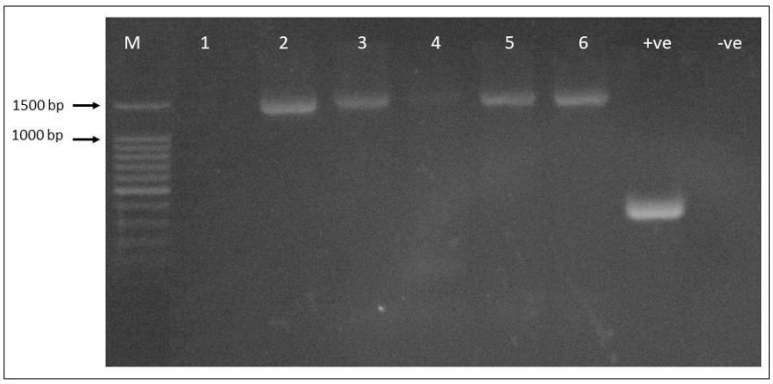


Figure 1. Agarose gel analyses (1% agarose, w/v) of amplified 16S RNA of the isolates. (Lane M = marker; lane 1 = J-111; lane 2 = J-112; lane 3 = J-122; lane 4 = J-221; lane 5 = K-311; lane 6 = L-112; lane +ve = positive control and lane -ve = negative control without any colony).

Among the 61 bacterial isolates, 35 solubilised phosphates were indicated by the distinct development of a halo zone growing around each colony on Pikovskaya's medium (Figure 2a). Bacterial strains with the highest phosphate solubilising efficiency were found in bacterial strain J11-1, with a solubilising phosphate index of 4.1. (Figure 2b).

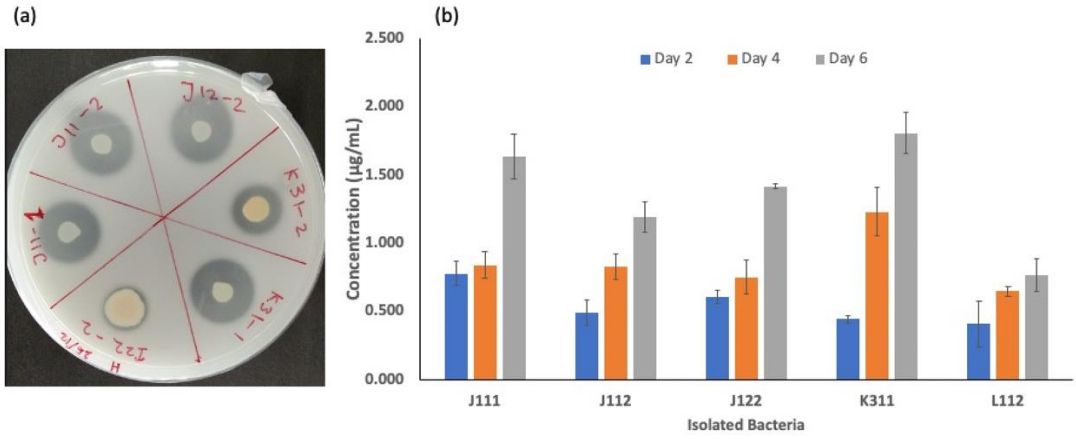


Figure 2. Phosphorous solubilisation activity of the isolated bacteria.

The 5 most potent bacteria strains were selected to test their ability to promote tomato plant growth. Tomato plants inoculated with strains J-111 and J-112 produced better roots formation, shoot length and fresh weight than tomato plants inoculated with J-122, K-311 and L-112 strains. This demonstrates that strains J-111 and J-112 had the greatest effect and greatly increased root biomass compared to a control plant without bacteria (Figure 3).

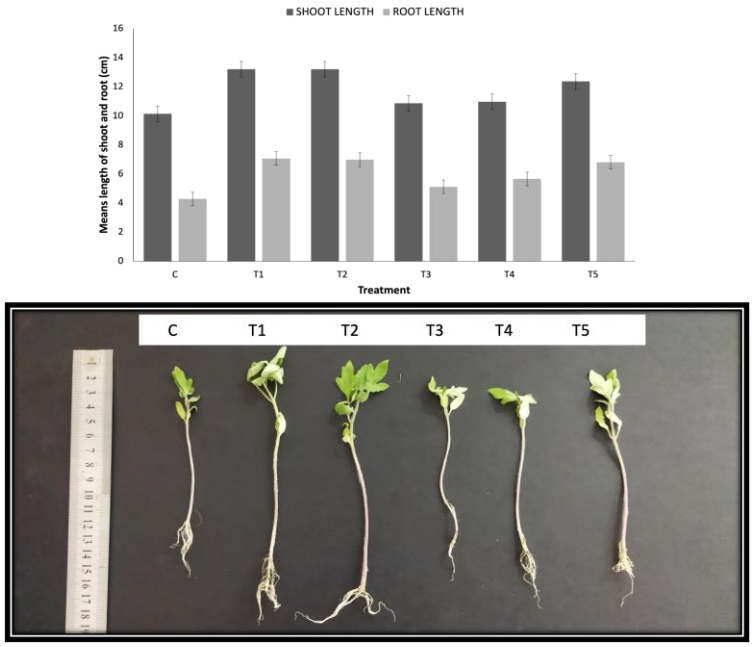


Figure 3. Root germination in tomato plants inoculated with selected bacterial strain. C = control; T1 = J-111; T2 = J-112; T3 = J122; T4 = K-311; T5 = L-112.

CONCLUSION

This study demonstrated the presence and diversity of culturable bacterial endophytes in the native *Uncaria borneensis* tree. Despite the economic significance and geographical distribution of native *Uncaria* trees, no research on endophytic bacteria associated with this plant has been found according to our best knowledge. Strains J-111 and J-112 have the potential to be used as agents that stimulate plant growth. According to best our knowledge, this is the first report on isolating bacterial endophytes from this host plant. The presence of these microbes suggests that they may be employed in the future for purposes such as promoting plant growth. Nonetheless, additional in vivo investigations and research under natural environmental settings are required to corroborate this idea.

ACKNOWLEDGEMENT

This work was supported financially by the Universiti Putra Malaysia Grant (UPM/800-3/3/1/GPIPM/2019/9681100).

REFERENCES

Dudeja, S.S., Giri, R., Saini, R., Suneja-Madan, P. and Kothe, E. 2012. Interaction of endophytic microbes with legumes. *Journal of Basic Microbiology*, *52*(3): 248-260.

Dutta, J. and Thakur, D. 2017. Evaluation of multifarious plant growth promoting traits, antagonistic potential and phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling, India. *PloS one*, *12*(8): e0182302.

Jasim, B., Jimtha, C. J., Jyothis, M. and Radhakrishnan, E.K. 2013. Plant growth promoting potential of endophytic bacteria isolated from Piper nigrum. *Plant Growth Regulation*, *71*(1): 1-11.

Musdja, M.Y., Elvita, L. and Rahayu, N. 2018. Effects of gambir (*Uncaria gambir* roxb) catechins on burn wound healing in male rats. In *Proceedings of BROMO Conference.*

Win, P. M., Matsumura, E. and Fukuda, K. 2018. Diversity of tea endophytic fungi: cultivar-and tissue preferences. *Applied Ecology and Environmental Research*, *16*(1): 677-695.

Yunarto, N., Sulistyaningrum, N., Kurniatri, A. A. and Elya, B. 2021. Gambir (*Uncaria gambir* Roxb.) as a potential alternative treatment for hyperlipidemia. *Media Penelitian dan Pengembangan Kesehatan*, *31*(3): 183-192.

Preliminary Evaluation of *Midin (Stenochlaena palustris)*, The Edible Fern of Sarawak, Malaysia as A Potential Prebiotic Ingredient

Tan Hui Yan[1], Pei Xin Lim[1], Lim Hui-Zi[1], Kathleen Michelle Mikal[1], Noorasmah Saupi[1], Juriah Kamaludeen[2], and Shahrul Razid Sarbini[1,3]*

[1] Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Kampus Bintulu Sarawak, 97008 Bintulu, Sarawak, Malaysia

[2] Department of Animal Science and Fishery, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Kampus Bintulu Sarawak, 97008 Bintulu, Sarawak, Malaysia

[3] Halal Products Research Institute, Universiti Putra Malaysia, 43400 Serdang, Malaysia

**Corresponding author:* shahrulrazid@upm.edu.my*

INTRODUCTION

Stenochlaena palustris, locally named as midin or paku midin is an indigenous edible fern found in Sarawak that have been commonly consumed locally (Chai, 2016). The earlier reports have identified the benefits of *S. palustris* consumption in the aspect of antioxidant, antibacterial and antifungal activities; natural food preservatives; steroids, flavonoids and alkaloids; and presence of phosphorus and potassium (Dash, 2016). As an indigenous food, the lack of scientific evidence for its prebiotic potential of *S. palustris* is the main reason for this present work. Therefore, in our present work, different parts of *S. palustris* including stem, fiddlehead, stem and fiddlehead, and mucilage extract were analysed using *in vitro* probiotic fermentation by *Bifidobacterium* spp and *Lactobacillus* spp*.* The bacterial enumeration was performed using Fluorescence *in situ* Hybridisation (FISH) technique. As a prebiotic ingredient, it is also important that fermentation of test substrates to produce beneficial metabolites such as short-chain fatty acids. The study therefore carried out quantification of organic acids using High-Performances Liquid Chromatography (HPLC). The preliminary findings of this study may therefore contribute to a new direction of finding local functional food, at the same time value-added the indigenous crop and broaden the local market in terms of agricultural and economic development.

MATERIALS AND METHODS

Sample preparation

Fresh *midin (Stenochlaena palustris)* was obtained from the local market of Sarawak, Malaysia. Sample was cleaned and divided into parts of fiddlehead, stem, fiddlehead and stem, and mucilage extracted from both fiddlehead and stem.

Boiling of plant sample

Sample (100 g) was cooked in boiling water (80-90°C) for 5 minutes then cooled down immediately in ice water. Excess water was removed and sample was cut into small pieces prior to analyses.

Extraction of mucilage from plant sample

Sample (100 g) was cut and cooked in boiling water at 80-90°C for 2 hours, then cooled down rapidly in ice water. Sample was left in cold water (8°C) for 24 hours for extraction of mucilage. The liquid was then collected and precipitated using isopropyl ethanol at a ratio of 1:1.5 (Yahia et al., 2007).

Isolation of probiotic and preparation of seed inoculum

Lactobacillus plantarum, L. sakei, L. acidophilus, and *Bifidobacteria spp.* were isolated from dietary supplement (*Lactopy SP, Korea* and *Blackmores*) using De Man, Rogosa and Sharpe (MRS) and *Bifidobacteria* Selective Media (BSM) from HiMedia, India. Seed inoculum was prepared by incubating the single bacteria colony in the specific media broth under anaerobic condition at 37°C and 220 RPM for 24 hours.

***In vitro* probiotic fermentation**

In vitro fermentation was performed using basal nutrient media (BNM, 45 mL), seed inoculum (5 mL), and test substrates (0.5 g). Fermentation was carried out anaerobically using Oxoid AnaeroGen sachets at 37°C, sampling was carried out at the interval of 0, 6, 12, 24 hours. Fructo-oligosaccharide (FOS) was used as positive control.

Bacterial enumeration

Fluorescent *in situ* hybridization (FISH) was performed as described elsewhere (Lee-Ling et al., 2022). The enumeration of bacteria was (*Bifidobacterium spp.* and *Lactobacillus-Enterococcus*) were performed by using 16S rRNA targeted oligonucleotide probes labelled with the fluorescent Cy3 dye.

Organic acids analyses

The quantification of short chain fatty acids (acetate, propionate, butyrate) was performed using High-Performances Liquid Chromatography (HPLC) equipped with C12 ion-exclusion silica column, UV detector at 210 nm, and 0.25 mM sulphuric acid as mobile phase.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) version 9.4. Tukey's Multiple Range Test ($p \leq 0.05$) was used to perform multiple comparisons between the means.

RESULTS AND DISCUSSION

Microbial enumeration

Figure 1 showed that population of *Lactobacilli-Enterococcus* and *Bifidobacteria spp.* increase throughout the fermentation of *midin,* except for negative control. The probiotic stimulation responses were similar to those in FOS as positive control. This may be related to abundant carbohydrate (3.4%) and protein (2.5%) present in *midin* (Voon and Kueh, 1999). Non-digestible oligosaccharide (NDO) in plants and vegetables contributes to support growth of probiotic (Muir et al., 2007). Besides, the proteolytic *Lactobacilli* and *Bifidobacteria* as well metabolise protein in *midin* (Zhang et al., 2020), and thus support the growth of probiotic. *Midin* also contains polyphenols and glycoprotein in mucilage that stimulate probiotic growth (Rawi et al., 2020).

Production of short-chain fatty acids (SCFA)

Figure 2 showed that probiotic fermentation of *midin* leads to a positive production of acetate, propionate, and butyrate. The production of SCFA is similar or even higher to those in FOS, the positive control. The acetogenic *Lactobaccilli* and *Bifidobacteria* contribute on acetate production (Flint et al., 2015). Yet, a decreasing trend was observed due to the conversion to butyrate via cross-feeding (Devaux et al., 2020). Thus, abundant amount of butyrate was observed in the fermentation of *midin*, particularly in M3 and M4 (fiddlehead and stem; mucilage). In short,

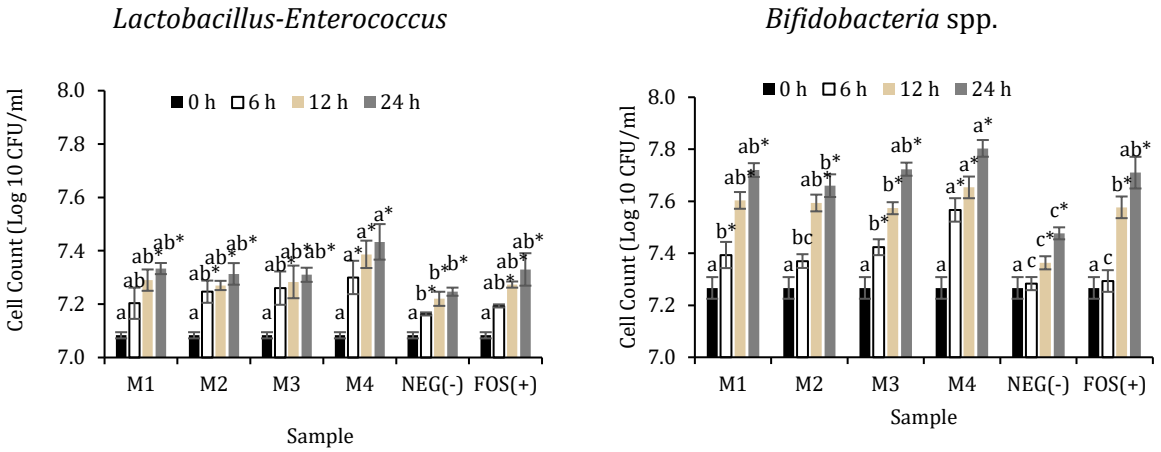


Figure 1. Population changes of the probiotic (i.e. *Lactobacilli-Enterococcus* and *Bifidobacteria spp.*) during the fermentation of *midin* at 0, 6, 12, and 24 hours. [a,b,c] Means with unlike letters is significantly higher/lower population when compare within the same sampling period (n=3, $p \leq 0.05$). * Mean values with significant difference when compare to 0 h fermentation (n=3, $p \leq 0.05$). M1 = stem of *midin,* M2 = fiddlehead of *midin*, M3 = stem and fiddlehead of *midin*, M4 = mucilage of *midin*, NEG = negative control, and FOS = fructo-oligosaccharide as positive control.

beneficial metabolites production from probiotic fermentation of *midin* may promotes gastrointestinal heath.

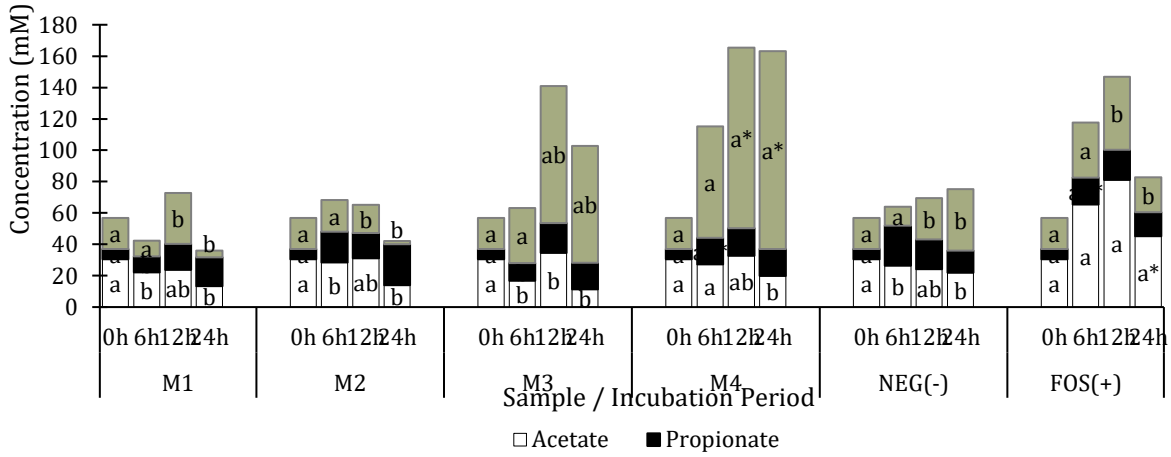


Figure 2. Concentration of short-chain fatty acids (acetate, propionate, and butyrate) during the fermentation of *midin* at 0, 6, 12, and 24 hours. [a,b,c] Means with unlike letters is significantly higher/lower population when compare within the same sampling period (n=3, $p \leq 0.05$). * Mean values with significant difference when compared to 0 h fermentation (n=3, $p \leq 0.05$). M1 = stem of *midin,* M2 = fiddlehead of *midin*, M3 = stem and fiddlehead of *midin*, M4 = mucilage of *midin*, NEG = negative control, and FOS = fructo-oligosaccharide as positive control.

CONCLUSION

Midin (Stenochlaena palustris) revealed a positive prebiotic potential activity by means of positive probiotic stimulation response and production of beneficial short-chain fatty acids, particularly butyrate in abundant amount.

ACKNOWLEDGEMENT

The authors would like to thank the support from University Consortium (UC) Seed Fund for Collaborative Research Grant (Ref. No. GBG22-0877) funded by the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA).

REFERENCES

Chai, P.P. 2016. Midin (*Stenochlaena palustris*), the popular wild vegetable of Sarawak. *UTAR Agriculture Science Journal, 2:* 18-20.

Dash, G.K. 2016. Pharmacognostic studies on *Stenochlaena palustris* (Burm. f) Bedd. *Scholars Research Journal, 8:* 132–137.

Devaux, C.A., Million, M. and Raoult, D. 2020. The Butyrogenic and lactic bacteria of the gut microbiota determine the outcome of allogenic hematopoietic cell transplant. *Frontiers in Microbiology, 11.*

Flint, H.J., Duncan, S.H., Scott, K.P. and Louis, P. 2015. Links between diet, gut microbiota composition and gut metabolism. *Proceedings of the Nutrition Society, 74*: 13–22.

Lee-Ling, C., Hui Yan, T., Saupi, N., Saari, N. and Sarbini, S.R. 2022. An *in vitro* study: prebiotic effects of edible palm hearts in batch human fecal fermentation system. *Journal of the Science of Food and Agriculture,* n/a. https://doi.org/10.1002/jsfa.12088.

Muir, J.G., Shepherd, S.J., Rosella, O., Rose, R., Barrett, J.S. and Gibson, P.R. 2007. Fructan and free fructose content of common Australian vegetables and fruit. *Journal of Agricultural and Food Chemistry, 55*: 6619-6627.

Rawi, M.H., Zaman, S.A., Pa'ee, K.F., Leong, S.S. and Sarbini, S.R. 2020. Prebiotics metabolism by gut-isolated probiotics. *Journal of Food Science and Technology, 57*: 2786-2799.

Voon, B.H. and Kueh, H.S. 1999. The nutritional value of indigenous fruits and vegetables in Sarawak. *Asia Pacific Journal of Clinical Nutrition*, *8*: 24–31.

Yahia, E.M., Ornelas, J. de J. and Anaya, A. 2007. Extraction and chemical characteristics of mucilage from mesquite, Aloe vera, maguey and prickly pear cactus cladodes (Nopal) and evaluation of its prebiotic effect on the growth of 2 probiotic bacteria, in: II International Symposium on Human Health Effects of Fruits and Vegetables: FAVHEALTH 2007 841. pp. 625–628.

Zhang, C., Zhang, Y., Li, H. and Liu, X. 2020. The potential of proteins, hydrolysates and peptides as growth factors for *Lactobacillus* and *Bifidobacterium*: current research and future perspectives. *Food Function, 11*: 1946-1957.