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Bridging Science and Forestry for A Sustainable Future

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Editors:
Asiah Osman, Huda Farhana Mohamad Muslim, Noor Hazmira Merous, Luqman Hakim Adzis

INSTITUT PENYELIDIKAN PERHUTANAN MALAYSIA
FRIM

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Preface

The Seminar on Project Evaluation and Monitoring (PEM) is an important annual event where researchers present their findings from projects completed within the current year. This seminar serves as a platform for discussing the issues, challenges, and opportunities in forestry research, with the hope of sparking collaboration among divisions within the Forest Research Institute Malaysia (FRIM). Researchers of selected projects will present their findings through oral, montage, or poster presentations.

The theme of the seminar is "Bridging Science and Forestry for a Sustainable Future". This theme emphasizes the essential integration of scientific knowledge and innovative forestry practices to promote environmental sustainability, biodiversity conservation, and responsible resource management. By operating at the intersection of science and forestry, we can ensure that forests continue to provide vital ecosystem services, support biodiversity, and contribute to the global economy in an environmentally responsible and sustainable manner for future generations.

A total of 40 projects were completed in 2023. However, due to limited presentation slots and capacity constraints, only selected projects were presented at the seminar. The proceedings encompass both upstream and downstream forestry research, covering topics such as forestry and the environment, forest biodiversity, forestry biotechnology, economic and social forestry, forest products, and natural products. In total, 25 articles were included in this publication.

Finally, we would like to express our gratitude and appreciation to all authors for their contributions to this publication. We extend our thanks to all project leaders and researchers for their support and cooperation during the seminar. Special acknowledgments go to the top management of FRIM, including Dato' Dr Ismail Hj Parlan (Director General), Dr Norwati Muhammad (Deputy Director General of Research), Dr Lillian Chua Swee Lian (Deputy Director General of Operations), and all division directors, particularly Dr Mohd Rosli Haron (Director of Research Planning Division), for their unwavering support and encouragement.
We hope that the proceedings will prove to be useful, exciting, and inspiring, especially for all FRIM researchers, motivating them to continue producing excellent results and to increase efforts in obtaining funding on both national and international levels.

DNA DATABASES OF *RUBROSHOREA CURTISII*, *DRYOBALANOPS AROMATICA* AND *D. OBLONGIFOLIA* FOR FORENSIC APPLICATION

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ABSTRACT

Illegal harvesting of forest resources poses a significant threat to the sustainability of forest ecosystems. At the Forest Research Institute Malaysia (FRIM), comprehensive DNA databases of important forest plant species have been developed since 2009 for timber tracking and source of origin identification. This paper describes the development of DNA databases of three major timber species in the tropics, i.e., *Rubroshorea curtisii*, *Dryobalanops aromatica*, and *D. oblongifolia*. A total of 57 populations throughout Peninsular Malaysia and about 2,019 individual samples were used to establish population and individual identification databases. The population identification database can be used to reveal the source of origin up to the population level. If the DNA profile of a suspected timber matches that of its original stump, by using the individual identification database, a random match probability can be estimated using subpopulation-cum inbreeding model to rule out the possibility of matching due to chance. The availability of DNA databases of important timber species for wood tracking enhances the capacity of forest officials to curb the problem of illegal logging. Besides, these databases can also be used to develop conservation and management guidelines to strike a balance between the conservation and utilisation of forest genetic resources.

Keywords: Dipterocarpaceae, Meranti Seraya, Kapur, Keladan, cpDNA haplotype, Short tandem repeat (STR), DNA forensics

INTRODUCTION

Malaysia is a megadiverse country rich in forest genetic resources. It is home to an estimated 15,000 species of vascular plants, including around 408 timber tree species with commercial values. However, illegal logging and the illegal timber trade have become significant challenges both domestically and internationally, undermining not only the development of the timber industry but also efforts toward the sustainable management and utilization of forest genetic resources. Innovative DNA-based techniques for detecting illegal logging play a crucial role in controlling illegal logging and ensuring accurate chain of custody certification. Recognizing the importance of DNA techniques for advancing the timber industry and promoting sustainable use of forest genetic resources, FRIM has been developing DNA technology since Malaysia 9th Development Plan. The output includes DNA profiling databases for timber tracking, developed for seven key tree species: Chengal (*Neobalanocarpus heimii*), Ramin Malawis (*Gonystylus bancanus*), Kempas (*Koompassia malaccensis*), Meranti Bukit (*Rubroshorea platyclados*), Karas (*Aquilaria malaccensis*), Merbau (*Intsia palembanica*) and Meranti Tembaga (*R. leprosula*). Additionally, in collaboration with the Forestry Department of Peninsular Malaysia (Jabatan Perhutanan Semenanjung Malaysia, JPSM) since 2017, DNA profiling databases have been developed for Bakau Minyak (*Rhizophora apiculata*) and Bakau Kurap (*R. mucronata*) specifically for Peninsular Malaysia. These DNA databases have supported investigation officers from JPSM, the State Forest Department and the Department of Wildlife, and National Parks Peninsular Malaysia in providing forensic evidence for illegal logging cases. Moreover, these DNA databases are ready to enhance the competitiveness of Malaysian wood products in the international market by supporting chain of custody certification to meet the international standards, including CITES, the US Lacey Act, and the EU and Australian Timber Regulations.

MATERIALS & METHODS

Sample collections of inner bark or leaves for *Rubroshorea curtisii*, *Dryobalanops aromatica* and *D. oblongifolia* were carried out from forest reserves in Peninsular Malaysia. Total genomic DNA was extracted using the 2x cetyltrimethylammonium bromide (CTAB) (Murray & Thompson, 1980) and purified using the High Pure PCR Template Preparation Kit. Polymorphic and specific universal chloroplast primers were used to develop the cpDNA haplotype database (Heinze, 2007; Taberlet et al., 1991). The simple sequence repeat (SSR) markers for *R. curtisii*, *D. aromatica*, and *D. oblongifolia* were developed using next-generation sequencing. Resequencing data for *R. curtisii* and transcriptome data for *D. aromatica* and *D. oblongifolia* were generated using the Illumina platform. The data was used to mine the SSR region using MISA (Thiel et al., 2003) and primers were designed using Primer3 (Rozen & Skaletsky, 2000).

To develop a haplotype distribution map, selected chloroplast regions were sequenced from samples of *R. curtisii*, *D. aromatica*, and *D. oblongifolia*. The DNA sequences were edited and aligned using Sequencher. Haplotypes were determined from nucleotide substitutions and indels (insertion and deletions). These haplotypes were used to plot the haplotype distribution map for *R. curtisii*, *D. aromatica*, and *D. oblongifolia*. For the development of DNA profiling databases, selected SSR markers were used for fragment analysis on samples of *R. curtisii*, *D. aromatica*, and *D. oblongifolia*. The genotyping was carried out using GeneMarker software. The genetic structure of populations was identified based on Bayesian clustering estimation in STRUCTURE (Pritchard et al., 2000). Self-assignment tests were used to evaluate the proportion of correctly assigned individuals to the designated population and region as implemented in GENECLASS2 (Piry et al., 2004).

RESULTS AND DISCUSSION

A DNA bank with 1,473 *Rubroshorea curtisii*, 385 *Dryobalanops aromatica*, and 161 *D. oblongifolia* samples throughout Peninsular Malaysia was established (Table 1). For *R. curtisii*, the DNA bank includes samples collected from 41 forest reserves in 10 states, i.e., Kedah (Bukit Enggang, Bukit Perangin, Gunung Jerai, Sungai Badak & Ulu Muda), Penang (Bukit Kerajaan), Perak (Behrang, Bubu, Bukit Larut, Gerik, Kledang Saiong, Sungai Pinang & Temenggor), Selangor (Hulu Langat & Semangkok), Negeri Sembilan (Angsi, Berembun, Kenaboi, Pasir Panjang & Pelangai), Melaka (Bukit Senggeh), Johor (Gunung Arong, Gunung Ledang, Gunung Pulai, Kluang & Panti), Pahang (Aur Gading, Berkelah, Beserah, Pulau Tioman, Tekai Tembeling & Ulu Jelai), Terengganu (Bukit Bauk, Cerul, Gunung Tebu, Pulau Redang & Tembat), and Kelantan (Gunung Basur, Gunung Rabong, Gunung Stong & Sungai Durian). For *D. aromatica*, sampling was carried out in 11 forest reserves in Johor (Gunung Arong, Kluang, LenggorA and LenggorB), Pahang (Balok & Taman Negeri Rompin), and Terengganu (Bukit Bandi, Bukit Bauk, Bukit Sai, Jengai & Rasau Kertih), while for *D. oblongifolia*, sampling was carried out in five forest reserves i.e., Kluang, Lenggor & Mersing (Johor), Taman Negeri Rompin (Pahang), and Sungai Nipah (Terengganu). In terms of natural distribution, *R. curtisii* can be found throughout Peninsular Malaysia in hilly and hillside areas while *D. aromatica* and *D. oblongifolia* are limited to the east coast of Peninsular Malaysia in three states, i.e., Johor, Pahang, and Terengganu. In addition, the distribution of *R. curtisii* and *D. aromatica* in natural habitats is clustered while *D. oblongifolia* is rare and can usually be found near rivers and swampy areas.

Table 1 Population of *Rubroshorea curtisii*, *Dryobalanops aromatica*, and *D. oblongifolia* from Peninsular Malaysia used to develop DNA database for population and individual identification.

No.	Forest Reserve	State	*R. curtisii*	*D. aromatica*	*D. oblongifolia*
1	Bukit Enggang	Kedah	35	-	-
2	Bukit Perangin	Kedah	35	-	-
3	Gunung Jerai	Kedah	42	-	-
4	Sungai Badak	Kedah	36	-	-
5	Ulu Muda	Kedah	35	-	-
6	Bukit Kerajaan	Pulau Pinang	35	-	-
7	Behrang	Perak	35	-	-
8	Bubu	Perak	41	-	-
9	Bukit Larut	Perak	53	-	-
10	Gerik	Perak	21	-	-
11	Kledang Saiong	Perak	32	-	-
12	Sungai Pinang	Perak	35	-	-
13	Temenggor	Perak	35	-	-
14	Hulu Langat	Selangor	30	-	-
15	Semangkok	Selangor	35	-	-
16	Angsi	Negeri Sembilan	35	-	-
17	Berembun	Negeri Sembilan	30	-	-
18	Kenaboi	Negeri Sembilan	34	-	-
19	Pasir Panjang	Negeri Sembilan	41	-	-
20	Pelangai	Negeri Sembilan	35	-	-
21	Bukit Senggeh	Melaka	35	-	-
22	Gunung Arong	Johor	35	35	-
23	Gunung Ledang	Johor	35	-	-
24	Gunung Pulai	Johor	35	-	-
25	Kluang	Johor	35	35	35
26	Lenggor	Johor	-	-	36
27	LenggorA	Johor	-	35	-
28	LenggorB	Johor	-	35	-
29	Mersing	Johor	-	-	35
30	Panti	Johor	35	-	-
31	Aur Gading	Pahang	35	-	-
32	Balok	Pahang	-	35	-
33	Berkelah	Pahang	35	-	-
34	Beserah	Pahang	35	-	-
35	Pulau Tioman	Pahang	35	-	-
36	Taman Negeri Rompin	Pahang	-	35	20

37	Tekai Tembeling	Pahang	35	-	-
38	Ulu Jelai	Pahang	35	-	-
39	Bukit Bandi	Terengganu	-	35	-
40	Bukit Bauk	Terengganu	35	35	-
41	Bukit Sai	Terengganu	-	35	-
42	Cerul	Terengganu	35	-	-
43	Gunung Tebu	Terengganu	35	-	-
44	Jengai	Terengganu	-	35	-
45	Pulau Redang	Terengganu	35	-	-
46	Rasau Kertih	Terengganu	-	35	-
47	Sungai Nipah	Terengganu	-	-	35
48	Tembat	Terengganu	35	-	-
49	Gunung Basur	Kelantan	52	-	-
50	Gunung Rabong	Kelantan	35	-	-
51	Gunung Stong	Kelantan	35	-	-
52	Sungai Durian	Kelantan	35	-	-

The haplotype distribution map of *R. curtisii* was developed based on 41 populations and 21 haplotypes (H1-H21) derived from 24 substitutions and 6 indels on three chloroplast regions, i.e., *trn*L, *trn*G, and intergenic spacers *trn*G-*atp*A (Figure 1A). Haplotypes H1 and H2 are the most dominant haplotypes (57.6% and 17.1% respectively) and are scattered throughout Peninsular Malaysia. Haplotype H3 can only be found in the states of Pahang, Kelantan and Terengganu while other haplotypes (H4 to H21) are endemic to a certain population. For *D. aromatica*, the haplotype distribution map was developed based on 11 populations and 16 haplotypes (H1-H16) derived from 15 substitutions and 4 indels on four chloroplast regions, i.e., *trn*V-*trn*M, *pet*L-*psb*E, *psb*J-*pet*A, and *trn*G-*atp*A (Figure 1B). Haplotype H1 is the dominant haplotype (71.6%) and can be found in all populations. Haplotype H2 (4.5%) can be found in Jengai, Bukit Sai, and Balok, while H3 (4.5%) can be found in Balok and Rompin. Haplotype H4 (3.4%) can be found in Gunung Arong, Kluang, and Lenggor2. Haplotype H5 (2.3%) can be found in Bukit Bauk and Rompin. Haplotype H6 (2.3%) can be found in Jengai and Bukit Bandi. Haplotypes H7 to H16 are rare haplotypes that are unique to a population. For *D. oblongifolia*, the haplotype distribution map was developed based on five populations and 12 haplotypes (H1-H12) derived from 8 substitutions and 7 indels on four chloroplast regions, i.e., *pet*G-*trn*P, *psb*K-*trn*S, *trn*D-*trn*E, dan *trn*M-*atp*E (Figure 1C). Haplotype H1 is the dominant haplotype (42.5%) and can be found in Rompin, Mersing, Lenggor, and Kluang. Haplotype H2 (12.5%) can be found in Mersing and Lenggor. Haplotypes H3 (10%) and H4 (7.5%) can only be found in the Sungai Nipah. Haplotype H5 (7.5%) can be found in Rompin, Lenggor, and Kluang. Haplotype H6 (5%) can be found in Rompin and Lenggor. Haplotypes H7 to H12 are rare haplotypes that are unique to a population.

A B C

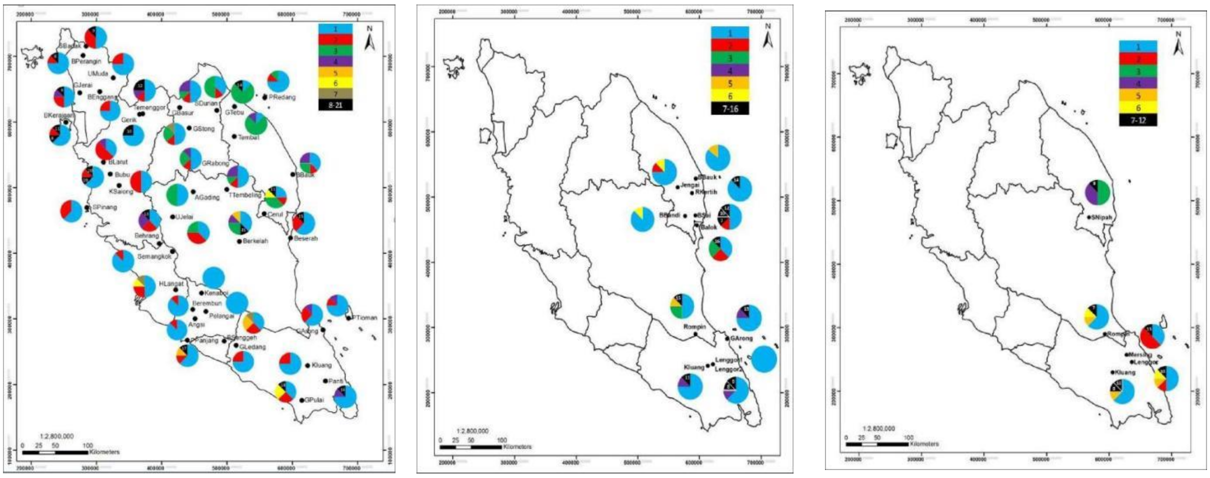

Figure 1 Haplotype distribution map for *Rubroshorea curtisii* (1A), *Dryobalanops aromatica* (1B), and *D. oblongifolia* (1C) in Peninsular Malaysia.

The DNA profiling database of *R. curtisii* was developed based on 10 highly polymorphic SSR markers (*Scu*03, *Scu*04, *Scu*11, *Scu*12, *Scu*32, *Scu*37, *Scu*41, *Scu*43, *Scu*44, and *Scu*59). Structure analysis partitioned the *R. curtisii* in Peninsular Malaysia into two main genetic clusters, namely Cluster 1 which includes the populations from Sungai Badak (Kedah) to Beserah (Pahang), and Cluster 2 which includes the populations from Gunung Arong (Johor) to Pulau Tioman (Pahang). If Cluster 1 is further analysed, it can be divided into two clusters, i.e., Cluster 1a which includes the populations from Sungai Badak (Kedah) to Gunung Ledang (Johor), and Cluster 1b which includes the populations from Cerul (Terengganu) to Beserah (Pahang). The structuring of *R. curtisii* in Peninsular Malaysia into Clusters 1a, 1b and 2 is further supported by Neighbor Joining tree analysis. Therefore, three DNA profiling databases named (1) Seraya Cluster 1a, (2) Seraya Cluster 1b, and (3) Seraya Cluster 2 were developed. For the estimation of profile frequency, to ensure that the databases are conservative, the values of theta (θ) are corrected from 0.0279 to 0.1800 for Seraya Cluster 1a, from 0.0183 to 0.1200 for Seraya Cluster 1b, and from 0.0435 to 0.1600 for Seraya Cluster 2. The self-assignment test shows that on average only 29.10% of individuals can be matched back to the original population.

For *D. aromatica*, the DNA profiling database was developed based on 10 highly polymorphic SSR markers (*Dar*44, *Dar*T01, *Dar*T15, *Dar*T24, *Dar*T29, *Dar*T57, *Dar*187, *Dar*426, *Dar*519, and *Dar*569). Structure analysis partitioned *D. aromatica* in Peninsular Malaysia into two main genetic clusters, i.e., Cluster 1 which includes populations from Bukit Bandi, Bukit Bauk, Bukit Sai, Jengai, Rasau Kertih, Balok, and Taman Negeri Rompin, and Cluster 2 includes populations from Kluang, LenggorA, LenggorB, and Gunung Arong. The Neighbor Joining tree analysis also divided the *D. aromatica* into two genetic clusters that correspond to Clusters 1 and 2. Hence, two DNA profiling databases named as Kapur Cluster 1 and Kapur Cluster 2 were developed. For the estimation of profile frequency, to ensure that the databases are conservative, the value of θ was corrected from 0.0322 to 0.1200 for Kapur Cluster 1, and from

0.0404 to 0.1400 for Kapur Cluster 2. The self-assignment test shows that on average 62.81% of individuals can be matched back to the original population.

For *D. oblongifolia*, the DNA profiling database was developed based on 10 highly polymorphic SSR markers (*Dob*T05, *Dob*T19, *Dob*T41, *Dob*T42, *Dob*T51, *Dob*T52, *Dob*T54, *Dob*T56, *Dob*T57, and *Dob*T75). Structure analysis divided the five *D. oblongifolia* populations into two main clusters, namely Cluster 1 which includes the populations of Rompin, Mersing, Lenggor, and Kluang, and Cluster 2 which includes the population of Sungai Nipah. Since Cluster 2 only includes the Sungai Nipah population, the DNA profiling database for *D. oblongifolia* was developed by combining the populations in Clusters 1 and 2. For the estimation of profile frequency, to ensure that the database is conservative, the value of θ is corrected from 0.0870 to 0.1850. The self-assignment test shows that on average 82.83% of individuals can be successfully assigned back to the original population.

CONCLUSION

In terms of forensic application, the population identification database can be used to reveal the source of origin up to the population level. If the DNA profile of a suspected timber matches that of its original stump, by using the individual identification database, a random match probability can be estimated using subpopulation-cum inbreeding model to rule out the possibility of matching due to chance. The availability of DNA databases of important timber species for wood tracking enhances the capacity of forest officials to curb the problem of illegal logging. Besides, these databases can also be used to develop conservation and management guidelines to strike a balance between the conservation and utilisation of forest genetic resources.

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PRODUCTION OF PROTOTYPE BIODEGRADABLE FACE MASK MADE FROM KENAF FIBRE

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ABSTRACT

The COVID-19 pandemic has led to the widespread use of facemasks to reduce the spread of infection, but their disposal has created a new environmental problem due to the non-degradable plastic used in their production. To address this issue, we propose using biodegradable materials such as kenaf fiber as a substitute for plastic in facemask production. In this study, we evaluated the potential of kenaf paper as a supporting layer for facemasks by analysing its mechanical properties, filtration efficiency, and antimicrobial properties. Our results showed that kenaf paper, with the inclusion of NFC and antimicrobial treatment, could be a promising material for replacing non-biodegradable plastic in facemask production. The samples exhibited good tear and tensile indices of 7.19 mNm^2/g and 42.99 Nm/g, respectively, a particle penetration rate of 33.63%, and a breathing resistance of 1.26 kPa, while also demonstrating a medium inhibition zone for Staphylococcus aureus and Escherichia coli growth. These findings suggest that kenaf paper has significant potential as a raw material for facemask production, offering a solution to mitigate the negative environmental impact of facemask disposal.

Keywords: kenaf paper, bio-degradable, NFC, antimicrobial, facemask

INTRODUCTION

The COVID-19 pandemic has had a devastating impact on public health worldwide, with over 2.5 billion people affected and a mortality rate of more than 10% for those with insufficient immunity (Khan et al., 2021). Wearing face masks has been identified as an effective way to reduce the spread of the virus by preventing respiratory droplets from entering the mouth and nose. However, the widespread use of face masks has resulted in large volumes of plastic waste, as most masks are made from petroleum-based polymers that are non-degradable (Chaowana, 2013). This has created an environmental issue that can be resolved by using biodegradable materials such as kenaf fiber.

Kenaf fiber is a sustainable and low-cost material that can be extracted from the entire plant, and has the potential to replace synthetic fibres in reinforcement composites (Akil et al., 2011). To improve the performance of kenaf fiber, nanofibrillated cellulose (NFC) was incorporated into the composite, which exhibits excellent mechanical strength and large specific surface area (Jonoobi et al., 2010). Additionally, the composite was treated with polyhexamethylene guanidine hydrochloride (PHGH) to provide antimicrobial properties. PHGH is a positively charged polymer that exhibits antibacterial activity against a wide range of bacteria and fungi, making it suitable for use in biodegradable face mask fabrication (Guan et al., 2007; Mei et al., 2014).

In this study, we evaluate the potential of kenaf paper with the inclusion of NFC and antimicrobial treatment as a supporting layer for face mask production. We investigate the mechanical properties, filtration efficiency, and antimicrobial properties of the composite, and compare its performance to conventional face masks made from non-biodegradable plastic. Our findings suggest that kenaf paper with NFC and PHGH treatment has excellent mechanical properties, effective filtration efficiency, and good antimicrobial properties, making it a promising material for replacing non-biodegradable plastic in face mask production.

MATERIALS AND METHODS

Materials

Kenaf V36 were supplied from Bionic Seeds Berhad. Sodium Hydroxide (NaOH) were purchased from Merck (German) while Hexamethylediamine, Guanidine Hydrochloride, Epichlorohydrin were purchased from Sigma Aldrich (United States).

Pulp Preparation

Pulp was prepared using chemical pulping method (Soda). Kenaf bast fiber was cooked with NaOH in digester at 170°C and 10 bar for 320 minutes. Then the pulp was beat for 1000 rev, 2000 rev, 3000 rev and 4000 rev for strength comparison.

NFC preparation

Cooked pulp was further treated with 40,000 revolution beat using PFI Mill Beater followed by ultrafine grinding with supermass colloider to get the nanofibrillated cellulose.

PHGH Preparation

In the preparation of the PHGH (Polyhexamethylene Guanidine Hydrochloride) solution, 116.21 g of hexamethylenediamine and 95.53 g of guanidine hydrochloride were combined in a 3-neck round bottom flask, heated sequentially at 100°C for 2 hours, 150°C for 2 hours, and 180°C for 1 hour. The resulting pre-polymer was dissolved in distilled water to achieve a 40% mass concentration. Subsequently, 50 g of the pre-polymer was mixed with 9.2 g of epichlorohydrin at 30°C over 30 minutes, followed by stirring for 4 hours. The mixture was then diluted to a 20% mass concentration and heated at 60°C for 6 hours to complete the reaction. The optimised pulp was treated with the PHGH solution by immersing it for 3, 6, 24, and 72 hours to determine the optimal contact time for the pulp to exhibit the most effective antimicrobial properties.

Production of Paper from Kenaf Pulp and NFC

The NFC was incorporated with the optimised (beat process) kenaf pulp. Two different ratios (5% NFC and 20% NFC) were produced in papermaking process for strength comparison.

Characterization

The tensile properties of the sample were tested in dry condition using a Horizontal Tensile Tester (Buchel Van Der Korput B.V) room temperature. The test was done with a constant elongation velocity of 10 mm/min. While the tearing properties were tested with L&W Tearing Tester. In order to determine the filtration efficiency of the sample, the samples were sent to Dusk Mask Laboratory (DML) at National Institute of Occupational Safety (NIOSH) Malaysia. The penetration and breathability test were tested with 0.3 micron of Sodium Chloride aerosol at 95 l/min by using TSI High Flow Automated Filter Tester 8130. Finally, the antimicrobial properties of the sample were tested at Antimicrobial Lab, Nature Products Department, FRIM using Disc Diffusion Assay method.

RESULTS AND DISCUSSION

Effect of beating process on Tensile Index and Tear Index

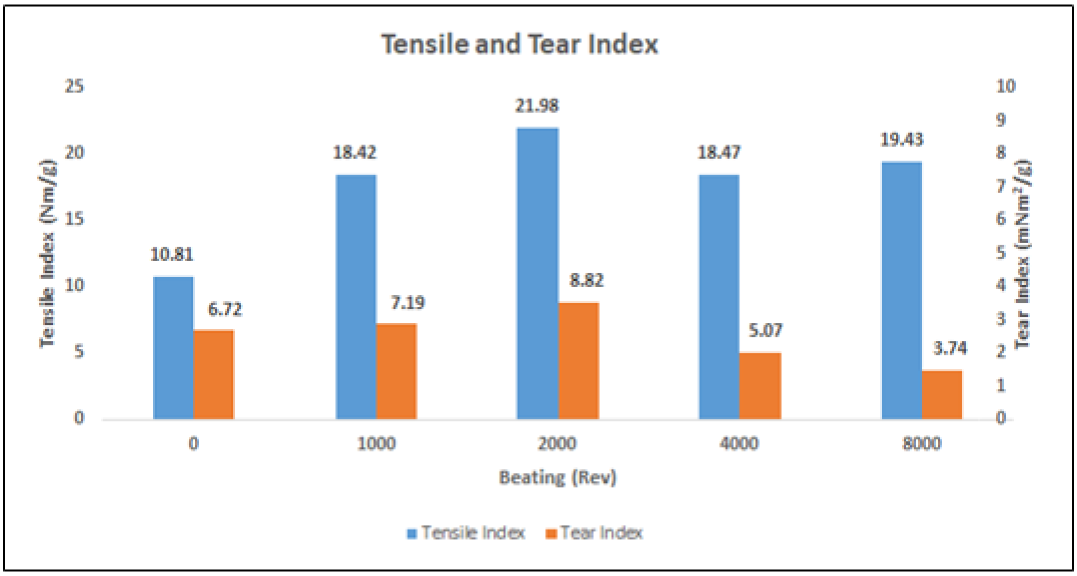

Figure 1 Tensile Index and Tear Index vs. Beating Graph

In this study, we observed a significant increase in the tensile index from 0 beating to 2000 rev beating, highlighting the paper's improved ability to withstand tensile stress. The maximum tensile index achieved was 21.98 Nm/g, indicating enhanced durability. However, as the beating continued at 4000 rev and 8000 rev, the tensile index exhibited a decline to 18.47 Nm/g and 19.43 Nm/g, respectively.

These findings underscore the critical role of fiber quality and fiber network structure in determining paper properties, as previously noted (González et al., 2012). Processes that enhance fiber interconnectivity, such as increased refining or wet pressing, contribute to the improvement in the paper's tensile index.

Similarly, in the context of tearing properties, our study reveals a comparable trend. We observed that the tearing index reached its peak at 2000 rev with a value of 8.82 m. Nm^2/g, but then experienced a decline at 4000 rev and 8000 rev, with tearing indices of 5.07 $m.Nm^2/g$ and 3.74 $m.Nm^2/g$, respectively.

This phenomenon can be attributed to the complex interplay of various parameters that influence tear strength, including fiber length, fiber strength, and bonding strength between the fibers. As the beating process progresses, fiber length tends to shorten, which in turn affects the overall tear strength. These findings emphasize the importance of considering multiple factors when evaluating and optimizing tearing properties in paper production processes.

Effect of NFC on Tensile Index and Tear Index

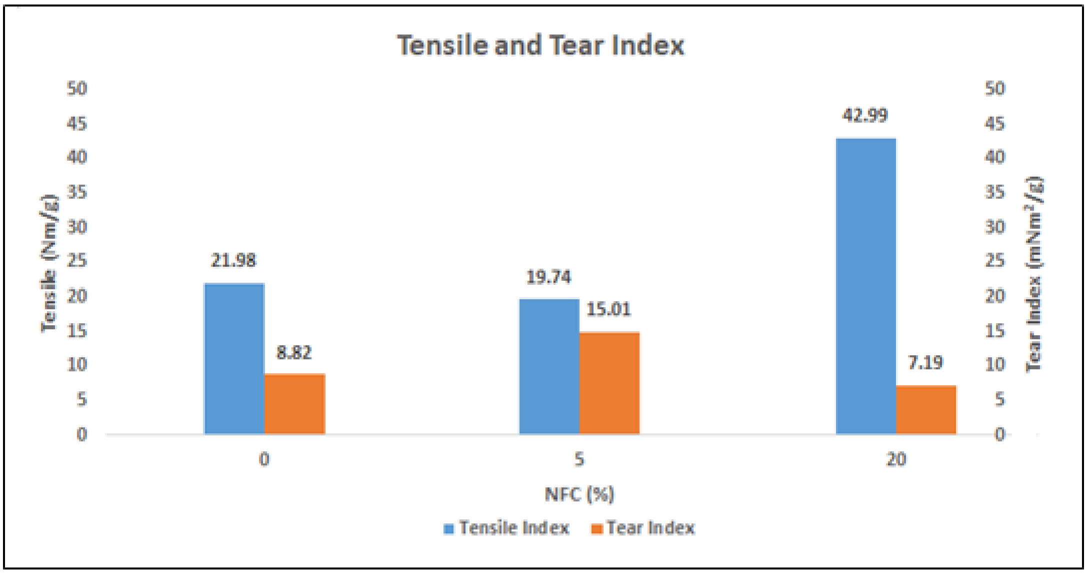

Figure 2 Tensile Index and Tear Index vs. NFC (%)

The incorporation of nanocellulose (NFC) in the pulp demonstrated a positive correlation with the tensile index, as observed in our study. As the concentration of NFC increased, so did the tensile index, highlighting the beneficial effects of NFC on paper strength.

This improvement can be attributed to the unique properties of nanocellulose. Particularly, when nanofiber dimension's decrease, there is a notable increase in the specific surface area of the cellulosic fibers. This increased surface area results in a greater number of accessible hydroxyl groups on the nanofiber surfaces. These hydroxyl groups play a crucial role in facilitating the formation of multiple hydrogen bonds with adjacent nanofibers, as highlighted in the work of (Yousefi et al., 2011). Consequently, this extensive nanofiber network formation contributes significantly to an overall increase in the fiber tensile strength.

The observed trend in tearing strength follows a fluctuating pattern, with the tearing index peaking at 15% concentration of nanocellulose (NFC) at 15.01 m. Nm^2/g but subsequently declining to 7.19 m.Nm^2/g at 20% NFC concentration.

This fluctuation can be attributed to the introduction of lignocellulosic nanofibers into the paper matrix. The incorporation of NFC enhances the level of hydrogen bonding within the paper structure. However, it's important to note that as NFC is introduced, it also tends to reduce the average fiber length within the paper. This combination of increased hydrogen bonding and reduced fiber length leads to the observed fluctuations in tear strength.

CONCLUSION AND RECOMMENDATIONS

In conclusion, our study explored the viability of using kenaf paper as a sustainable alternative to non-biodegradable plastics in facemask production, given the environmental concerns posed by mask disposal during the COVID-19 pandemic. Through comprehensive analysis, including assessments of mechanical properties, filtration efficiency, and antimicrobial capabilities, our findings support the potential of kenaf paper, especially when combined with NFC and antimicrobial treatment, as a promising material for facemask construction. The material exhibited favorable tear and tensile indices, a particle penetration rate comparable to existing masks, and reasonable breathing resistance. Additionally, its antimicrobial properties, as indicated by a medium inhibition zone for *Staphylococcus aureus* and *Escherichia coli* growth, further enhance its suitability. This research offers an eco-friendly avenue to address the environmental impact of disposable facemasks, emphasizing the potential of kenaf paper in contributing to a more sustainable future.

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BIODIVERSITY ENRICHMENT OF AN EX-TIN MINE WITH THREATENED AND ENDEMIC TREE SPECIES

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ABSTRACT

Land degradation caused by anthropogenic activities has led to serious and sometimes irreversible consequences, resulting in extensive environmental and economic losses. Among the activities that leave severe environmental footprints include industrialisation, deforestation, unsustainable agriculture activities and mining. Tin mining was once a lucrative industry in Peninsular Malaysia but had completely changed the landscape and soil characteristics in these areas. Ex-tin mines were usually barren with problematic soil and colonised by unimportant plants like ferns and grasses. Infertile soil and lack of seed sources have called for human intervention to green and increase the biodiversity of these areas. This project is a tripartite collaboration from 2016 to 2022 among FRIM, the Royal Forest Department Thailand and the Korea Forest Service under the ASEAN-ROK Forestry Cooperation (AFoCo) programme. The project aims to increase the biodiversity of degraded mined areas with threatened and endemic tree species in Malaysia and Thailand. For Malaysia, a total of 3,650 trees from 24 species comprising families from Dipterocarpaceae, Thymelaeaceae, Lythraceae and Sapotaceae were planted over 7.6 ha in FRIM Research Station Bidor, Perak, under existing mixed tree stands. These trees were planted in three phases in 2017, 2019 and 2021 with survival rates of 80%, 81% and 90%, respectively, when enumerated in the year 2022. Our observations showed that *Hopea ferruginea* and *Dryobalanops aromatica* were frequently attacked by wild boars, with survival of the former declined drastically to 37% and the latter to 56% at one year after planting. This project has successfully increased tree biodiversity of an ex-tin mine using valuable threatened species and will be maintained as a demonstration plot for further studies.

Keywords: tin mine, afforestation, biodiversity, endangered, endemic

INTRODUCTION

Tin mining was a lucrative industry in Malaya in the late 19th century. By 1883, Malaya had emerged as the world's largest tin-producing country as a consequence of industrialisation in Europe (Wong, 1959). Expansion of the tin mining industry up to the early 20th century had not only spurned the economy of the country but totally changed the landscape of the mined areas. During mining operations, soils were separated using the gravitational method to obtain the tin ores, resulting in two major soil types, namely, sand and slime tailings. Sand tailings have low nutrient and low water retention capacity. When compounded with high silica in the sand that causes high soil temperature, this can cause severe damage to seedlings with shallow root systems. Meanwhile, slime tailings can be water-logged during the rainy season, limiting the ability of plants to establish in these areas.

Mined areas are thus notorious for their poor site qualities. Mining sites have been converted into housing areas, recreational parks and agricultural areas. Due to poor soil nutrients, high operational costs for the application of fertilisers and irrigation required for turning ex-mines into agriculture purposes may limit the large-scale and long-term use. In addition, the discovery of high concentrations of potentially toxic elements, including heavy metals that exceed safe consumption levels in food crops grown on tin tailings, has rendered it an unsuitable site for food production (Ang & Ng, 2000). On the other hand, these unproductive ex-tin mines away from urban areas are usually abandoned or used for cattle grazing, which cannot recover into forest naturally due to adverse changes brought to this ecosystem by mining activities. These adverse changes include i) lack of mother trees or seed sources, ii) hostile microclimate and unfavourable soil properties, and iii) repeated disturbances by the same agents (for instance, grazing) that preclude the process of regeneration.

Human intervention is thus crucial to ameliorate the soil conditions in these degraded areas and to provide a suitable microclimate for growing trees particularly tropical rainforest species, as in afforestation. The IPCC Guidelines define afforestation as the "planting of new forests on lands which, historically, have not contained forests" (Watson et al., 2000). The establishment of a new forest stand can take many forms depending on the purpose of afforestation. New forests can be planted for either recovery of ecological integrity as in conservation and preservation or for extraction. As such, these new forests can be monoculture plantations, old-growth forests, logged forests, multispecies restoration, or secondary forests.

According to Yong et al. (2021), of the 1,292 taxa assessed in Peninsular Malaysia, 77 taxa (5.96%) are Critically Endangered (CR), 120 taxa (9.29%) are Endangered (EN), and 173 taxa (13.39%) are Vulnerable (VU), bringing the total number of threatened taxa to 370 taxa (28.64%). These figures are alarming and will require both *in-situ* and *ex-situ* conservation efforts to prevent the numbers from increasing, especially due to land conversion as well as forest loss and degradation.

ASEAN-ROK Forestry Cooperation (AFoCo) is a flagship initiative developed in response to the Republic of Korea (ROK)'s vision on “Low Carbon Green Growth” and is committed to addressing the impact of global climate change as announced by the President of the ROK during the ASEAN-ROK Commemorative Summit held in Jeju Island in June 2009. The programme promotes technical cooperation, policy development, capacity building and partnership in areas related to sustainable forest management (SFM) and, in particular, the rehabilitation of degraded forest land and prevention of deforestation and degradation. One of the projects under the AFoCo programme is a tripartite collaboration between Forest Research Institute Malaysia (FRIM) and the Royal Forest Department Thailand (RFD) as implementing agencies, and the Korea Forest Service (KFS) as the donor agency. This six-year project titled ‘Domestication of Endangered, Endemic and Threatened Plant Species in Disturbed Terrestrial Ecosystem in Malaysia and Thailand’ was initiated in 2016 with the aim to plant endemic and threatened species in ex-mining areas in both countries besides organising activities for technology transfer and capacity building. This paper describes the survival and growth performance of the trees planted in an ex-tin mine in Malaysia.

MATERIALS AND METHODS

The project site is an ex-mining area and is located at FRIM Research Station Bidor, Perak (4°05'16.3" N, 101°14'25.9" E). There are a total of three planting phases (Table 1) where plants from the IUCN Red List and an endemic species were chosen to be planted. All three phases were established on slime tailings through line planting under tree stands of *Acacia mangium* and/or *Hopea odorata*. All trees must have a minimum height of 1.5 m upon planting. The maintenance regime includes the application of fertiliser, soil loosening, blanket weeding, and irrigation, which were provided for all plants until December 2022. Total height and diameter at breast height (dbh) were measured, and survival was counted every year after planting except for phase 3, which was planted in the year 2021.

Table 1 Three planting phases of AFoCo project in FRIM Research Station Bidor

Phase	1	2	3
Year planted	2017	2019	2021
Area (ha)	3.0	3.6	1.0
No. of trees	1,500	1,650	500
No. of species	14	19	10

RESULTS AND DISCUSSION

A total of 3,650 trees were planted in three phases from 2017 to 2021, covering an area of 7.6 ha under *H. odorata* and *A. mangium* stands (Table 1). With the aim to enrich biodiversity as well as conserve threatened and endemic tree species, a total of 24 species were selected and planted from four different families of Dipterocarpaceae, Thymelaeaceae, Lythraceae and Sapotaceae.

Figures 1-3 show the survival of all species planted in the three phases. As of the final count and measurement conducted in November 2022, the survival rates of trees from phases 1, 2 and 3 are 80%, 81% and 90%, respectively. Extremely low survival rates were recorded for *H. ferruginea* and *Dryobalanops aromatica* primarily due to attacks by wild boars that peeled off tree barks, resulting in mortality of the trees. The former species was thus only planted in phase 1. Among the species planted, *Palaquium maingayi, D. aromatica* and *Rubroshorea leprosula* exhibited better growth in terms of height and dbh. In phase 1, *P. maingayi* recorded the best height and dbh after five years at 9.35 ± 2.46 m and 10.98 ± 3.67 cm, respectively. Despite the frequent attacks by wild boars, *D. aromatica* in phase 2 attained an average height of 6.35 ± 1.39 m and 5.40 ± 1.62 cm three years after planting. Meanwhile, *R. leprosula*, one year after planting in phase 3, showed average height of 2.29 ± 0.66 m and dbh of 0.87 ± 0.85 cm. Building on the success of planting in degraded areas, FRIM was invited by the Royal Forest Department of Thailand to provide technical expertise for the rehabilitation of an ex-tin mine located at Phang Nga Forestry Research Station of Kasetsart University.

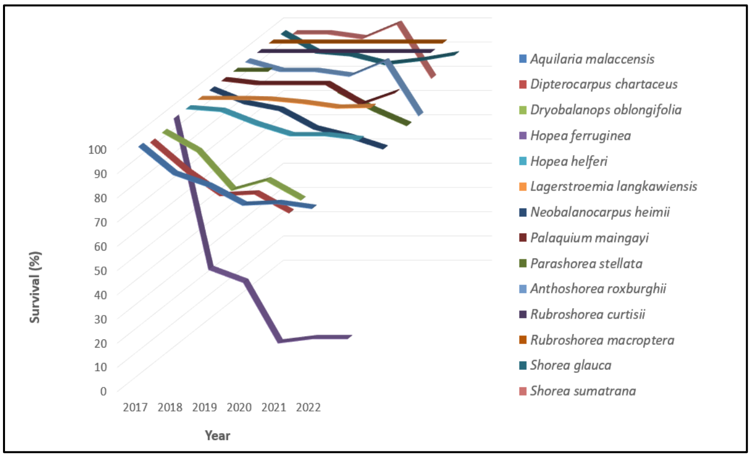

Figure 1 Survival of tree species planted in Phase 1

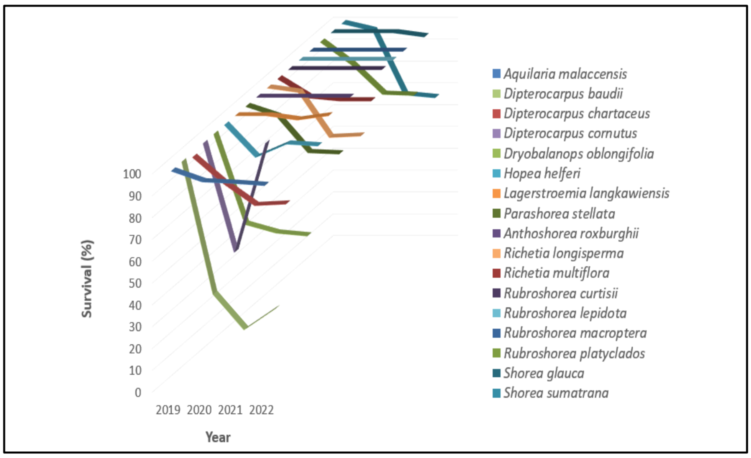

Figure 2 Survival of tree species planted in Phase 2

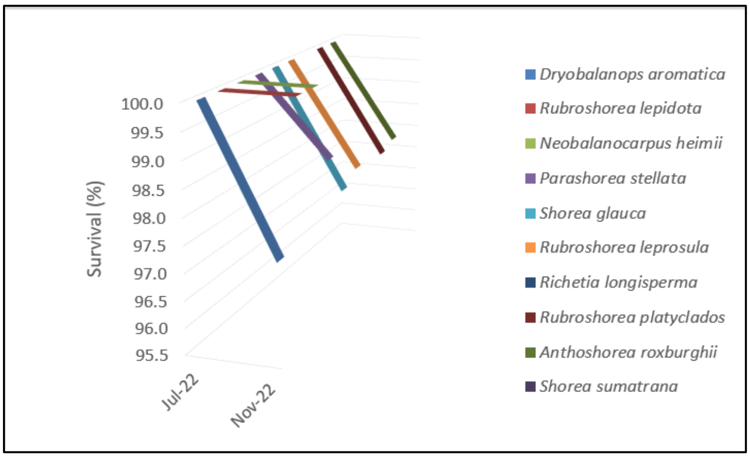

Figure 3 Survival of tree species planted in Phase 3

In addition to biodiversity conservation, the project placed significant emphasis on information dissemination and capacity building. With support from AFoCo, FRIM organised a series of events to promote knowledge sharing and skill development: a national seminar in 2016, a regional seminar in 2017, a regional workshop in 2019, and a regional webinar in 2021. The webinar was conducted online due to the global COVID-19 pandemic and was also shared via YouTube and FRIM's Facebook. As part of the programmes in all physical seminars and workshops, participants were brought to FRIM Research Station in Bidor to witness the successful conservation efforts of threatened and endemic trees of this project. The project has also published two journals, four books and eight proceedings, among others.

CONCLUSION AND RECOMMENDATIONS

This regional project has not only promoted biological conservation but also facilitated extensive opportunities for networking, collaboration, and knowledge-sharing, particularly among ASEAN countries. Conservation of endangered and endemic species requires careful species selection and soil improvement prior to planting in degraded areas like ex-tin mines. Additionally, the use of larger trees, ideally with a height exceeding 1.5 meters, enhances overall survival rates, although this will incur higher costs. Vulnerable species, such as *H. ferruginea*, as in this project, should be avoided to reduce mortality and financial loss. The planted areas have now become popular among visitors who are keen on rehabilitation and conservation efforts. The project site will continue to serve as a research and educational area with the potential to become a seed production area for threatened species in the future.

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UNLOCKING THE POTENTIAL OF JUNIOR RANGERS FOR GREEN NATURE SOLUTIONS AND NATURE-BASED COMMUNICATION

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ABSTRACT

Community-based ecotourism (CBET) is a unique type of tourism to alleviate the impact of tourism activities on the environment and striking a balance between local people and the natural environment. The benefit of CBET should be extended to young children's roles in serving tourists. Experiencing nature at an early age might critically affect one's perception of nature. Childhood nature experience is an important factor in navigating these individuals toward conservation activities. As people's alienation from nature could be a significant threat to the conservation of natural environments, the junior ranger programme in Ulu Tampik Waterfall called Tampik Junior Rangers is an ideal tool to foster strong and lasting bonds between local communities and their protected areas. The research aims to educate and empower school children in rural areas for greater support and engagement in natural resource conservation. This research focused on providing information, education, and training among the local communities, especially young children. They were trained in several series at the nature trails as young local nature guides, and learning new skills, connecting with their local communities, risk management, and emergency procedures indirectly empowered their forest survival knowledge. This research has been undertaken at Compartment 51, Lentang Forest Reserve, Pahang, with the active participation of young children aged seven to 14 who live within or adjacent to the protected area. This gives them a sense that they have a role to play and that their actions have an impact on improving their surroundings while also developing strong interpersonal skills. That skills will contribute to better relationships in education, recreation business services and society. The journey of the junior rangers' training, capacity building, and experiences has been compiled as published module guidelines. The guideline provides the steps and measurements taken as a procedure or model to establish a junior ranger group in other forests and protected areas. The establishment of junior rangers contributes to bridging the science communication gap between scientists and the public, especially the forest communities, for a better sustainable future.

Keywords: Human-nature interactions, environmental education, community, recreation, extinction of experience

INTRODUCTION

The responsibility of conserving the forest environment is getting more and more attention from all parties including the local community. Forests and their associated products have remained essential in sustaining livelihoods (Mukul et al., 2016) and these benefits made forests become fundamental to the livelihoods and well-being of people, not only for the people who live in them, but also for those living in the peripheral landscapes. Many alternatives were developed to improve habitat destruction in forest environments, including empowerment of local communities' participation. Community-based ecotourism (CBET) is often regarded as a panacea by creating an alternative source of livelihood indirectly provide a medium or platform for local communities to actively participate in various stages of CBET development. Community-based ecotourism (CBET) in Malaysia is an option especially for the rural children adjacent to the protected areas. The benefit of CBET should be extended to young children's roles in serving tourists. Furthermore, the local communities' involvement continuously associated to the nature conservation.

More and more people, especially children, have less and less contact with nature, an ongoing alienation termed "the extinction of experience". For the majority of people today, outdoor nature experiences are vanishing and being replaced by virtual alternatives (Clements, 2004; Pergams & Zaradic, 2006; Hofferth, 2009; Ballouard et al., 2011). Arguably, the root driver of the loss of human-nature interactions is the loss of opportunity to experience nature (Soga et al., 2016; Sun et al., 2023). Experiencing nature at an early age might critically affect one's perception of nature. Childhood nature experience is an important factor in navigating these individuals toward conservation activities. As people's alienation from nature could be a significant threat to the conservation of natural environments, the junior ranger programme in Ulu Tampik Waterfall called Tampik Junior Rangers is an ideal tool to foster strong and lasting bonds between local communities and their protected areas. This study was undertaken to (i) to educate and empower the public especially school children in rural area for greater support and engagement in natural resource conservation and to (ii) to provide information, education and training among local people around Ulu Tampik Waterfall (UTW) especially the young children known as Tampik Junior Rangers (TJR).

RESEARCH METHODOLOGY

Study area

This project was conducted in Ulu Tampik Waterfall (UTW), located at Kampung Janda Baik, Bentong district, Pahang, Malaysia. The area was approximately 45-minute drive from downtown Kuala Lumpur. It was located in compartment 51 of LFR and managed by Bentong District Forest Office. The environment was a unique and exciting recreation area. The main physical component was the uniqueness of the waterfall and its clear water purity (Amirnodin, 2020). The UTW and its environment was a popular leisure area for local residents of Janda Baik, visitors and tourists from other areas as well as foreigners. As of April 2020, currently this area was within the Use Permit area, operated by the local society as an association known as Persatuan Sahabat Alam Tampik Janda Baik (SATJB). The society was the official registered society under The Registry of Societies Malaysia (ROS) managed by the local communities and the land was protected water catchment area. The SATJB initiated the establishment of TJR to enhance the support for the environmental conservation of UTW.

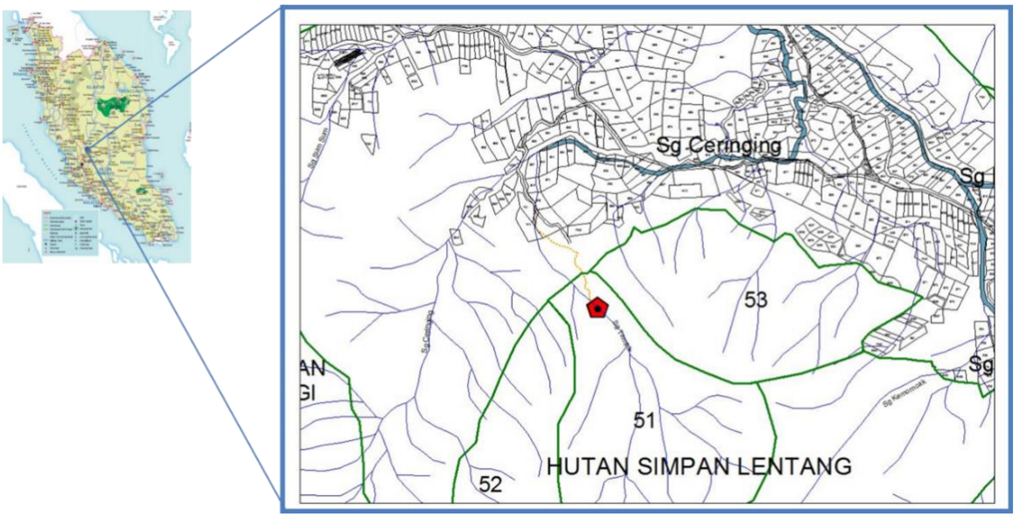

Figure 1 Map of Ulu Tampik Waterfall (UTW) as a project site location

RESULTS AND DISCUSSION

Junior ranger has been trained and gain experienced as skilled young nature guides or environmental interpreters. This opportunity has exposed the juniors and young school children on the importance and preservation of natural resources. This programme is open and on volunteer-based to the young schoolchildren. They were trained in the field site as a young local nature guide and learning new skills, connecting with their local communities, risk management and emergency procedures indirectly empowered their forest survival knowledge. Module of trainings and capacity building involved three different series with specific approach. The table listed the series of capacity building that involved active participation of young children aged seven to 14 who live within or adjacent to the protected area.

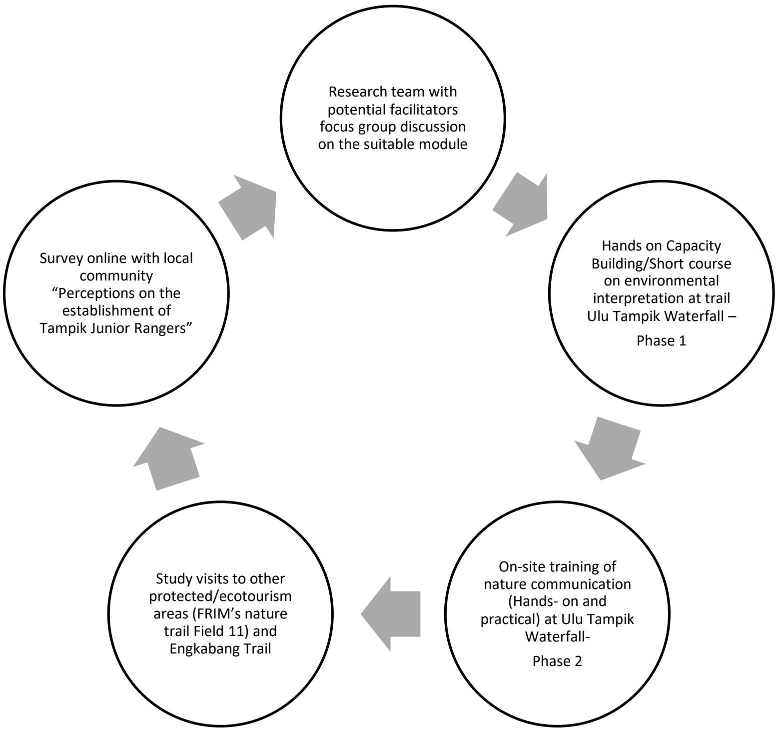

Figure 1 The community development programme through Tampik Junior Rangers in 2022 to 2023

The first series was a workshop to train the TJR on environmental interpretation. This workshop was held on 6 to 7 August 2022 in Kampung Janda Baik and was organized jointly with the local community and the SATJB- Tampik Janda Baik Friends of Nature Association. In the first series, the junior rangers assigned a group tasks that correspond to the four different storytelling theme. In the "Oh My Trees!" activity, the participants have planted five trees and the planted trees will be monitored based on the manual of instructions in Tree Planting Eco Kit. Each of the junior ranger also had special task assignments that particularly on practical training in the field. The practical held at the Ulu Tampik Waterfall trail in Lentang Forest Reserve. The instructors and facilitators briefed the participants on the information of the selected forest trees including bamboo along the nature trail to the peak of waterfall at Tier five. From the information and their knowledge especially on five selected species on forest trees the junior rangers had to interpret and present their findings in the closing session through the "Let's Hear My Stories" segment.

The community development "Oh My Forests" (Series 2) organized on 3rd of December 2023 has exposed junior rangers on two main elements. The first element was to inform the junior on the ethics as a tourist nature guide. The second element involved on the presentation in "What and Who is the Trees" segment. Through this session it trained junior rangers to recognize tree species such as Meranti bukit, Perah and Pulai including several types of bamboo. Junior rangers have been evaluated through their individual presentation held at the end of the series with the theme "Listen to...Trees, Nature and Us".

Series 3 of community development involves a learning visit session to the FRIM campus held on 7 March 2023 to explore several attractions such as the Keruing trail, crown shyness and a visit to Field 11. The third series involved 13 junior rangers and two FRIM's nature guides. Junior rangers had the opportunities exchanged knowldege such as do and dont's as a nature guides and ethics to become nature guides through this exchange learning session. The visits also exposed them to the forest tree species at Engkabang trail and crown shyness as FRIM's main attractions.

CONCLUSION

TJR programme is open and on volunteer-based to the young schoolchildren. The establishment of a guide group in Kampung Janda Baik, Bentong, Pahang is one of the local community initiatives under the guidance of FRIM researchers to ensure that the local community also plays a role in the preservation and conservation of forest treasures. Tampik Junior Rangers (TJR) were formed as part of the association initiative to increase support for UTW environmental conservation. This opportunity exposed the juniors and school children to the importance and preservation of natural resources. With the continuous support from Forest Research Institute Malaysia (FRIM) as a technical advisor to the community-based ecotourism project (CBET), various activities have been carried out. Indirectly will influence their attitudes to nature conservation and provide direction to frame engaging conservation messages and connect young children with nearby nature.

The young or junior rangers' establishment improved and upscale their communication skills on the forest-based and natural resources through three difference series of community development programme. The journey of the junior rangers' training, capacity building and study site visit activities has been compiled as a published guideline. Thus, this alternative is one of the best practice in bridging the science and forestry for a sustainable future. The guideline caters an up-to-date evidence and measurements taken as a procedure or model to establish a junior ranger group in other forest and protected areas.

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BIOMASS PRODUCTION AND ALLOCATION OF TONGKAT ALI (*EURYCOMA LONGIFOLIA*) FROM VARIOUS AGES IN SELECTED TONGKAT ALI PLANTATIONS IN PENINSULAR MALAYSIA

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ABSTRACT

It is known that growth parameters are proportional to age and are also influenced by environmental factors and genotype. This study was conducted to evaluate the biomass production of tongkat ali (*Eurycoma longifolia*) plants. The target studied were as young as 5 years old up to 25 years old. These 5-year-old plants were chosen based on the initial harvest age when tongkat ali was planted, whereas those 25-year-old plants were the age when tongkat ali was first introduced in Malaysia. Data collection was carried out in five (5) selected locations, namely SPF Mata Ayer, Perlis (7 years old), KESEDAR, Kelantan (12 years old), Malaysian Rubber Board Bandar Penawar, Johor (17 years old), MARDI Kluang, Johor (22 years old) and Kuala Nerang, Kedah (27 years old) which were intercropped with other trees. The selection included an additional age range of around two (2) to three (3) years from the age of the targeted plants. Results of this post-harvest report showed that tongkat ali in Kluang, Johor, had the highest average in diameter, root length and biomass, while on average, the tallest and longest stem length of tongkat ali were recorded in Kuala Nerang, Kedah. Based on the above data, the growth of tongkat ali depends on the age, type of soil and spacing of the plants. This study shows that the best harvest results were in Kluang, Johor, compared to Kuala Nerang, Kedah, which was older. This is due to the combination of coconut plants with tongkat ali compared to teak with tongkat ali using different planting designs and distances. Integrated cultivation is suggested to give better results compared to monoculture.

Keywords: Tongkat ali, biomass production, plant age, agroforestry, biomass allocation

INTRODUCTION

Eurycoma longifolia is one of the famous herbal medicinal plants found in Southeast Asia (Malaysia, Vietnam, Java, Sumatra, Thailand). Commonly known as tongkat ali, its medicinal properties have been known for centuries. Traditionally, it is used as an afterbirth tonic, reducing fevers, curing mouth ulcers and treating intestinal worms (Burkill, 1993).

Until now, the commercial use of *E. longifolia*, the raw materials are mostly obtained from forest reserves without knowledge of their age. This leads to the different quality of growth and phytochemical contents. According to Abewoy (2021), the yield of plant material and the composition are strongly dependent on the development stage of the plant, and therefore, harvesting age is one of the most important factors that affect the quality and quantity of plant material.

Ex-situ conservation approach with a proper planting method can ensure the harvesting age with targeted compounds. In the late1990's, planting activities began to control the quality of compounds and biomass.

MATERIALS AND METHODS

Tongkat ali trees were collected from an existing farm in Stesen Penyelidikan FRIM (SPF) Mata Ayer, Perlis, KESEDAR, Kelantan, Bandar Penawar and Kluang, Johor, Setiu, Terengganu and Kuala Nerang, Kedah.

Eurycoma longifolia were harvested at various ages from a total of five locations. Three replicates were harvested to represent each age group studied. Physical parameters studied were stem length, stem diameter, root length and weight of *E. longifolia* plant parts (leaf, stem and root).

RESULTS AND DISCUSSION

Effect of Harvesting Age on Physical Characteristics of *Eurycoma longifolia*

Based on Table 1, the physical characteristics were obtained from five different locations according to age, soil type and integrated plants involved. Branching characteristics were also observed besides the measurement of fresh weight, stem diameter, and length of stem and root.

Figure 1 shows the mean stem length is 3.4 m in trees aged ±5 years and starts to increase up to 11 m in length at the oldest age of the study (±25 years). This is in line with the height over stem diameter ratio as shown in Figure 2, which starts at 3.6 inches (±5 years), 6.3 inches (±10 years), and slightly reduced at ±15 years to 5.7 inches, 7.7 inches (±20 years) and 7.5 inches (±25 years), respectively.

Table 1 Physical characterization of *Eurycoma longifolia* trees based on different ages

Characterization	Harvest Age (Years)				
	±5	±10	±15	±20	±25
Locations	SPF Mata Ayer, Perlis	KESEDAR, Kelantan	Bandar Penawar, Johor	Kuala Nerang, Kedah	Kluang, Johor
Trees Integration	Under Pine tree	Rare tree	Rubber	Teak	Coconut
Soil Type	Minerals	Minerals	Minerals	Minerals	Minerals
Soil Series		Bungor/ Tai tak	Gajah Mati	Bungor	Renggam
Branching Characteristic					
Branching	50%	100%	100%	84%	66%
Unbranched	50%	0%	0%	16%	33%
Fresh Weight (kg)					
Root	1.8	3.1	3.8	6.7	11.2
Stem	4.43	17.8	21.5	37.2	60.5
Leaf	1.18	1.2	3.9	2.5	8.5
Biomass of Each Part					
Stem Length (m)	3.4	6.1	7.8	10.9	11.0
Stem Diameter (cm)	3.6	6.3	5.7	7.7	7.5
Root Length (m)	0.5	0.5	0.6	0.8	1.3

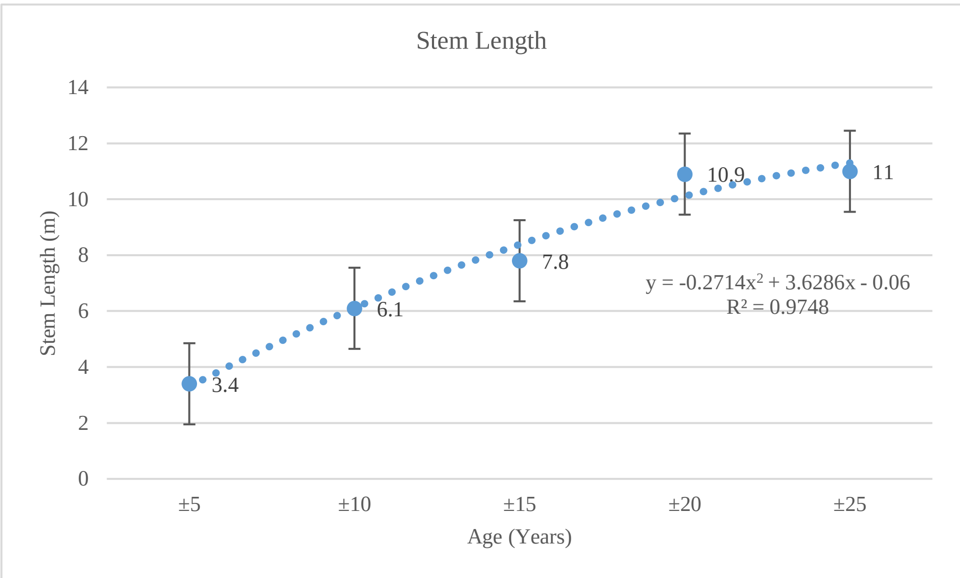

Figure 1 Changes of *Eurycoma longifolia* stem length at different ages

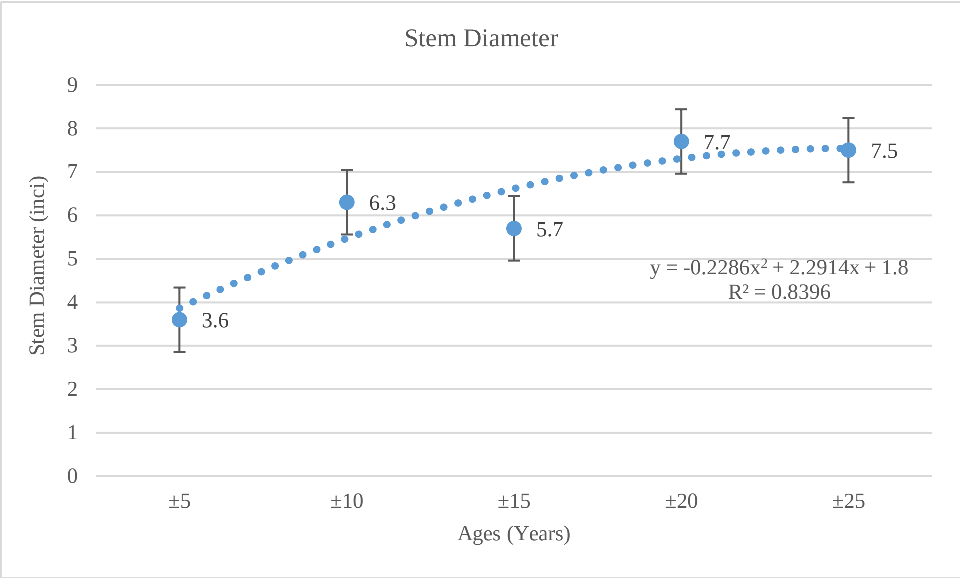

Figure 2 Changes of *Eurycoma longifolia* stem diameter at different ages

Root growth is also seen to increase with stem growth. This is to balance the ratio of stem length to root so that the tree can grow to get enough light. Based on Figure 3, root length is between 0.5 m at the age of ±5 years to 1.3 m at ±20 years and 0.8 m at ±25 years. The biomass of the tree probably influences a decrease in root length at that age which can be observed in Figure 4.

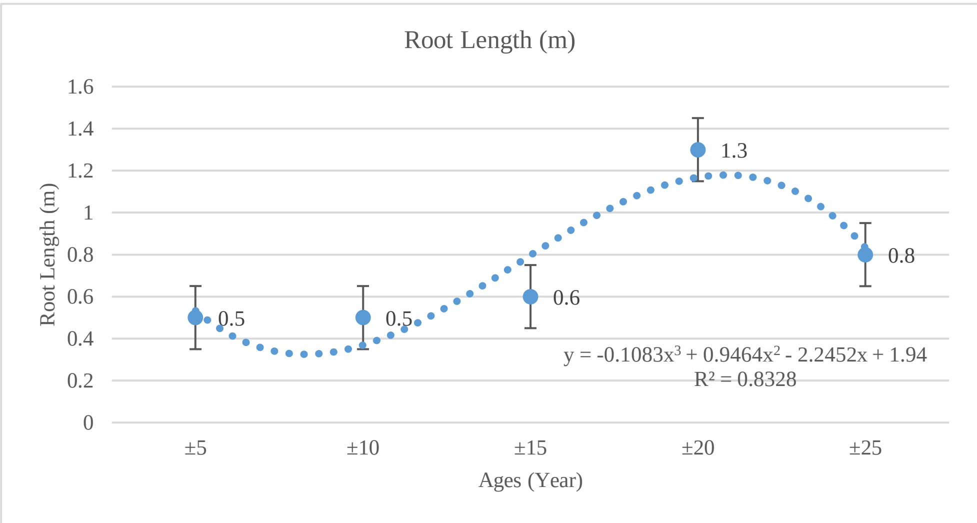

Figure 3 Changes of *Eurycoma longifolia* root length at different ages

Figure 4 shows that stem biomass contributes to the highest biomass proportion, averaging 73.8%, followed by root and leaves. Average root and leaf biomass proportions were 13.9% and 12.3%, respectively.

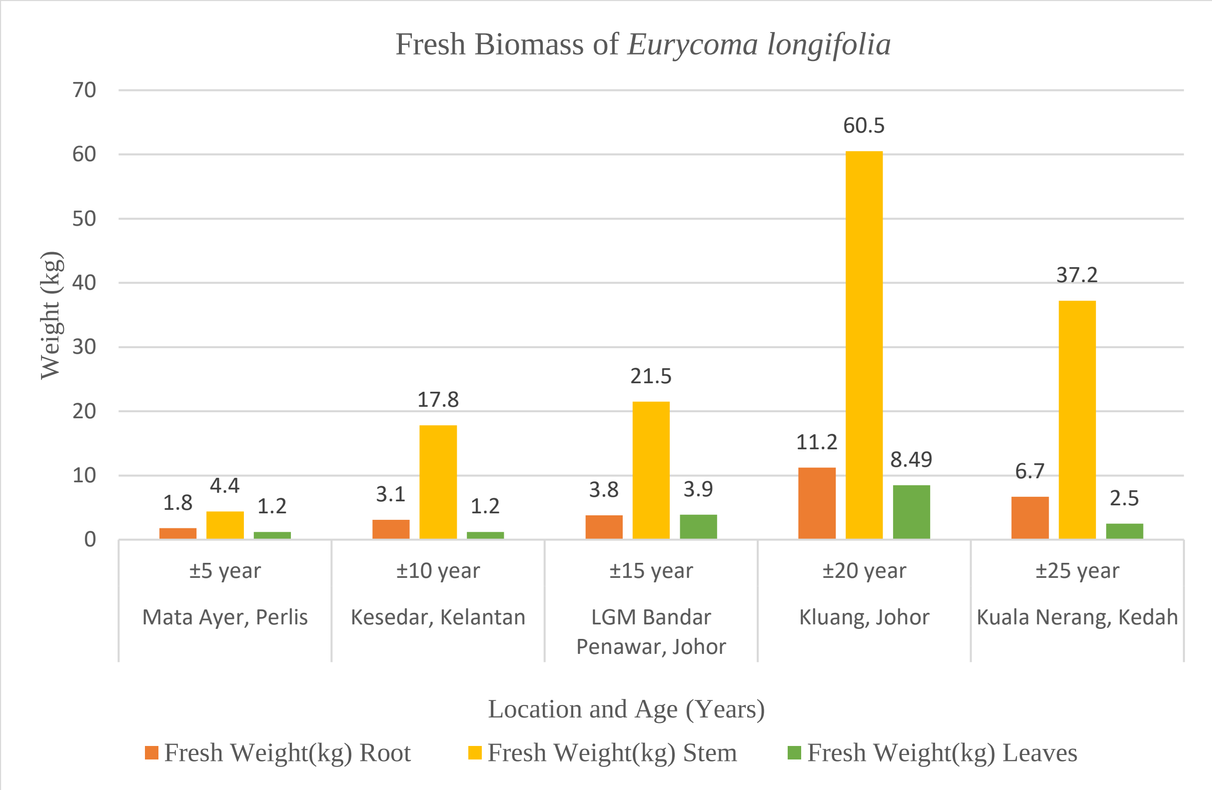

Figure 4 Fresh biomass of *Eurycoma longifolia* according to location and age

Based on the data above, the growth of tongkat ali depended on the age, type of soil and also spacing of the plants. The results showed that the highest harvest was recorded in MARDI Kluang, Johor, compared to the older plants in Kuala Nerang, Kedah. This is due to the combination of coconut plants with tongkat ali in MARDI compared to teak with tongkat ali in Kuala Nerang using different designs and spacings of plants. The results obtained from the study are in line with other studies on some plants. Ma et al., (2021) emphasized that harvest age is interrelated with the optimization of biomass production and the importance of determining the appropriate harvest stage before any deterioration in biomass, active ingredient content and quality as a guideline for harvesting and processing. This shows that the harvesting age is one of the most important factors influencing the yield of raw materials.

CONCLUSION

Results obtained during this study showed that the root and stem growth rates are influenced by age. For this study, *E. longifolia* should be harvested at the age of around 10 to 20 years to ensure optimum yield. However, this also depends on the type of soil and whether it involves planting as monoculture or integration with other plants. This new information is needed in future research for the production of high-quality planting materials for the global market. In fact, it serves as a guide for farmers to cultivate high-quality *E. longifolia* to produce healthcare products.

ACKNOWLEDGMENTS

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ASSESSMENT OF THE EFFECTS OF CHANGE IN FOREST ECOSYSTEMS THROUGH THE TekamFACE® SYSTEM

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ABSTRACT

The TekamFACE (Free Air Carbon Dioxide Enrichment) system was developed to study forest ecosystem changes due to free enrichment of carbon dioxide gas in production forests. The system built in Compartment 84, Tekam Forest Reserve, Jerantut, Pahang near the FRIM Jengka Research Station (SPF Jengka) was developed by the Forest Research Institute Malaysia (FRIM) with the cooperation of the National University of Malaysia (UKM), the Pahang State Forestry Department (JPNPhg) through the Jerantut District Forest Office (PHDJ), and the Forestry Department Peninsular Malaysia (JPSM). The study involving the TekamFACE System began with the signing ceremony of the Memorandum of Understanding (MoA) between FRIM and UKM on 12 April 2016 at UKM, followed by the installation of the steel structure on 10 October 2016 until the completion of the construction of the TekamFACE system on 16 February 2017. This was followed by a launch ceremony held during a Memorandum of Understanding on 3 August 2017. This study published many non-technical and technical articles, including journals, books, and manuals, received wide coverage through the mass media, received recognition, and received awards. In addition, it was the focus of studies involving flora, fauna, hydrology, phenology, physiology, seeds, soil, microbes, and genetics. The study received allocation through the development project of the 11th Malaysia Plan under the title Assessing the Impact of Climate Change on Forest Ecosystems to Form Appropriate Adaptation Strategies and continued in the 12th Malaysia Plan under the title Strengthening the Role of Forests in Addressing Climate Change.

Keywords: GHG, adaptation, forest ecosystem, species

INTRODUCTION

The concentration of carbon dioxide (CO_2)) in the earth's atmosphere has increased to almost 30% since the mid-1800s because of daily human activities (Barnola et al., 1995; Cha et al., 2017; Lachgar et al., 2022; Trenberth, 2018; Wang et al., 2020). The increase in CO_2 concentration is a global concern because it affects the forest ecosystem, especially forest productivity (Karnosky, 2003). Several studies have shown an increase and decrease in forest productivity or individual species due to increased CO_2 (Ainsworth & Long, 2005; Boisvenue & Running, 2006; Ellsworth et al., 2017; Morin et al., 2018; Norby et al., 2010). A study by Ainsworth & Long (2005) showed that a high increase in CO_2 in the 12 *Free Air CO_2 Enrichment* (FACE) study areas has helped to increase carbon intake during the photosynthesis process, which is an increase in light saturated carbon intake (Asat) by 31% and 28%, respectively, increasing the carbon assimilation rate of diurnal photosynthesis (A').

Ellsworth et al. (2017) showed that the productivity of *Eucalyptus* oil did not significantly increase despite the increase in CO_2 over a period of three years. It only helps to increase the process of leaf photosynthesis by 19% and 35%, respectively, when there is an increase in P in the soil. Norby & Zak (2011) and Norby et al. (2010) have reported that increased CO_2 and limited nitrogen (N) will decrease the productivity of deciduous forests of *Liquidambar styraciflua* in 2001 - 2003 from 24% to 9% in 2008. Decreased forest productivity can also occur in high CO_2 conditions. However, the phosphorous content of soil and water is limited, which prevents tree growth (Boisvenue & Running, 2006).

In addition, studies using computer modelling projections by Azian et al. (2018) showed that the productivity of the forest increased by about 13% or 12.8 GgC per ha if the CO_2 and temperature rise under the RCP 8.5 scenario (worst case scenario), as well as decrease in forest productivity and affect the soil by 7% or 8 GgC per ha if the temperature rise under the RCP 8.5 with the carbon of 1990 scenario by 2099 (Azian et al., 2022).

Most FACE systems have been constructed for agricultural crop studies, such as soybean, native grassland, wheat , and rice (Izaurralde et al., 2003; Kim et al., 2003; Kimball, 1983). Additionally, the first forest research FACE system was developed in North America to study the effects of high CO_2 elevation on l15-year-old trees (Hendrey et al., 1999). The system has three levels of high atmospheric CO2 at 200 ppm and above normal ambient levels and is administered 24 hours per day. However, most FACE systems built for forest research focus only on temperate ecosystems, whereas tropical, boreal, and Arctic ecosystems remain unexplored (Ainsworth & Long, 2005).

Since the current knowledge and understanding of the response of increase of CO_2 towards tropical forests is poorly understood, especially in Malaysia, the Forest Research Institute Malaysia (FRIM) has taken the initiative through allocations under the 11th Malaysia Plan (RMK11) and 12th (RMK12) to carry out study entitled "Evaluation of the Impact of Climate Change on Forest Ecosystems and the Formation of Appropriate Adaptation Strategies" as well as "Strengthening the Role of Forests in Addressing Climate Change". This study was supported by the cooperation of Universiti Kebangsaan Malaysia (UKM), the Pahang State Forestry Department (JPN Phg) through the Jerantut District Forest Office (PHDJ), and the Peninsular Malaysia Forestry Department (JPSM). The objective of this study and collaboration is to assess the impact of an increase in CO_2 on the composition of forest species. This information will help provide additional information on the sustainable forest management system and its impact on climate change.

METHODOLOGY

Study Area

The FACE study on forest ecosystems in Malaysia was first developed in 2010. The study was initiate by a researcher from Universiti Kebangsaan Malaysia (UKM) Bangi, Selangor, in their research forest reserved. To ensure that this project can be carried out in an active forest still harvesting, FRIM took the initiative to establish a similar but larger FACE system in Compartment 84, Tekam Forest Reserve, Jerantut, Pahang, which has an area surrounding of 228 ha. The study area is close to the FRIM Research Station (SPF) in Jengka, Pahang. This forest is regulated under the management of the PHDJ. This area was chosen after considering several issues, especially the implications of the construction work and development of the system, selection of forest types, travel distance, historical data collection, easy access routes, electricity sources, long-term studies, safety of the equipment, data collection, approval, and costs.

Establishment Plots and the TekamFACE System

Two study plots have been established in the study area, i) FACE plots and ii) control plots, which are both square shaped with a size of 25 m by 25 m or 0.0625 ha of each plot (Figure 1). The FACE infrastructure was constructed in the form of a hexagon structure with a height of 12 m, sides of 6 m sides, 50 m flow pipes, and a control house where all automated systems can control the release of CO_2 in the plots. The construction of this system started on 10 October 2016 and was completed on 16 February 2017. The first release of CO_2 was held on 3 August 2017, during the opening ceremony of the TekamFACE system. However, CO_2 emissions officially started on June 1, 2018.

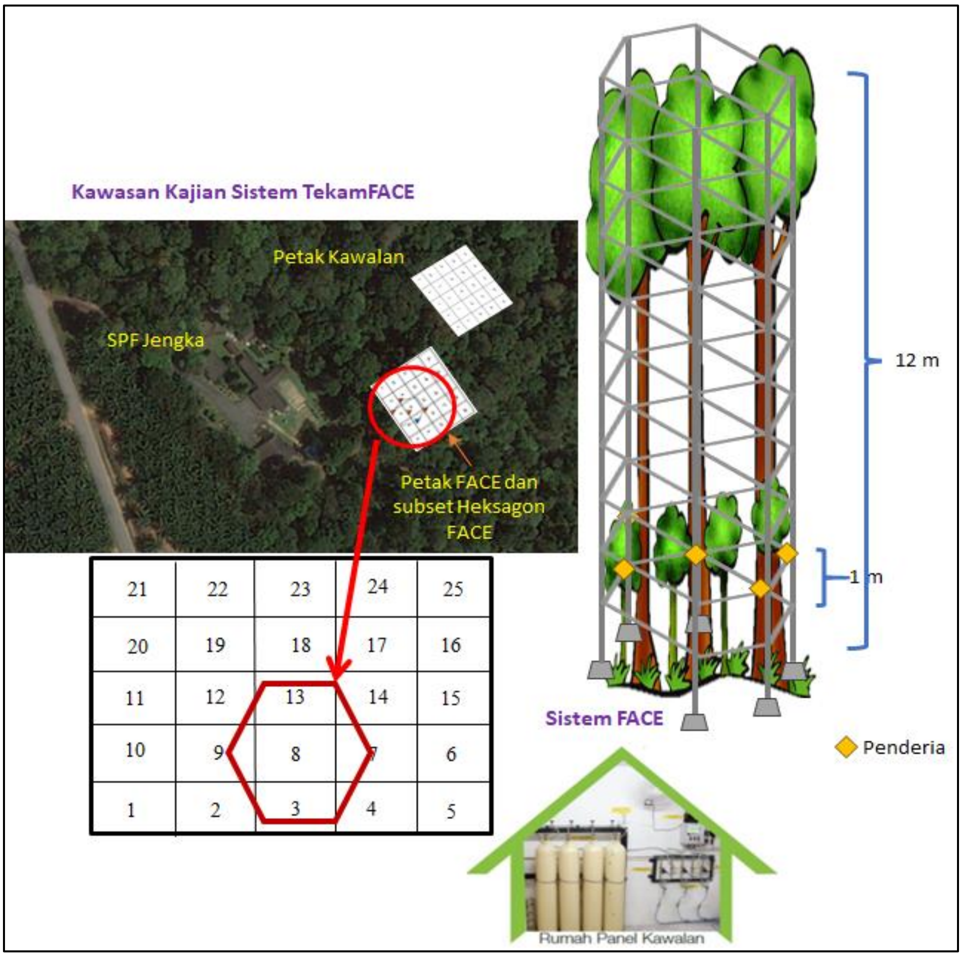

Figure 1 The position of the FACE plot and the control plot (top left), an illustration of the hexagonal position of the FACE inside the FACE compartment (bottom left), and an illustration of the TekamFACE® system (right) in Compartment 84, Tekam Forest Reserve, Jerantut, Pahang

In general, of the operational journey of the TekamFACE system starts with the release of CO_2 gas from the gas barrel in the control house, through the flow pipe to the environment area inside the hexagon, then the transmission of data from a CLTM® sensor device via LAN/Wifi, storage the data captured about five parameters into the computer through EZ ICMS software, and monitoring through an android phone as shown in Figure 2 (Azian et al., 2020).

The CLTM® sensor was installed in the hexagon to measure the forest environment. Among the parameters measured are (i) CO_2 (ppm), (ii) air humidity (%), (iii) light intensity (Lux), and (iv) temperature (°C) measured every 30 min over a period of 1 hour. The injection of CO_2 gas into hexagons in the normal environment is about ± 50 ml per injection.

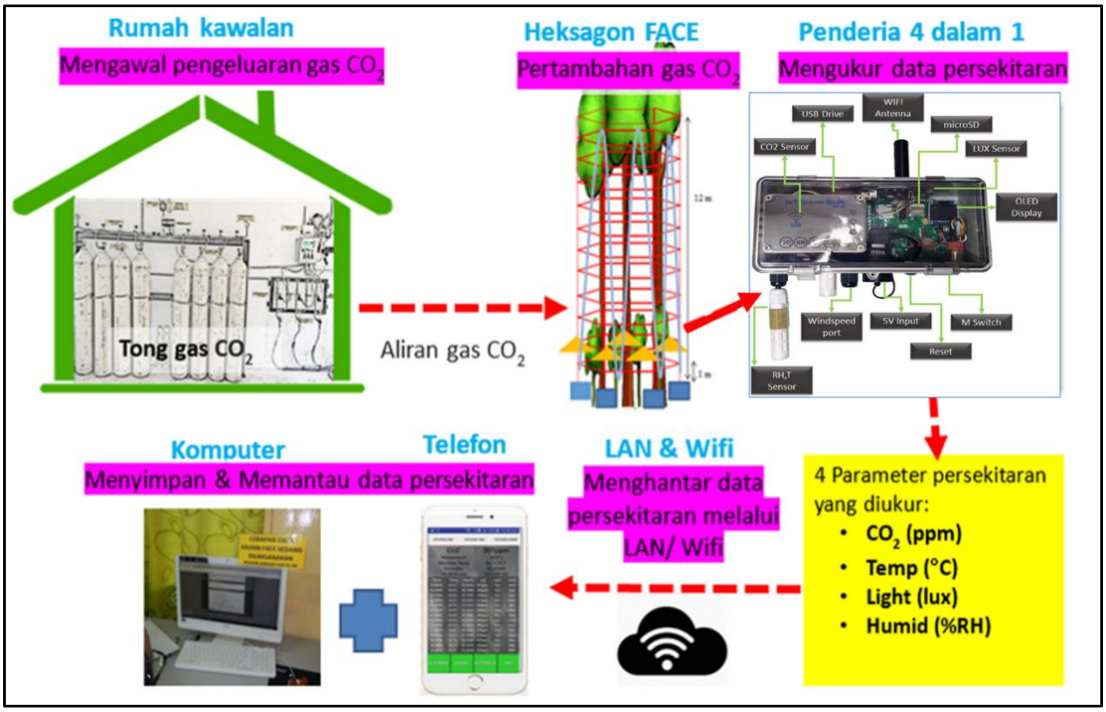

Figure 2 TekamFACE System operation

Advantages of the TekamFACE® System

This fully automated system is designed to ensure that forest environment data can be received in many ways in real time, even when the system was developed in the forest. The system can also be monitored with just one tool, which is captured through the CLTM® censor. In addition, the raw data are stored centrally in the EZ ICMS database in *.csv format. Data are also easy to store, access, and manage.

Data Usage Target of TekamFACE

The TekamFACE system can be adapted for any use of research activities, especially those involved and needing information for forest ecosystem adaptation studies towards increasing CO2. This study is essential for the forestry sector to remain relevant in dealing with climate change issues. Among the users of the TekamFACE system are to study the effects of CO_2 level in the forest ecosystem through the study of flora productivity, physiology, fauna, phenology, soil, microbes, seeds, hydrology, and the level of gene expression in plants.

RESULTS AND DISCUSSION

Environmental Data

Figures 3 to 6 show the environmental data obtained in the hexagonal area from 2017 to 2022. The data obtained for carbon dioxide from 2017 to 2022 show an average level of 507 ppm inside FACE plots compared with the control (438 ppm) (Figure 3). The average temperature was approximately 26.8 °C in the FACE plot and 26.3 °C in the control plot (Figure 4). The average light intensity rate was approximately 101.4 lux (Figure 5), and the relative humidity was 88.5 Lux (Figure 6).

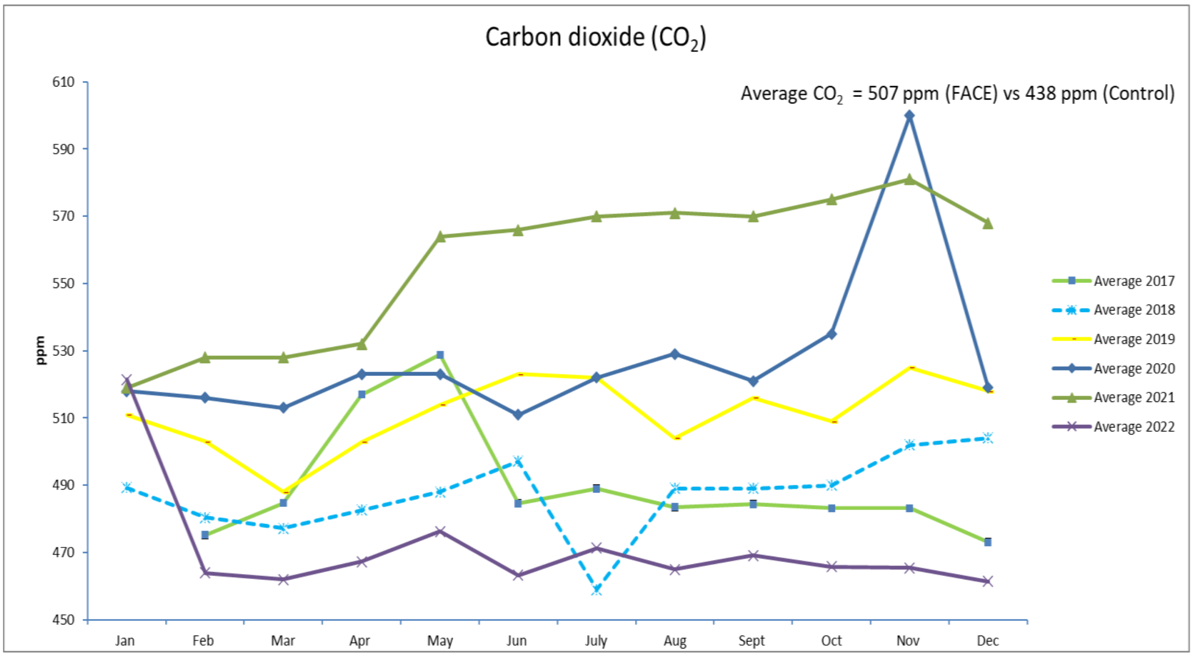

Figure 3 Level of CO_2 from 2017 to 2022

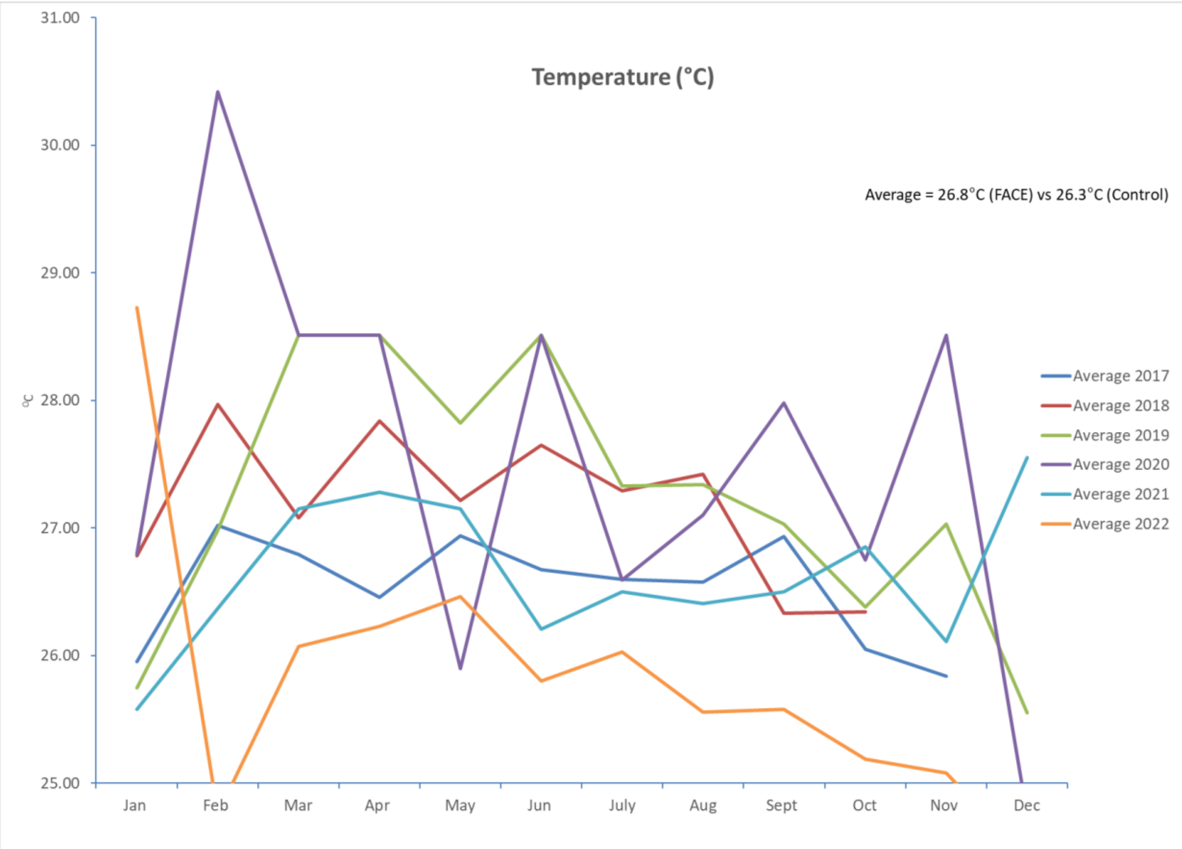

Figure 4 Level of temperature from 2017 to 2022

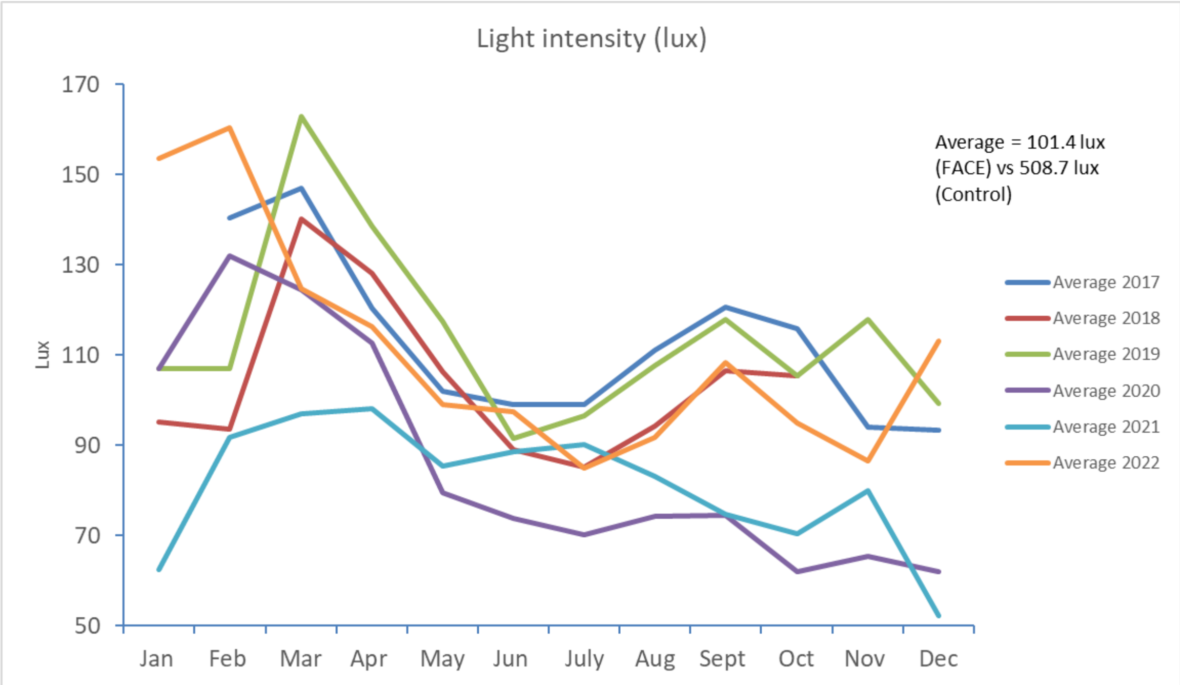

Figure 5 Level of light intensity in lux from 2017 to 2022

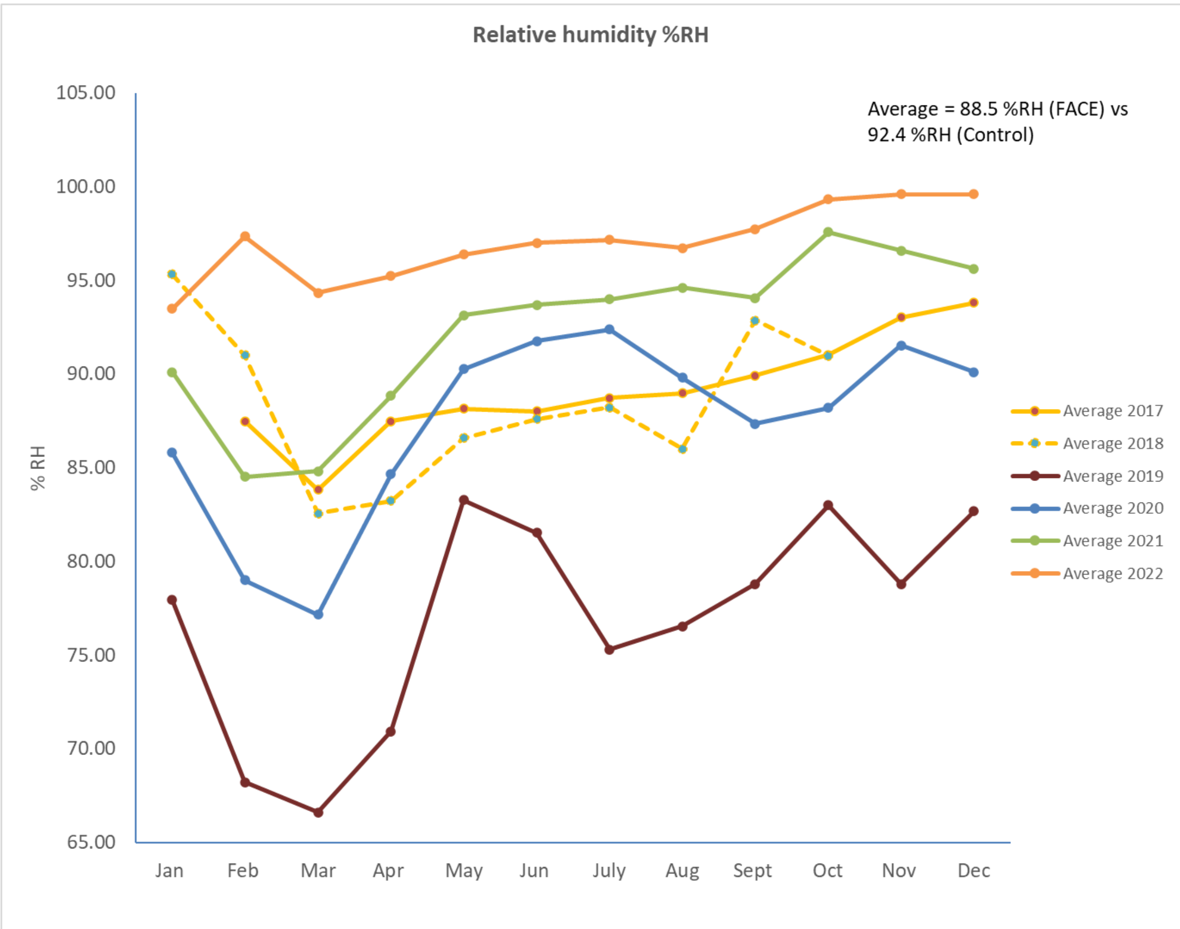

Figure 6 Level of humidity (%) from 2017 to 2022

CONCLUSION AND RECOMMENDATIONS

The study conducted in the TekamFACE system, Tekam Forest Reserve, Jerantut, Pahang, is one of the efforts to answer the question of the effects of climate change, especially carbon dioxide gas, on the forest ecosystem based on actual observations carried out in the field. Good forest management and the changes that occur need to be identified, and there is a strategy to identify those changes. The results of this study are expected to attract outside scientists to conduct research together as well as help forest managers in managing, preserving, and conserving the forest ecosystem if changes occur with the increase of CO2 in the future. In addition, this study can help institutions provide technical and scientific input to policy makers.

However, it is still too early to obtain the effects that have an impact on the forest ecosystem because this kind of study requires a long time, which is more than 10 years or the period of one cycle of forest exploitation. The observations and results from this study are expected to only help in preparing an initial report on the forestry sector's adaptation measures strategy based on initial observations of the effects of CO_2 increase seen in the short term.

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HCD1 AS A SAFE AND EFFICACIOUS ANTI-MRSA AGENT

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been known to display multidrug-resistance properties towards a wide range of structurally-unrelated antimicrobial agents. Currently, only a handful of antibiotics can inhibit this dangerous nosocomial pathogen. Thus, a dire need for an alternative source of antimicrobials is crucial to reduce MRSA infections worldwide. Previously, we have successfully synthesized a plant-based halogenated codonopsinine derivative (HCD1) that showed good inhibitory activity against a panel of MRSA isolates with MIC values of less than 8 µg/ml. In this study, HCD1 exhibited low cytotoxicity with IC_{50} values of more than 300 µg/ml against three normal mammalian cell lines. Following that, all three toxicity studies (acute, sub-acute, and sub-chronic) in Sprague-Dawley (SD) rats displayed no significant physical, biochemical, and hematological toxicity. In the mice protection assay, HCD1 provided an 83% survival rate via the intraperitoneal route against an MRSA infection at 62.5 mg/kg. Based on this study, the mean effective dose (ED_{50}) was calculated at 38.76 mg/kg. Transcriptomic analysis revealed that HCD1 may disrupt MRSA survival by repressing its energy production hence abolishing the regulation of various important transmembrane transporters and formation of biofilm in the cell. Six selected down-regulated genes were also validated using RT-PCR. Lastly, scanning electron microscopy (SEM) showed structural and cytoplasmic membrane disruptions in treated MRSA cells against HCD1. In conclusion, HCD1 is highly recommended to be developed further as a safe and efficacious novel antibacterial agent against MRSA.

Keywords: MRSA, plant-based synthetic compound, *in vivo* toxicity, mice infection

INTRODUCTION

MRSA is a nosocomial-related, Gram-positive bacteria that have been known to display multidrug-resistance properties toward a wide range of structurally-unrelated antibiotics and antimicrobial agents (Johari et al., 2023). Currently, MRSA is one of the most common antibiotic-resistant and economically relevant pathogens while maintaining its reigning status as one of the main causes of hospital-acquired infections in all regions of the world (Xu et al., 2021, Gajdács, 2019). Ironically, the development of novel antibiotics has been dwindling almost to a halt as only two new classes of anti-MRSA drugs (linezolid and tedizolid from the novel oxazolidinone class of antibiotics and daptomycin; a lipopeptide-class antibiotic) have been approved by the FDA in the last 50 years (Hindy et al., 2022; Scarafile, 2016, Gadakh & Van, 2015).

Hence, a dire need for a novel and alternative class of antimicrobials is very crucial to prevent or at least reduce MRSA infections worldwide. Previously, a group of scientists from IOS, UiTM have successfully synthesized novel derivatives of codonopsinine which represent interesting electron rich-functional groups believed to be responsible for many pharmacological properties using an economical one-pot reaction scheme (Mohammat et al., 2009). Initial minimum inhibitory concentration (MIC) assay revealed that one of the halogenated codonopsinine derivatives, HCD1, exhibited good inhibitory action with MIC value of less than 8 µg/ml against a panel of MRSA isolates.

In this study, *in vivo* toxicological evaluations, mice protection assay, molecular determination of mechanism-of-action, and SEM analysis were conducted towards the initial development of HCD1 as a potential novel inhibitory agent against MRSA. It is hoped that HCD1 will serve as an important addition to the depleting armament against this dangerous 'superbug'.

MATERIALS AND METHODS

HCD1 was synthesized at the Synthesis and Chemical Biology Laboratory, IOS, UiTM Puncak Alam and identified using NMR and FTIR methods as generally described previously with minor modifications (Mohammat et al., 2009). An *in vitro* cytotoxic effect of HCD1 against two normal mammalian cell lines (liver and kidney-like) was determined as described previously (Johari et al., 2023).

In vivo toxicity (acute, sub-acute, and sub-chronic) using SD rats was conducted as per the OECD 420, 407, and 408 guidelines, respectively (OECD 2002, 2008, and 2018). The survival rate and ED_{50} of HCD1 against MRSA systemic infection in ICR mice was determined as described previously with minor modifications (Im et al., 2011). SEM analysis was employed to observe the effect of HCD1 at 1x MIC value on the cell integrity and structural changes against an MRSA isolate (Johari et al., 2017).

Lastly, transcriptomic analysis via next-generation sequencing technique was used on the treated and non-treated MRSA isolate against HCD1 as described previously (Adnan et al., 2017). The generated transcriptomic data revealed which MRSA bacterial genes were affected once exposed to HCD1. Validation of affected genes was carried out using RT-PCR.

RESULTS AND DISCUSSION

Three batches of HCD1 were successfully synthesized and identified. The one-pot synthesis protocol follows the green chemistry initiative and has an eco-friendly production approach (Ryzhkova et al., 2022, Hayashi, 2016). HCD1 was produced using a one-step reaction that utilizes fewer solvents, and less chemical waste was released for the environment as compared to conventional multi-step reactions (Ryzhkova et al., 2022; Hayashi, 2016). Additionally, HCD1 is easier to synthesize due to its less complex structure as compared to the other current MRSA antibiotics, such as vancomycin and linezolid. Technically, HCD1 can easily be prepared on a multigram scale in a relatively short time and at a moderate 60% yield, making it an economically attractive compound for further development as a new antibiotic.

The fact that HCD1 portrayed a five-membered carbon ring as opposed to the conventional four-carbon ring of β-lactam might give way to a new class of antibiotics against MRSA infections. Since HCD1 is only active against *S. aureus* isolates, it can be inferred that HCD1 has a narrow-spectrum microbiological activity. Previous studies showed that narrow-spectrum antibiotics are more favourable as compared to broad-spectrum antibiotics since this type of drug would be less likely to develop antimicrobial resistance and kill 'good' bacteria in the human body (Melander et al., 2018).

In the cytotoxicity study, HCD1 showed low toxicity with mean inhibitory concentration (IC_{50}) values of 335 µg/ml and more than 500 µg/ml against Vero (kidney-like) and WRL-68 (liver-like) cell lines, respectively. Based on this result, toxicity studies using animal models are warranted. In the *in vivo* acute toxicity study, no mortality or behavioural changes were observed in SD rats treated with 300 mg/kg and 2000 mg/kg of HCD1, indicating that the mean lethal dose (LD_{50}) is higher than these doses.

Based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) scheme, the estimated LD_{50} for HCD1 was categorized in Category 4 (> 300 mg/kg < 2000 mg/kg). This classification is for chemicals that are harmful if swallowed while carrying a "Warning" signal word (United Nations, 2019). Although HCD1 would be categorized as harmful, other common antibiotics such as amikacin and clindamycin also have LD_{50} values of more than 2000 mg/kg (Cayman Chemicals 2022a, b).

Subsequently, a sub-acute toxicity test in SD rats at a repeated dose of 125 mg/kg for 28 days, showed no signs of toxicity in the treated animals. Histological examination also supports the non-toxicity observations. Following that, the mice protection assay revealed that HCD1 provided an 83% survival rate via the intraperitoneal route against an MRSA infection in ICR mice at 62.5 mg/kg. Based on this study, the mean effective dose (ED_{50}) was calculated at 38.76 mg/kg. Since it was discovered that HCD1 was effective at 62.5 mg/kg, a lower concentration of HCD1 (70 mg/kg) is used for the sub-chronic toxicity study (repeated dose for 90 days) as opposed to the sub-acute evaluation. In this additional toxicity study, no significant physical, biochemical, and hematological toxicity was observed in the treated rats.

In the transcriptomic analysis, HCD1 had a huge impact on the treated MRSA transcriptome by significantly down-regulating 50 annotated genes associated with ATP binding, biofilm formation, virulence, toxin activity, iron binding and transport, and arginine biosynthetic process. In contrast, most of the up-regulated 40 annotated genes corresponded to various biosynthesis processes such as urea, pyrimidine, histidine, and riboflavin were up-regulated. This analysis revealed that HCD1 may disrupt MRSA survival by repressing its energy production hence abolishing the regulation of various important transmembrane transporters and formation of biofilm in the cell.

To verify the down-regulated genes involved, six genes were selected for RT-PCR (*graS*: ATP binding; *ssl7*: staphylococcal superantigen-like; *mnhB2*: ion transport; *sarT*; arginine expression system; *selX*: toxin activity and *sasG*: biofilm formation). As the results, all of the selected resistance-related genes showed a similar correlation as the results obtained from the transcriptomic analysis. Lastly, SEM analysis revealed structural and cytoplasmic membrane disruptions amongst the treated MRSA cells with HCD1 as compared to untreated bacterial cells.

CONCLUSION AND RECOMMENDATIONS

HCD1 is highly recommended to be developed further as a new safe and efficacious antibacterial agent against MRSA. It is hoped that HCD1 will serve as an important addition to the depleting armament against this dangerous 'superbug'.

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BUTTERFLIES OF FRIM – AN UPDATED DOCUMENTATION OF THEIR DIVERSITY: A PATH TO UNESCO RECOGNITION

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ABSTRACT

The grounds of the Forest Research Institute Malaysia (FRIM) are a patchwork of natural and man-made forests established on land consisting of farms and tin mines. The forested grounds host an interesting community of butterflies. The first inventory of butterflies of FRIM was carried out 41 years ago in 1982 and recorded 147 species. In a 2018 FRIM Compendium, the number of species reported was 206. A new project to re-inventory the butterflies of FRIM was carried out from February 2020 to November 2022. Formal cataloguing of butterfly specimens that had been collected or bred in FRIM and deposited in the FRIM Entomological Reference Collection indicated the total number of species recorded to 279. A field inventory resulted in the addition of approximately 2,330 butterfly specimens to the collection, of which 96% have been identified. It recorded 324 species, of which 98.5% were verified by at least one specimen deposited in the collection, and added a further 137 species to the total number recorded from FRIM. Many of the new species added to the checklist are likely to have been overlooked in the past. Among the species previously reported from FRIM, 92 were not recorded in the latest field inventory, and 19 of these lack specimen vouchers in the collection. Several of these could have been due to misidentifications, and a few are almost certainly so. Although the combined number of species recorded in FRIM in past and present inventories is 416, we estimate the number currently in FRIM to be closer to 395. It is likely that as the forests of FRIM continue to mature and succession takes place in the understorey, changes have occurred to the butterfly species composition, with some species becoming rarer or even extinct and others becoming more abundant. Some species may even be recent colonisers. Based on this inventory, good butterfly-watching areas have been identified in FRIM for the promotion of nature awareness and education. Important butterfly areas that are a habitat for rare species or have greater diversity have been identified, and suitable sites for annual monitoring are recommended. Among some of the interesting discoveries in FRIM were the previously unknown female of the very rare *Erionota hislopi*, and the rare *Elymnias dara,* for which the life history was documented for the first time in Malaysia. Articles on these findings are being drafted. A report listing and mapping important butterfly areas for inclusion in the Conservation Management Plan of the FRIM grounds has been prepared for the World Heritage Site (WHS) secretariat and Campus Committee. Grounds management practices that promote butterfly conservation are suggested. An article updating the FRIM butterfly checklist and a guidebook for butterfly watching in FRIM are being prepared. The findings and recommendations stemming from this project strengthen FRIM's position in obtaining world heritage site recognition under UNESCO and also serve to showcase FRIM as an organisation active in butterfly research and conservation.

Keywords: butterfly diversity, field inventory, nature conservation, education, awareness

INTRODUCTION

The forests of FRIM have an interesting history and composition. Much of it is forest that has been replanted on land that had been farmed and mined. Surrounded by natural forest, FRIM stands out as unusual because of its diverse plantings, including about 100 indigenous and exotic timber species in plantations and arboreta (Yong et al., 2011). The Dipterocarp Arboretum, established in 1929, hosts around 120 Dipterocarpaceae species, while the Non-Dipterocarp Arboretum, established in the same year, contains 169 species from 45 families such as Leguminosae and Meliaceae. The Coniferatum, established in 1949, has 23 gymnosperm species from the families Araucariaceae, Cupressaceae, Cycadaceae, Gnetaceae, Pinaceae, Podocarpaceae and Taxodiaceae. The Fruit Tree Arboretum I & II, established in 1979, includes 89 species of Anacardiaceae, Guttiferae, Meliaceae and Moraceae. The Monocot Garden, planted in 1981, comprises herbaceous plants from the families Araceae, Graminea, Marantaceae, Pandanaceae, Taccaceae and the dominant family Palmae. This botanical diversity supports an interesting butterfly community. However, the sole butterfly checklist for the FRIM grounds prior to this study was published 42 years ago by Tho & Mahyudin (1982). Since then, there have been changes in the forests of FRIM.

As the trees mature, the microhabitat changes and subsequently, the composition of butterflies may change too (Sant'Anna et al., 2017). In addition, there have been new developments in the FRIM grounds. Some examples are the establishment of the Bambusetum and the Ethnobotanical Garden in 1994, the Kepong Botanical Gardens in the late 1990s (Yong et al., 2011), and a Bamboo Garden within the Kepong Botanical Gardens in 2008 (Khairuddin, 2012). These developments have created heterogeneous habitats, influencing butterfly communities.

Since the first inventory by Tho & Mahyudin (1982), a further 59 species have been added, bringing the total recorded in the FRIM compendium to 206 species (FRIM, 2018). Most of the new additions were recorded during a 2013 survey along the TNB reserve line (57 species). However, this figure of 206 species in FRIM did not include many species collected and bred in FRIM after the first inventory, as many such specimens in the Entomological Reference Collection had not been identified and catalogued. Identifications of older specimens had also not been adequately verified. Furthermore, the first inventory focused primarily on larger and more easily seen species, and many of the deeper trails and forest areas in the outer boundaries of FRIM were not explored. To illustrate the point, a one-day survey conducted along parts of the Rover Track shortly after the publication of the 2018 compendium added a further 13 species new to the FRIM checklist. Given the ease with which new records were added, we believed that extensive sampling across the entire FRIM grounds and surrounding forest areas would reveal the presence of even more previously unrecorded species. Therefore, we re-identified, verified, and catalogued all butterfly specimens collected in the FRIM grounds in the past, and conducted a comprehensive butterfly inventory to further document butterfly species diversity in FRIM. In addition, good butterfly-watching areas and important butterfly areas were also identified based on the information obtained in this project.

MATERIALS AND METHODS

All butterfly specimens collected in FRIM before the 2020 inventory commenced were re-identified and assigned accession numbers, and their collection data were entered into the Entomological Reference Collection butterfly database. These included the specimens collected by Tho & Mahyudin (1982) and many specimens collected or bred by L.G. Kirton, M.W. Tan, A. Mahyudin, A. Saimas and C.-K. Phon.

New butterfly inventories were conducted in nature trails on the campus and the surrounding forest from February 2020 to November 2022. Two to three days of field collecting and observation were carried out nearly every month in good weather from about 10.00 am to 4.00 pm. Shrimp paste was used as bait on a few occasions. Butterflies were collected selectively to avoid over-collecting of unnecessary duplicates. Specimens were deposited in the FRIM Entomological Reference Collection. References used for the identification of the butterflies were Corbet & Pendlebury (1992), Eliot (2006) and Kirton (2014).

Each trail or habitat visited was evaluated for potential butterfly-watching sites. The criteria used were butterfly richness, abundance and visibility, the presence of interesting or rare species, the relative ease of walking the trail and the duration required to complete the trail. Qualitative categories were used to describe each criterion.

Sampling sites were geolocated using a handheld GPS device during inventories. Information on the butterflies collected at each site was compiled and used to determine important butterfly areas. Areas with high butterfly diversity, rare species, or in which certain species were very localised were considered important butterfly areas.

RESULTS AND DISCUSSION

A total of 279 species were recorded from FRIM after cataloguing existing collections of butterflies in the FRIM Entomological Reference Collection that had been collected or bred from FRIM before the commencement of this project. This was 73 species more than the original 206 species reported in FRIM (2018). The field inventory conducted during the current project between 2020 and 2022 added approximately 2,330 butterfly specimens to the Entomological Reference Collection, of which 96% have been identified. A total of 324 species were collected or seen in FRIM during the inventory. A high percentage of species recorded were from the three largest families, Nymphalidae, Lycaenidae and Hesperiidae, which accounted for 33.7%, 28.7% and 24.8%, respectively, or collectively 87.2% of all species. Of the 324 species recorded, 98.5% were verified by at least one specimen. A total of 137 species were new records to FRIM, many of which were probably overlooked in the past. However, 92 species previously reported from FRIM were not found in the latest inventory. Among them are 19 species records that are not verifiable by a specimen in the Entomological Reference Collection. Some of these records may have been based on past misidentifications. A few records are almost certainly incorrect identifications as they are from genera that are very difficult to identify at the species level and are unlikely to occur in the forest habitats of FRIM. The combined number of species recorded in FRIM was 416, taking into consideration the previous inventory (Tho & Mahyudin, 1982), the identification and cataloguing of more recent collections, and the present inventory. However, we estimate that the actual number that can be reliably confirmed to occur in FRIM is about 395 species. Besides possible misidentifications in the past and overlooked species, some of the differences between the present and past inventories could be due to changes within the forests of FRIM. As the forests of FRIM continue to grow and mature, and as succession occurs in the understorey vegetation, changes are also expected to occur to the composition and abundance of butterfly species. Some species may have become rarer or even extinct, while others may have become more abundant, and other species may have recently colonised the forests of FRIM.

Among the interesting discoveries made during the current inventory was the female of the very rare *Erionota hislopi*, which had remained unknown until it was collected for the first time on the grounds of FRIM. The life history of the rare *Elymnias dara*, which is also a new species record for FRIM, was also documented for the first time in Malaysia. Several good butterfly-watching areas were identified based on the six criteria used. They are the Rover Track, Bukit Bujang Trail, the Dipterocarp and Non-Dipterocarp Arboreta, the Sungai Kroh Waterfall area, Syabas Trail, the FRIM nursery, and the FRIM wetland area. These areas could be promoted for butterfly-watching activities to raise nature awareness and foster education.

Additionally, we identified approximately 23 forestry fields as important butterfly areas. These areas had rare or localised species present or had a great diversity of species. A report listing these important butterfly areas and a map were prepared for the World Heritage Site (WHS) secretariat and Campus Committee (*Jawatankuasa Kampus*). In the report, recommendations are also made for grounds management practices that promote butterfly conservation, such as minimising development in these areas and avoiding extensive edge clearing along wide forest trails.

A further recommendation is made for long-term monitoring programmes in five important butterfly areas, which are the Rover Track, Bukit Bujang, Sungai Kroh Waterfall, the Dipterocarp Arboretum, and the Non-Dipterocarp Arboretum. It is envisaged that these recommendations will be incorporated into the Conservation Management Plan of the FRIM grounds. The full results and species checklist of butterflies in FRIM based on this study are being prepared in journal article format. A guidebook for butterfly watching in FRIM, featuring about 60 butterfly species, is also being prepared. Frequently encountered or interesting species, their habitats, and some aspects of their behaviour will be described in the guidebook.

CONCLUSION

The current field inventory of butterflies recorded 137 species not previously known to occur in FRIM, and together with a re-identification of all butterfly specimens collected or bred from FRIM that are housed in the Entomological Reference Collection, brings the total number of species known to occur in FRIM to nearly 400 species. The grounds of FRIM are, therefore, rich in butterfly species, being home to nearly 40% of the total number of butterfly species known from Peninsular Malaysia. The location of FRIM near the city and its high butterfly diversity make it a suitable centre for public education on butterfly diversity and conservation.

The findings of this study provide information and recommendations that will strengthen FRIM's position in obtaining World Heritage site status. They also showcase FRIM as an organisation that is actively involved in butterfly research and conservation.

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GENETIC DIVERSITY ANALYSES OF *PTEROPTYX TENER* (COLEOPTERA: LAMPYRIDAE) IN THE SELANGOR RIVER

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ABSTRACT

The estuarine firefly, *Pteroptyx tener* (Coleoptera: Lampyridae), is a key inhabitant of the Pasangan sub-district in Kuala Selangor. The population here, which numbers in the thousands, depends on Berembang trees (*Sonneratia caseolaris*) lining the Selangor River for its existence. Apart from providing them with shelter, the trees also provide them with a platform to perform their nightly displays of synchronised flashing all year round, making them an entomotourism attraction in both Selangor and Malaysia. Although Lembaga Urus Air Selangor and the Selangor government have enacted legislation, policies, and programs to mitigate the threats faced by *P. tener*, we took the approach of applying molecular biology techniques, viz. mitochondrial DNA gene sequencing and population genotyping of firefly individuals from the Selangor River to describe/assess genetic diversity and structure here. Early microsatellite results indicate that the population in Kuala Selangor is suffering from an excess of homozygous or genetically identical individuals, while mitochondrial DNA marker analysis showed the presence of numerous rare alleles, which, if not protected or conserved, may be at risk of becoming lost from the population.

Keywords: *Pteroptyx tener*, estuarine, microsatellite DNA, mitochondrial DNA

INTRODUCTION

Genetic Conservation of the Selangor River Fireflies (*Pteroptyx tener*)

Populations of *Pteroptyx tene*r inhabiting the Selangor River are restricted to the swatches/belts of vegetation on the landward side of its riverbanks (Figure 1). The inability of *P. tener* fireflies to interact or breed with individuals on the opposite side of the river, coupled with threats to their habitat, may have an impact on their genetic diversity. In order to verify this, an assessment of their genetic diversity is required.

Codominant nuclear markers such as microsatellites are frequently used in population genetic studies (Wang et al., 2021) to assess genetic diversity in endangered populations (Vashistha et al., 2020). Microsatellite markers contain repetitive DNA sequences, which can sometimes result in variation in repeat numbers between individuals and populations due to polymerase slippage during replication (Shlotterer & Tautz, 1992). Apart from being neutral markers, microsatellites have several advantages over other types of markers. They are highly polymorphic, easily amplified due to their short length (a few to several hundred nucleotides long), and codominant, which enables differentiation between heterozygotes and homozygotes (Viera et al., 2016). These advantages provide relatively higher resolution for detecting genetic diversity within and among populations, population structure, and geographic distance (Selkoe & Toonen, 2006; Sheriff & Alemayehu, 2018). Past studies generally only used mtDNA markers for phylogeographic and phylogenetic analysis (Cheng et al., 2019; Jusoh et al., 2020; Yaakop et al., 2023).

Although microsatellite markers must be isolated *de novo* for each species or group of closely related species, Next Generation Sequencing has sped up our ability to sequence genomes and isolate microsatellites with much greater accuracy and at a fraction of the cost than it would have been a couple of decades ago. Understanding the genetic relationship between *P. tener* populations living along the riverbanks of the Selangor River is important so that strategies can be formulated to preserve and enhance biological diversity in this species. It will also allow us to assess if firefly populations inhabiting the Selangor River are becoming increasingly isolated from one another, especially when they begin to breed with closely related individuals or even siblings. Closely related individuals could simply mean firefly individuals from the same section of the river, riverbank, or even tree along the Selangor River.

As individuals of the species become increasingly identical to one another, they may respond negatively to new and emerging environmental pressures, such as diseases or changing climate conditions, causing individuals within the population to succumb or die. The Selangor Waters Management Authority, the agency entrusted with conserving synchronous fireflies in the Selangor River, has enacted laws (the Selangor Waters Management Authority Enactment 1999, the Zone of Protection Notification 2009), guidelines, and initiatives to address these issues. In this project, however, we applied molecular biology techniques, viz. mitochondrial DNA gene sequencing, and microsatellite genotyping of firefly individuals from a section of the Selangor River, to describe and assess genetic diversity and population genetic structure in firefly populations inhabiting adjacent banks of the river.

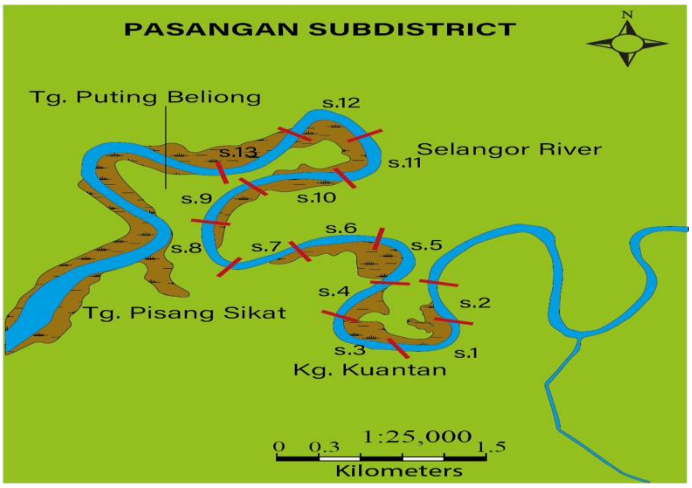

Figure 1 Location of areas sampled for fireflies along the Selangor River

MATERIALS AND METHOD

Firefly Sampling

A total of seventy-one (71) adult *P. tener* specimens were collected from both sides of the riverbank on their display tree, *Sonneratia caseolaris,* between July 2022 and July 2023. In addition, we obtained GPS coordinates of trees where fireflies were sampled from host and habitat information, as well as notes on the sex of the individuals that were collected. Samples were kept alive in Falcon tubes until they were brought back to the Genetics Lab in FRIM, Kepong. Identification of fireflies is largely based on male specimens, and in cases where female samples were obtained, information on the identity of male specimens collected alongside females was used for species identification. The presence or observation of a single photic organ/band denotes a female, while the presence of two photic organs/bands indicates a male.

Bioinformatics Analyses: Mining for Microsatellites

The partially assembled genome assembly of *P. tener* was mined for microsatellite-containing loci with FullSSR (Metz et al., 2016), a bioinformatics tool that combines microsatellite discovery and primer design capabilities using the Primer3 module or algorithm by Untergasser et al. (2012). FullSSR returned an output containing a unique simple sequence repeat identification number, microsatellite motif, number of tandem repeats, loci/sequence length, as well as the start and end of each SSR-containing loci. We used the random or 'rand' function in Microsoft Office Excel to select primers, after which they were synthesised by Bio Basic Inc. (Ontario, Canada).

DNA Extraction and Purity Analysis and PCR

Firefly DNA was extracted with the Wizard Genomic DNA Purification Kit (Promega, USA). Sample purity and concentration were then measured with NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific, USA) and visualised on a 0.85% agarose gel stained with GelRed® Nucleic Acid Gel Stain (Biotium, USA). Primers synthesised by Bio Basic Inc. (Ontario, Canada) were reconstituted to a working concentration of 10 µM while DNA extracts were normalised to 10 ng/µl. Polymerase Chain Reactions (PCR) was prepared with Q5 High Fidelity 2× Master Mix (New England Biolabs, USA); forward and reverse primers; and UltraPure DNase/RNase-Free Distilled Water (Invitrogen, USA) in a final reaction volume of 10 µL. An initial annealing temperature of 48 °C was used for PCR on a SimpliAmp thermal cycler (Applied Biosystems, USA). PCR products were screened on 1.5 % agarose gels stained with GelRed® (Biotium, USA) alongside a 100 bp–1 kb DNA ladder. Touchdown PCR was then employed to amplify the markers using the average melt temperature (T_m) of our primers as the final annealing temperature to use in the second stage of our thermal cycling program. The Oligo Analyzer Tool at the Integrated DNA Technologies (IDT) website (IDT, USA) was used to calculate the T_m of our primers. PCR products were once again electrophoresed on 1.5% agarose gel stained with GelRed® alongside Quick-Load 100 bp DNA Ladder (New England Biolabs, USA) at 120 V for approximately 20 minutes. The size of the amplicons was assessed/compared to the expected size of the loci obtained from

FullSSR (Metz et al., 2016). Highly specific primer pairs with the criteria of single-locus amplification and that fell within expected amplicon sizes were selected for ensuing genotyping experiments.

Data Analysis

Popgene v1.32 (Yeh et al., 1999) and GDA v1.1 (Lewis & Zaykin, 2002) were used to compute the genetic diversity statistics of the *P. tener* population situated at the right and left river banks. The FSTAT v2.9.4 (Goudet, 2003) was used to identify the inbreeding coefficient (F_{IS}) between the populations. The GenAlEx v6.5 (Peakall & Smouse, 2012) was then used to assess the pairwise population genetic differentiation coefficient (F_{ST}), gene flow (N_m), unbiased Nei genetic distance (D), and Nei genetic identity (I).

RESULTS AND DISCUSSION

Genetic Analysis of *Pteroptyx tener* Using Mitochondrial DNA Markers

Early results from sequences of the *cox1* gene showed differences in the genetic makeup of samples collected from the pilot study site in the Selangor River. At nucleotide position no. 2108, which corresponds to a locus on the *cox1* gene, an indel (insertion/deletion) event appeared among individuals inhabiting the Selangor River (Figure 2). The indel event, where a 'T' base was inserted in position no. 2108, appeared in several samples from the right bank of the Selangor River (individuals PTZ3–7 and PTZ10–11) (Figure 2).

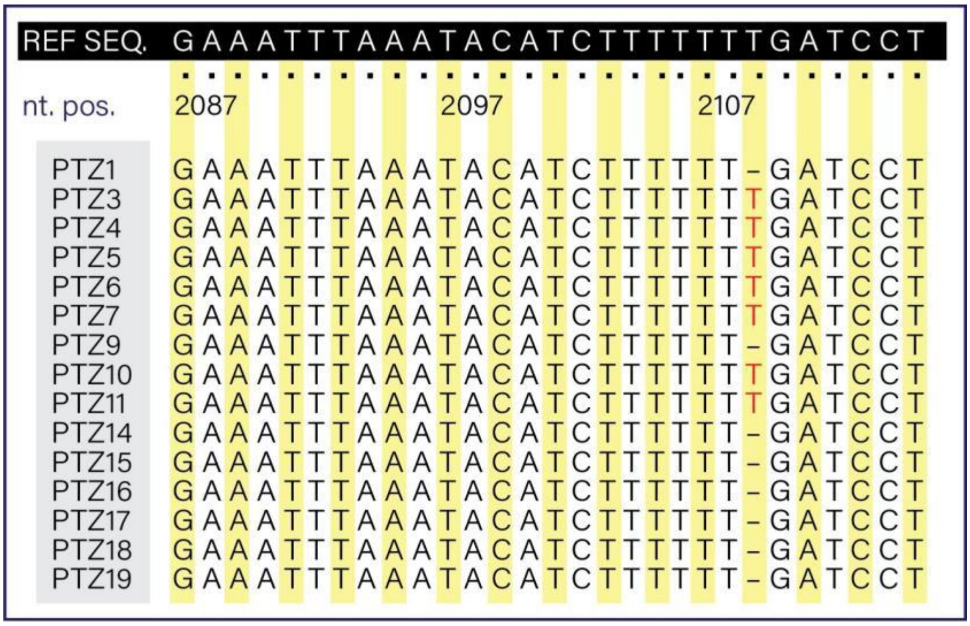

Figure 2 Mutation event in the *cox1* gene at nucleotide position no. 2108 where an indel (insertion/deletion event) had taken place

The indel event where the 'T' nucleotide arose was, however, in a low complexity region. Low-complexity regions are characterised by the presence of mononucleotide repeats or single amino acids. In *cox1* gene sequences of *P. tener* samples from our pilot study site, mononucleotide repeats of the ‘T’ base occurred between nucleotide positions 2102 and 2109. Although this indel event could well be real, we do not discount the possibility that the insertion event could also be caused by errors during DNA sequencing or slippage during PCR. We also analyse the DNA sequences through haplotype network analysis and characterise mitochondrial DNA (mtDNA) diversity in *P. tener* through haplotype identification with the software Population Analysis with Reticulate Trees or PopArt (Leigh & Bryant, 2015) in a subset of the samples from our pilot study site in the Selangor River (Figure 3).

We were able to identify eight (8) different haplotypes from our pilot study site in the Selangor River (Figure 3) with PopArt (Leigh and Bryant, 2015). We detected two haplotypes that were abundant, that is, haplotypes ‘A’ and ‘B’, and which were found on both banks of the Selangor River here. Haplotypes ‘A’ and ‘B’ accounted for close to 59% of cox1 alleles or variants found in our study site. We also detected six (6) rare haplotypes, viz. haplotypes ‘C’ to ‘H’ in our pilot study site, which requires urgent conservation and/or translocation to other parts of the study site or the Selangor River as part of our effort to increase firefly diversity (Figure 3). We detected more diversity on the right bank compared to the left bank of the Selangor River (Figure 3). There were also rarer alleles on the right bank compared to the left bank of the river.

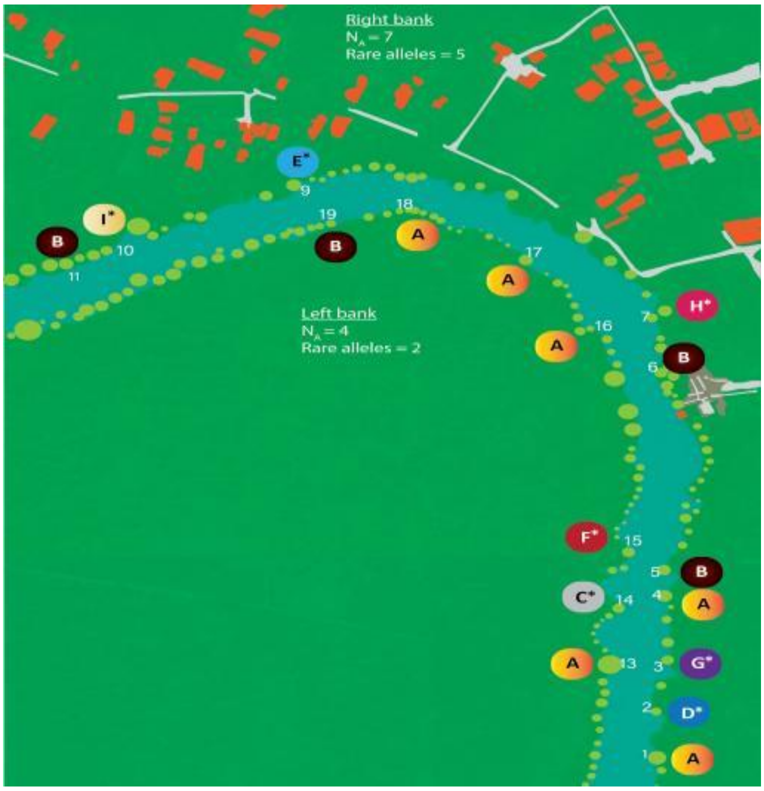

Figure 3 Distribution of mtDNA haplotypes along Sector 1 of the Selangor River. Haplotypes indicated with an asterisk are rare alleles. GPS coordinates for every haplotype were also collected

Genetic Analysis of *Pteroptyx tener* Using Microsatellite DNA Markers

The results of genotyping experiments on *P. tener* at locus no. MZ22 showed an excess of homozygotes compared to heterozygotes (Figure 4). Eight individuals were homozygous, while four were heterozygous (Figure 4). The homozygous individuals likely received the same allele from each of their closely related parents, while the heterozygous individuals received a different allele from each of their genetically unrelated parents. At least one allele, that is, allele 139 bp, was found on both sides of the pilot study site in the Selangor River and from individuals' numbers 4 and 14 (Figure 4). However, a majority of the alleles appear to be restricted to only one side of the river. Alleles 137 and 138 (from individuals no. 15–17) were found only in fireflies on the left bank of the Selangor River, while alleles 140, 141, and 145 (from individuals 1, 3, and 10) were only found in individuals from the right side of the Selangor River/pilot study site (Figure 4).

Genotyping at locus no. MZ29 similarly showed an excess of homozygotes compared to heterozygotes, with eight individuals having a homozygous constitution compared to three heterozygous individuals (Figure 5). One allele was, however, found on both sides of the river, that is, allele 210 bp (Figure 5). Similar to allele 139 bp from locus 22, the presence of the allele in individuals no. 3, 9, and 18 in firefly samples from both sides of the river indicates that gene flow had occurred in the past. One particular firefly individual, that is, individual no. 19, had a unique genotype or set of alleles that were not found in other individuals at the pilot study site. Although, likely, individuals from the section of the river or firefly display from which individual no. 19 was sampled may also possess the same alleles, resampling of fireflies from the very same location is necessary to verify this finding.

On average, we detected only 2.3 alleles per locus in the entire population (Table 1). This indicated that genetic diversity in the firefly population in Sector 1 was very low. The effective number of alleles (Ne) in the firefly populations sampled from the left and right banks of the Selangor River, as well as that calculated from the total population, was lower than the observed number of alleles (Table 1). The effective number of alleles (Ne) is the number of equally frequent alleles that it would take to achieve the same expected heterozygosity in a population that is under investigation/study. As a rule/theoretically, this number should generally be lower than the actual/observed number of alleles (N_A).

Meanwhile, observed heterozygosity (H_o) in firefly populations inhabiting each riverbank was lower than expected heterozygosity (H_e), indicating that the population was in an inbred state. H_e generally ranges from '0', indicating no heterozygosity, to nearly '1', which indicates the presence of many equally frequent alleles (Wright, 1931). The low F_{ST} value for the total population indicates as well that there was very little genetic variation between populations living on both sides of the river (Table 1). In addition, we detected a low F_{IS} coefficient, which once again indicated inbreeding in the firefly population in the Selangor River.

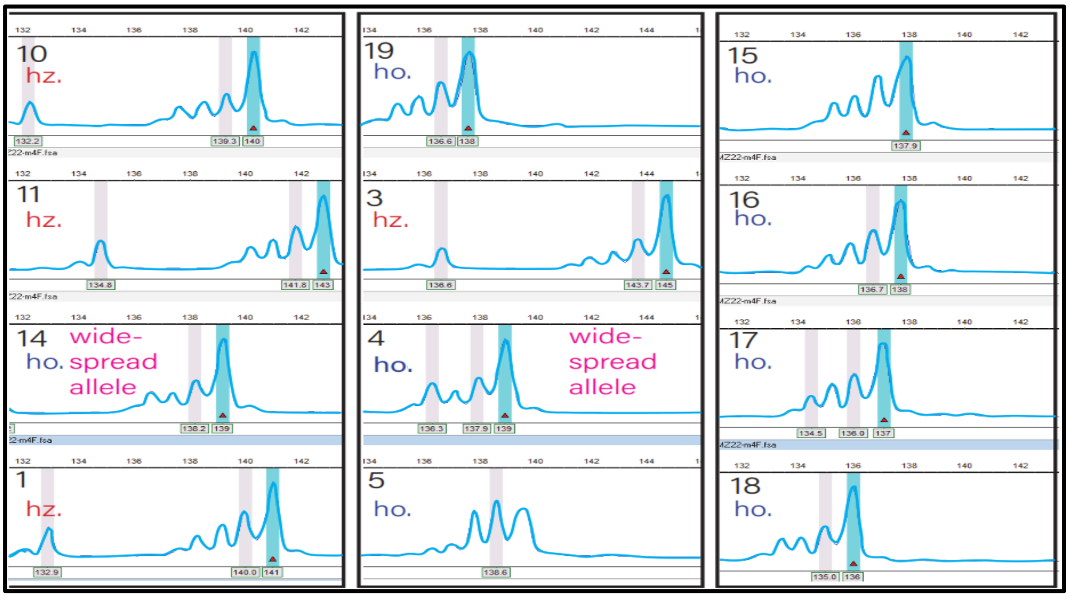

Figure 4 Electropherograms obtained from 12 firefly individual genotypes at locus no. MZ22 that were labelled with the 'FAM' fluorophore. Electrophoregram line thickness was digitally enhanced only for clarity. Peak height and shape were not adjusted/affected by the enhancement. 'Ho.' is used to indicate individuals that were in the homozygote state, while 'hz.' indicates those in the heterozygote state. Cross-reference **Figure 3**, for locations of where samples came from

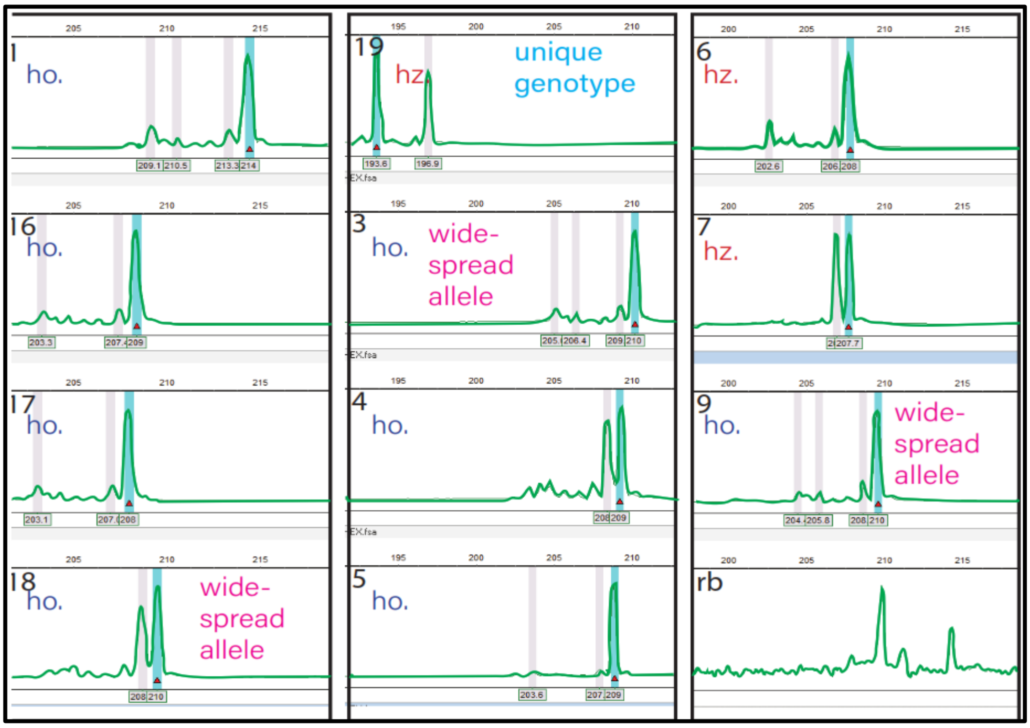

Figure 5 Electropherograms obtained from 11 firefly individuals genotyped at locus no. MZ29 that were labelled with the 'HEX' fluorophore. 'rb' stands for reagent blank which functions to ensure that there was no contamination in PCR experiments. Peak height and shape were not adjusted/affected by the enhancement. 'Ho.' was used to indicate individuals that were in the homozygous state, while 'hz.' indicated those in the heterozygous state. Cross-reference **Figure 3**, for locations of where samples came from

Table 1 Genetic diversity parameters for left and right bank locations of *P. tener* along the Selangor River

Population genetic parameters	**Right bank**	**Left bank**	**Total Population**
Sample Size, N	9	6	7.289
Number of alleles per locus, N_A	2.57	2.0	2.289
Number of effective alleles, N_E	1.773	1.040	1.699
Information Index, I	0.576	0.350	0.515
Observed heterozygosity, H_o	0.272	0.262	0.259
Expected heterozygosity, H_e	0.336	0.291	0.307
Unbiased expected heterozygosity, mH_e	0.356	0.304	0.330
Fixation index, F	0.141	0.211	0.174
Wright fixation index, F_{st}	-	-	0.021
Inbreeding coefficient, F_{IS}	-	-	0.133

CONCLUSION

Sequencing mtDNA genes, such as the *cytochrome oxidase subunit I (cox1)* gene, is the quickest way of understanding the relationships between firefly populations inhabiting opposite sides/banks of the Selangor River. The drawback, however, is that these genes evolve slowly and provide only matrilineal information on species history. This is because the mtDNA is inherited from the mother in almost all animal species. Nuclear DNA markers such as microsatellites provide higher resolution and sensitivity because they take into account the genetic information obtained from both parents in the individuals being analysed. Microsatellites are still the marker of choice for paternity and forensic analyses in humans. Although microsatellite markers have been used in the study of other animal species and populations, this is the first use of microsatellites to analyse diversity in *P. tener*. The information generated from this pioneering study is likely to benefit researchers in Southeast Asia and contribute broadly to firefly conservation in the region.

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BIOASSAY-GUIDED ISOLATION OF CYTOTOXIC COMPOUNDS FROM THE LEAVES OF *TECTONA GRANDIS*

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ABSTRACT

The bioassay-guided cytotoxicity study of *Tectona grandis* leaves was carried out using Sulforhodamine B assay. Preliminary evaluation of methanol crude extract showed moderate cytotoxic effects against two ovarian cancer cell lines: A2780 and SKOV-3 with IC_{50} values of 34.38 ± 0.94 and 37.73 ± 1.11 µg/mL, respectively. Fractionation via a tannin removal procedure using a polyamide column yielded 5 fractions (TGF1-TGF5) and all fractions were tested for cytotoxic effects against ovary (A2780 and SKOV-3), breast (MCF-7), colorectal (HT-29), and cervical (HeLa) cancer cell lines. One of the fractions, TGF4 showed moderate cytotoxic effects with IC_{50} 25.21 ± 0.52, 33.49 ± 0.074, 32.80 ± 0.17, 33.05 ± 0.20, and 41.65 ± 1.31 µg/mL, respectively. Purification of TGF4 successfully isolated three compounds, suspected to be terpenoids based on its NMR spectrum. One compound has been successfully identified as copalic acid, and the structure was confirmed by NMR spectroscopy and HREIMS. Unfortunately, these compounds did not exhibit any cytotoxic activity. Therefore, the study suggested that TGF4 should be considered as one of the potential compositions in the development of novel agents in chemotherapeutic treatment.

Keywords: *Tectona grandis*, cytotoxicity, bioassay-guided study

INTRODUCTION

In 2020, Malaysia recorded 48,639 new cancer cases and this number is expected to double by 2040. According to the Malaysia National Cancer Registry Report, there was an 11% increase in new cancer cases and almost 30% more deaths from cancer reported in 2012-2016 compared to the 2007-2011 report. (MNCR, 2019). According to WHO, the most common cancers in Malaysia are breast cancer, followed by colorectal, lung cancer, nasopharyngeal cancer, and liver cancer (WHO, 2021). One of the methods in cancer treatment is chemotherapy, the treatment that uses one or more drugs to destroy the cancer cells and prevent tumor growth. This technique is one of the effective treatments but has caused severe side effects and reduced the quality of patient's life. In many cases, the tumor itself possibly develops a resistance effect towards particular drugs. Therefore, the discovery of less toxic effects of effective anticancer medicine from botanical-based products should be continued for the benefit of the people.

Herbal medicine has received increasing attention in cancer treatment due to the complexity of its phyto-metabolite and their numerous reported biological and therapeutic properties. The analysis and understanding of the complex metabolites in the natural product sources will require reliable, robust, selective, and high-resolution analytical methods. The application of different chromatographic and spectroscopic methods in this bioassay-guided study will be beneficial in the prioritising of active fractions that need isolation and purification of the targeted cytotoxic compounds. *Tectona grandis* is one of the famous timber species renowned for its stability, extreme durability, and hardness which also resist decay. However, our literature search revealed no articles or research reported the use of any part of T. grandis as a traditional medicine in Malaysia. Our previous bioprospecting study on the methanol extract of the leaves showed potential cytotoxicity on the ovarian (SKOV-3), colorectal (HT-29), and breast (MCF-7) cancer cell lines. Identification and quantification of metabolites in natural products is a challenging task and time-consuming. Therefore, the present study aimed at the bioassay-guided approach for the identification of the fractions/bioactive compound(s) that could contribute to the cytotoxicity activity, and then could be used as an active ingredient for the development of novel agents in chemotherapeutic treatment.

MATERIALS AND METHODS

Dried and ground leaves of *T. grandis* (TG) collected from Bukit Hari FRIM were soaked in methanol to get the methanol crude extract (TGM). This extract was then subjected into column chromatography packed with polyamide and eluted with mobile phase consisted of water, 50% methanol and water, 100% methanol, 5% acetic acid and 0.1N sodium hydroxide to produced 5 fractions, TGF1-TGF5. The *in vitro* cytotoxicity of methanol crude extract and all fractions were tested using Sulforhodamine B assay as described by Nurhanan Murni et al., 2017 and the phytochemical profiles were analysed using HPLC. The fraction with high cytotoxic activity was then subjected into separation and purification using flash chromatography and prep-TLC. The structural elucidation and the confirmation of isolated compounds was analysed using NMR and LCMS.

RESULTS AND DISCUSSION

The methanol crude extract has showed moderate cytotoxic effects against two ovarian cancer cell lines; A2780 and SKOV-3 with IC_{50} values 34.38 ± 0.94 and 37.73 ± 1.11 μg/mL, respectively. The fractionation process successfully separated the methanol crude extract into 5 fractions. A fraction of TGF4, showed moderate active cytotoxicity effects against five cancer lines included ovary (A2780 and SKOV-3), breast (MCF-7), colorectal (HT-29 and cervical (HeLa) with IC_{50} of 25.21 ± 0.52, 33.49 ± 0.074, 32.80 ± 0.17, 33.05 ± 0.20, and 41.65 ± 1.31 μg/mL, respectively compared to cisplatin (Table 1). Chromatographic isolation and purification of TGF4 was successfully isolated three compounds TGF4.1.1, TGF4.1.2.3 and TGF4.1.3. Based on the analysis on 1D and 2D NMR and HREIMS data, to date only TGF4.1.3 was successfully characterised and identified as copalic acid or ent-8(17),13-labdadien-15-oic acid (Figure 1). However, all compounds did not show cytotoxic effects against tested cancer cell lines. Phytochemical analysis HPLC on TGM and TGF4 showed the presence of peaks with UV_{max} values at 329.6 nm. Based on the UV spectra pattern and UV_{max} value in the range of 320-330 nm, we suggested the presence of the compounds with caffeic acid derivatives in these fractions (Chen et al., 2012). The HPLC profile TGF4 also showed the presence of a few other peaks, with different UV spectral patterns with UV_{max} range of 250-270 nm and 330-350 nm, which typically characterized for the presence of compounds with flavonoids skeleton (Chen et al., 2012).

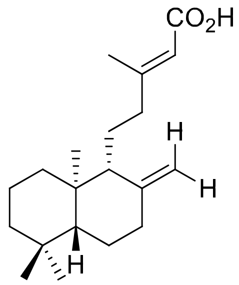

Figure 1 Structure of copalic acid

Table 1 IC_{50} (µg/mL) values of extract, fractions and compound in 5 cancer cell lines evaluated using Sulforhodamine B assay

Cancer cell lines/Extracts/ Fractions/ compound	**Ovary**		**Breast**	**Colorectal**	**Cervical**
	A2780	SKOV-3	MCF-7	HT-29	HeLa
TGM	34.38 ± 0.94	37.73 ± 1.11	50.94 ± 0.78	49.67 ± 0.78	71.12 ± 0.38
TGM-F4	25.21 ± 0.52	33.49 ± 0.074	32.80 ± 0.17	33.05 ± 0.20	41.65 ± 1.31
TGM-F4.1	45.04 ± 2.82	59.01 ± 0.90	52.86 ± 1.38	58.47 ± 0.67	60.92 ± 0.77
TGM-F4.1.1	>100	>100	>100	>100	>100
TGM-F4.1.2	>100	>100	>100	>100	>100
TGM-F4.1.3	>100	>100	>100	>100	>100
Cisplatin	0.68 ± 0.048	0.60 ± 0.016	0.60 ± 0.023	0.55 ± 0.037	1.66 ± 0.055

Notes: Cytotoxic effect category (Atjanasuppat et al., 2009; Baharum et al., 2014).

1. very active - (IC50 ≤ 20 µg/mL)
2. moderately active (IC50 > 20–100 µg/mL)
3. weakly active (IC50 > 100–1000 µg/mL)
4. inactive (IC50 > 1000 µg/mL).

CONCLUSION AND RECOMMENDATIONS

The present study has successfully identified one compound from the fraction, TGF4 of *T. grandis* leaves that showed moderate cytotoxicity activity against five cancer lines included ovary (A2780 and SKOV-3), breast (MCF-7), colorectal (HT-29), and cervical (HeLa). However, this compound known as copalic acid did not displayed cytotoxicity activity against tested cancer cell lines. A patent search using the keywords *T. grandis* leaves shows its potential as one of the compositions in the formulation for the treatment of insomnia, knee joint synovitis, thyroid gland diseases, liver-stomach disharmony type haitus hernia, cervical cancer, and colorectal cancer. Therefore, this fraction should be considered as one of the potential composition in the development of novel agents in chemotherapeutic treatment.

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CARBON STOCK ASSESSMENT IN EAST COAST TERENGGANU ECOLOGICAL TRIANGLE (ECTET) IN THE SETIU AND KUALA NERUS DISTRICTS, TERENGGANU

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ABSTRACT

East Coast Terengganu Ecological Triangle (ECTET) in Setiu and Kuala Nerus, Terengganu covers about 138,637 ha and comprises a diverse array of dry terrains, upper stream rivers, inter-connected freshwater, brackish and coastal ecosystems. The key habitats include rivers, Melaleuca swamp forests, riparian forests, seasonal swamp forests, peat swamp forests, mangrove forests, lagoons, and seagrass beds. The Setiu Wetlands, which is listed as a wetland of national importance in the Malaysian Wetland Directory, is also located within the ECTET area. They provide various ecosystem services, including food, water security and carbon sinks, especially in encountering the unexpected impacts of climate change. However, the importance of the ecological functions of these ecosystems here is rarely described. Therefore, this study was conducted to profile the ecological areas and develop a baseline of the carbon stock (C) within the ECTET area. The study used multiple satellite images from Landsat-8 Operational Land Imaging (OLI), Sentinel-2, Pléiades and images from drones. The forests within ECTET were divided into four major types, which are dry lowland dipterocarp forest, melaleuca swamp forest, mangrove forest and peat swamp forest. Based on the image classification, the total forest area within the ECTET area was about 42,314 ha, covering some 30.5% of the ECTET area. The study demonstrated that the aboveground carbon (AGC) stock in all types of forest was at 6,319,977 Mg C. This is equivalent to 23.3 million Mg carbon dioxide (CO_2) that the forests have sequestered. The study also found that the soil organic carbon (SOC) stored in these ecosystems varies from one to another, characterised by the forest types.

Keywords: Carbon stock, wetlands, ecology, forest landscape, Setiu

INTRODUCTION

Forests play a crucial role in sequestering carbon dioxide (CO_2) from the atmosphere, making them a vital component in efforts to mitigate climate change. The rate of carbon sequestration in forests can vary based on factors such as climate, geographical areas, forest types, tree species, forest age, and management practices. Additionally, forests can also become a carbon source if they are subjected to deforestation, wildfires, or degradation, releasing stored carbon back into the atmosphere. Efforts to protect and restore forests, promote sustainable forestry practices, and reduce deforestation are essential components of global climate change mitigation strategies, as they help maintain and enhance the carbon sequestration capacity of forests.

The East Coast Terengganu Ecological Triangle (ECTET) was proposed as a conservation area as it has a unique geographical setting and comprises all major types of forests in Malaysia, especially wetlands. The ECTET is located in the Setiu and Kuala Nerus districts of Terengganu and has an aerial extent of about 138,637 ha. This area not only serves various ecological functions to the surrounding environment but also supports various socio-economic activities of the local communities living in its vicinities. However, the importance of these functions is not measured scientifically. Information for advocacy and policy influencing the protection and sustainable management of the wetlands resources are scarce in the ECTET area. An assessment to determine the conservation value, primarily carbon stock in this area, needs to be carried out. Previous observations revealed that a large clearing of Melaleuca swamp forest (gelam) swamp forest occurred in this area. This is followed by the mangroves, lowland dipterocarp, and peat swamp forests. Anthropogenic activities are the main driving cause of land clearing and deforestation, mainly associated with agriculture activities, including oil palm plantation, cash crop, and aquaculture activities. Mining and infrastructure projects are also threatening the ecosystems, hence resulting in the depletion of forested areas there.

Therefore, the carbon stock assessment study has been carried out in this area. The study has produced comprehensive information in terms of the forest profiles, the stored carbon stock (C) and the sequestered carbon dioxide (CO_2) within the ECTET area. While the information is crucial to identify and prioritise the protection of forests of high conservation value, understanding the carbon storage in the forested areas and degraded forests could also help to dynamics of the carbon sequestration and emission in this area. The findings serve as a reference that would help support relevant stakeholders in understanding and managing forest resources, especially the wetlands ecosystems in this area. Several recommendations and interventions could be presented to the Terengganu State Government, which is looking for financial solutions that potentially generate economic income using a nature-based approach with a positive impact on the wetlands and local communities.

MATERIALS AND METHODS

Satellite images were acquired in this study to conduct land use/ land cover classification and to stratify forests into several categories of forest density. The stratification results were later used for the estimation of carbon stock corresponding to the density classes. Generally, the classification of the forests throughout ECTET was carried out by using medium-resolution images, which are Landsat-8 Operational Land Imaging (OLI) and Sentinel-2 multispectral images. However, due to some details of land features being required, higher-resolution images were purchased. This dataset was used to complement the existing classification results and to enhance the classification details, especially the forests covered with relatively small patches that were unable to be recognised by coarse-resolution data. It was also utilised for stratification, i.e., to classify the density and stand structure of the forests into several categories. Table 1 summarises the satellite images that were used for this exercise. In addition to the satellite imagery data, drone photos were captured at certain locations accessed during the fieldwork activities conducted.

Table 1 List of satellite images used for classification and stratification

No.	Satellite	Date of acquisition	Resolution
1.	Landsat-8 OLI	18 July 2022	30.0 m
2.	Sentinel-2 Multispectral	14 June 2022	10.0 m
3.	Pléiades	10 July 2021	0.50 m

Stratification is a process of classifying forest cover into several further detailed categories that explain the types and/or conditions of the forests. It is the most crucial process in any forest resources inventory project, as the accuracy of this process will influence the accuracy of the final inventory results. This stratification was also used for locating sampling plots for the carbon assessment at the field.

Forest canopy density (FCD) that was derived from the Landsat-8 OLI satellite images was used as an indicator for the stratification (Figure 1). FCD data can be utilised to indicate the degree of degradation of certain areas. The FCD model comprises bio-physical phenomenon modelling and analysis utilising data derived from four indices: Advanced Vegetation Index (AVI), Bare Soil Index (BI), Shadow Index or Scaled Shadow Index (SI, SSI) and Thermal Index (TI). It determines FCD by modelling operations obtained from these indices. The resulting FCD was an image containing pixels with FCD values in the form of an index (0 – 1), which later can be translated as a percentage (0 – 100%). These values have been divided into several thresholds, which become strata of a forest type. In this study, each forest type was stratified into three general categories according to the canopy density, which are low density (< 30%), medium density (30 – 70%), and high density (> 70%).

RESULTS AND DISCUSSION

Based on the stratification process that has been conducted on the satellite images, all forests were divided into three categories according to density. Table 2 and Figure 1 summarise the extent of forest types and their strata. This information was further used for field sampling and carbon stock estimation.

Table 2 Forest types and strata within the ECTET area

Forest Type	Strata (Density)	Area (ha)	Total Area (ha)
Melaleuca Swamp Forest	Low	2,733.48	6,552.41
	Medium	1,697.88	
	High	2,121.05	
Mangrove Forest	Low	113.25	509.64
	Medium	226.51	
	High	169.88	
Peat Swamp Forest	Low	n.a	1,303.83
	Medium	800.51	
	High	503.32	
Inland Dipterocarp Forest	Low	10,826.02	33,947.69
	Medium	3,617.22	
	High	19,504.45	
Total			**42,313.57**

By using the information on the forest stratification and the carbon stock measured at the sampling plots, the total carbon stock was then quantified for all forest types. In this case, the carbon stock is referred to as aboveground carbon (AGC), which is expressed in the unit of Mg C ha^{-1} as average and Mg C as the total (Table 3). The total AGC was estimated at 6,319,977 Mg C. The study also found that different types of forests have varying AGC. This variation can be illustrated in Figure 3, where the lowest average of AGC was in the Melaleuca swamp forest, and the highest was in the inland dipterocarp forest.

Table 3 The estimated AGC within the ECTET area

Forest Type	Carbon Density	Average AGC (Mg C ha^{-1})	Area (ha)	Total AGC (Mg C)
Melaleuca Swamp Forest	Low	29.03	2,733.48	79,349.24
	Medium	52.31	1,697.88	88,818.20
	High	91.38	2,121.05	193,830.06
Mangrove Forest	Low	39.23	113.25	4,442.45
	Medium	73.13	226.51	16,563.63
	High	121.25	169.88	20,597.94
Peat Swamp Forest	Low	n.a	n.a	n.a
	Medium	115.73	800.51	92,646.52
	High	205.18	503.32	103,269.65
Inland Dipterocarp Forest	Low	81.20	10,826.02	879,054.59
	Medium	144.42	3,617.22	522,390.06
	High	221.44	19,504.45	4,319,015.07
			Total AGC	**6,319,977.41**

Soil organic carbon (SOC) was used as the indicator to determine carbon stock within the topsoil (up to 30 cm depth from the surface). In this study samples were collected in Melaleuca swamp forest only. Secondary information from previous studies was used to report the SOC within peat swamps, mangroves, and inland dipterocarp forests. The analysis revealed that the soil OC in this forest was at 139.35 Mg C ha^{-1}. Tran et al. (2015) found that the SOC in Melaleuca swamp forest in sandy soil in Vietnam ranged from 75.81 to 275.98 Mg C ha^{-1}, with an average of 159.36 Mg C ha^{-1}. This indicates that the finding of this study was within the range, assuming that the condition of the Melaleuca swamp forest in Vietnam has a similar habitat as that found in Setiu, Terengganu.

Hamdan et al. (2018) reported that the average SOC in lowland dipterocarp forest in Peninsular Malaysia was at 48.80 Mg C ha^{-1}, regardless of the condition of the forest. Another study found that logging activities in the production forest will affect the carbon content of the soil. The SOC ranged from 14.03 to 67.88 Mg C ha^{-1}, with an average of 35.61 Mg C ha^{-1} in this forest (Hamdan et al., 2022). The study also found that the average SOC for mangroves with sandy coast was at 54.87 Mg C ha^{-1}. This figure is higher in muddy soil for mangroves in the western part of Peninsular Malaysia.

In terms of peat swamp forest, a previous study indicated that the SOC at Mak Jintan peat swamp forest ranged from 83.49 to 258.88 Mg C ha^{-1} with an average of 163.20 (Hyrul et al., 2021). The study concluded that each type of forest in ECTET has a different size pool in terms of SOC contents. The ecosystem type, soil type, habitat, geographical settings and tree densities, species and composition play a role in determining the contents of SOC within the forests. Table 4 summarises the variations of SOC in the forests within the ECTET area. Melaleuca swamp forest has great capability to store a considerable amount of SOC, almost similar to that of peat soil.

Table 4 Summary of soil organic carbon contents in various forest types in ECTET

Ecosystem Type	Soil Category	Average Soil OC (Mg C ha^{-1})
Melaleuca Swamp Forest	BRIS/ Sandy	139.35
Mangrove Forest	Sandy	54.87
Peat Swamp Forest	Peat	163.20
Inland Dipterocarp Forest	Clay/ Mineral soil	35.61

Figure 1 Landsat-8 OLI images over the study area

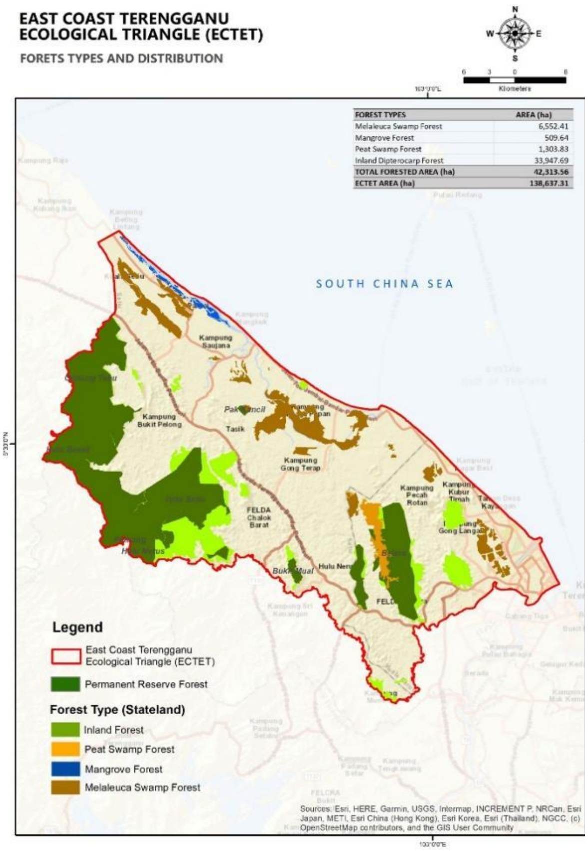

Figure 2 Distribution of forest types within the ECTET area

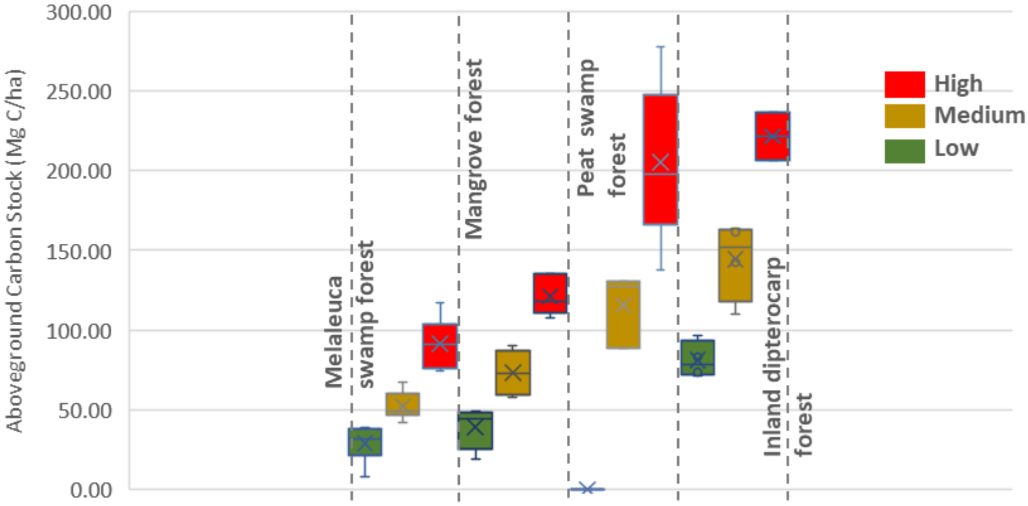

Figure 3 Variation of AGC in different types and strata of forests within ECTET

CONCLUSION

The study demonstrated that the AGC in all types of forest within the ECTET area was at 6,319,977 Mg C, which is equivalent to about 23.3 million Mg CO_2. The study also found that the SOC stored in these ecosystems varies from one to another, scharacterised by the habitat. Peat swamp and Melaleuca swamp forests indicated higher SOC compared to inland dipterocarp and mangrove forests, which were about 163 and 139, respectively. Moreover, the estimated AGC from this study serves as a baseline for carbon-related interventions in the future. This information is crucial to identify and sprioritise the protecting forests of high conservation value and understanding the carbon storage in forested and degraded areas. The results provide important information that will support the management and wise use of wetland ecosystems, allowing for the introduction and promotion of recommendations and interventions to the State government, particularly Terengganu, which is searching for nature-based solutions that could potentially generate economic income while maintaining positive effects on the wetlands, climate, and local communities.

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EVALUATION OF HYDROPHOBIC NANOCELLULOSE CHARACTERISTIC AND ITS REACTION MECHANISM VIA FACILE SURFACE MODIFICATION STRATEGY ON NANOCELLULOSE EXTRACTED FROM *MACARANGA GIGANTEA*

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ABSTRACT

The use of nanocellulose is currently restricted to water-based application systems as a result of its inherent hydrophilic properties. Surface modification of nanocellulose is commonly performed to enhance its dispersibility and expand its range of potential applications in non-aqueous settings. The process entails converting nanocellulose into a hydrophobic form, which exhibits water-repellent properties. Numerous hydrophobic modifications performed on nanocellulose present various challenges for commercial adoption. The modification reaction is usually time-consuming and requires the use of organic solvents and expensive reagents. The objective of this project is to conduct a facile method (either one-step or two-step), to synthesise hydrophobic nanocellulose. The hydrophobic modification strategy was conducted via two distinct reaction routes, namely Schiff base/Michael addition and esterification, employing a combination of various corresponding chemicals. The results of the study revealed that a combination of tannic acid and decylamine at the following regimes: molar ratio of 1:2 (tannic acid: decylamine), reaction time of 12 hours gave the highest yield (degree of substitution of 0.95), moderate crystallinity (55%) and high hydrophobicity (131°). The hydrophobicity was verified through a contact angle test, where a value exceeding 90° is indicative of a highly hydrophobic material. Hydrophobic nanocellulose has the potential for extensive use as a reinforcement material, film, and emulsion stabiliser in diverse industries, such as polymer composites, drug delivery, energy storage, cosmetics, pharmaceuticals, and various other sectors.

Keywords: Mahang, nanofibril, hydrophobic, modification, characterisation

INTRODUCTION

Cellulose is the most prevalent biopolymer on the planet, estimated at 75 billion tons, and has been utilised in various structural applications for ages (Du et al., 2019; Habibi, 2014). Nanocellulose refers to cellulose at the molecular level, a material with a nanoscale (10^{-9} of a meter) structure derived from renewable natural resources such as woody biomass, algae, marine organisms, and microbes. Nanocellulose has garnered increasing interest over the past decade due to the following benefits: high stiffness, high surface area, biodegradable, non-toxic, renewable, and intriguing optical properties that make it a versatile material for a wide range of applications (Le Gars et al., 2020; Habibi et al., 2010).

Nanocellulose is an inherently hydrophilic nanomaterial, thus it can only be used in aqueous systems. To be an effective nanomaterial in polymer matrices or non-aqueous conditions, nanocellulose must have excellent dispersibility not only in water-soluble polymeric systems but also in non-polar systems. Hydrophobic modification is thus conducted on the nanocellulose to improve its dispersibility and broaden its application in those non-aqueous (non-water) applications. Numerous modification methods for hydrophobic nanocellulose have been reported, the majority of which involve esterification (acetylation), silylation, amidation, "grafting to" and "grafting from" polymer chains (Habibi, 2014; Eyley & Thielemans, 2014). Most of these methods require extensive preparation, extensive purification, and the use of an organic solvent. In addition, the degree of substitution attained is typically low, resulting in ineffective hydrophobization.

This study focuses on developing a simple chemical modification method for making nanocellulose surface hydrophobic via two types of chemical modification methods i.e. Schiff base/Michael addition and esterification with carboxylic acid. The approach is based on using an aqueous workup and a relatively easy purification/separation process. The use of different chemical combinations at different reaction conditions will be the basis of this fundamental study toward understanding each reaction mechanism.

MATERIALS AND METHODS

Mahang gajah samples were obtained from Forest Research Institute Malaysia (FRIM) campus. Kraft pulping and bleaching were conducted to produce bleached pulp; being the feedstock for NFC preparation.

Preparation of Nanofibrillated Cellulose (NFC)

Cellulase enzyme was added into the bleached pulp and shaken in a water bath for 72 h at 25ºC. The reaction was stopped by adding boiled distilled water. The pre-treated pulp was then beaten by PFI mill at 40,000 rev, followed by ultrafine grinding using Masuko grinding machine until the suspension turned into gels.

Preparation of Modified NFC

The NFC was synthesized using two-step Schiff base and one-step esterification pathways. For Schiff base method, the NFC suspension was adjusted to pH 8 by NaOH solutions. Then, tannic acid was added and continuously stirred for 6 h at room temperature, followed by adding amine groups to the suspension and continuously stirred for another 3 h. The suspension was then centrifuged at 5°C for 10 min at 1000 rpm. The collected samples were washed using distilled water and ethanol three times each. The modified NFC was oven-dried at 80°C and stored for further analysis and characterisation. The esterification technique was carried out by adjusting the NFC to pH 4 with diluted hydrochloric acid. The NFC suspension was attached to a closed distillation system equipped with a condenser and placed in an oil bath at 130^{o}C. An excess of carboxylic acid was added to the suspension slowly and the reaction will take place for 8 h. The hydrophobe acted as both solvent and grafting agent for the esterification of NFC surface. After the reaction, the modified NFC was centrifuged with ethanol to remove a large excess of ethanol at room temperature. Different variables were studied which include molar ratio, type of chemicals, and reaction time.

Characterisation

The hydrophobic NFC was characterized using various analytical techniques namely Thermogravimetric Analysis (TGA), X-Ray Diffraction analysis (XRD), Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), elemental analysis, and contact angle measurements.

RESULTS AND DISCUSSION

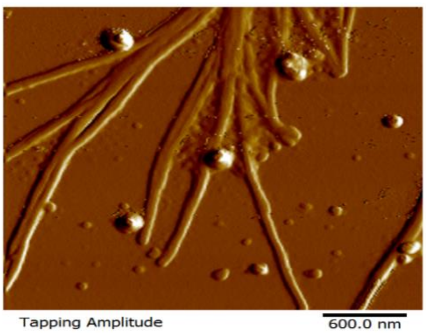
Despite many reaction conditions investigated in this project, this paper only highlights the modified NFCs that have undergone successful hydrophobization with high degree of substitutions and acceptable crystallinity values. The sample label and their respective reaction parameter are shown in Table 1.

Table 1 Different reaction time for surface hydrophobization

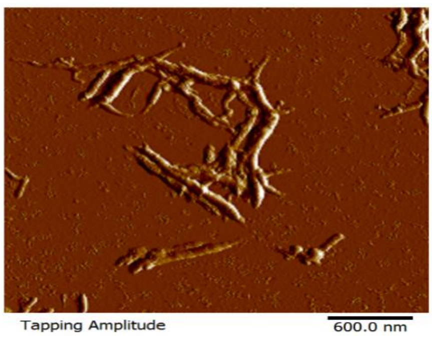
NFC	Tannic acid (hours):decylamine (hours)
SB18	3:1
SB17	6:2
SB16	9:3
SB15	12:4
SB14	15:5

After modification, the morphology of the modified NFC is shown in Figure 1. Based on the AFM images, the modified NFC seems to maintain a web-like structure indicating a relatively minimal effect of modification on the NFC integrity. However, such observation can only be confirmed by the crystallinity value of each modified NFC estimated via XRD analysis. The diameter of the modified NFC varied from 16.34 to 7.48 nm.

a)



b)



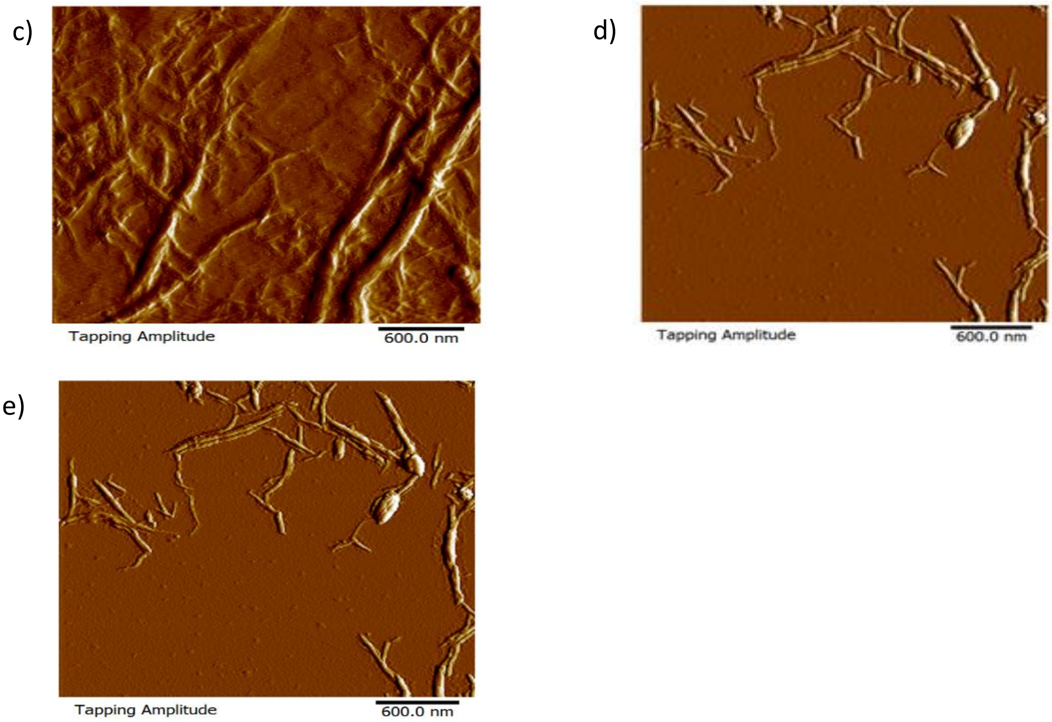


Figure 1 AFM images of modified NFC a) SB18, b) SB17, c) SB16, d) SB15, and e) SB14

Looking at FTIR spectra displayed in Figure 2, the presence of a stretching band at 2850 cm^{-1} and 2930 cm^{-1} was observed in all the modified NFC. This band was assigned to the CH_2 stretches from C10 chain of decylamine, which suggests its successful attachment to NFC. Furthermore, a notable peak at 1490 cm^{-1} was attributed to the secondary N-H bending which could prove that Schiff base reaction conducted on NFC was a success.

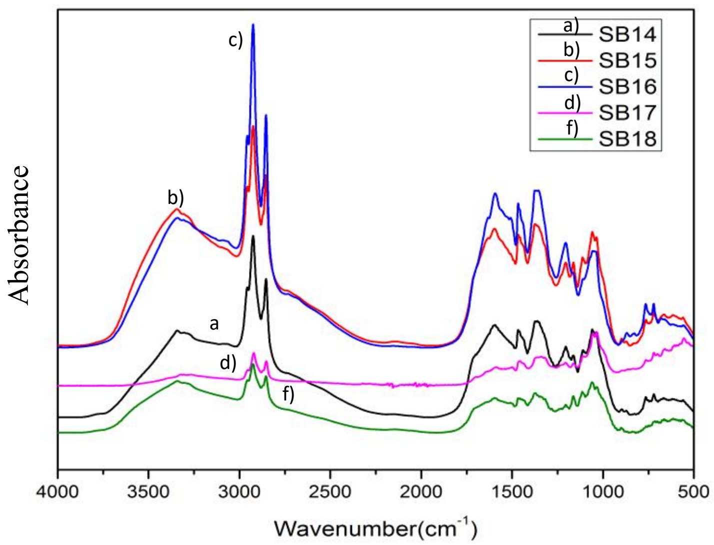


Figure 2 FTIR spectra of modified NFC a) SB14, b) SB15, c) SB16, d) SB17, and e) SB18

From elemental analysis results (Table 2), the presence of nitrogen was evident in all the samples. The DS can be estimated based on the amount of nitrogen in which SB16 showed the highest DS value (0.95). Given that the accessible hydroxyl group per cellobiose (at C6, C2, and C3 positions) on the surface is 3, the maximum DS per unit anhydroglucose can be obtained is 1.5, assuming complete surface substitution. If only one primary hydroxyl group is substituted, then the maximum DS is 0.5. Relating this to the present finding, the DS obtained ranged from 0.30 – 0.95; as to which some already underwent further substitution at secondary hydroxyl groups on the NFC surface. The trend for increased DS is due to the longer reaction time. However, it started to reduce remarkably if the reaction time was prolonged for more than 16 hours.

Table 2 DS value for SB18, SB17, SB 16, SB15, and SB14

NFC	C %	H %	N %	S %	DS
SB18	55.36	7.99	1.99	-	0.30
SB17	58.32	8.38	2.43	-	0.41
SB16	66.19	9.41	4.26	-	0.95
SB15	61.87	8.95	3.34	-	0.64
SB14	59.52	8.48	2.63	-	0.43

XRD analysis of the modified NFC reveals the crystallinity of the modified NFC (Figure 3 and Table 3). Overall, the crystallinity for modified NFC varied from 55 to 69% with a specific trend observed; which interestingly followed the DS trend. The crystallinity of modified NFC may be affected by modifications beyond the primary hydroxyl group which can be correlated with the DS obtained for each modified NFC.

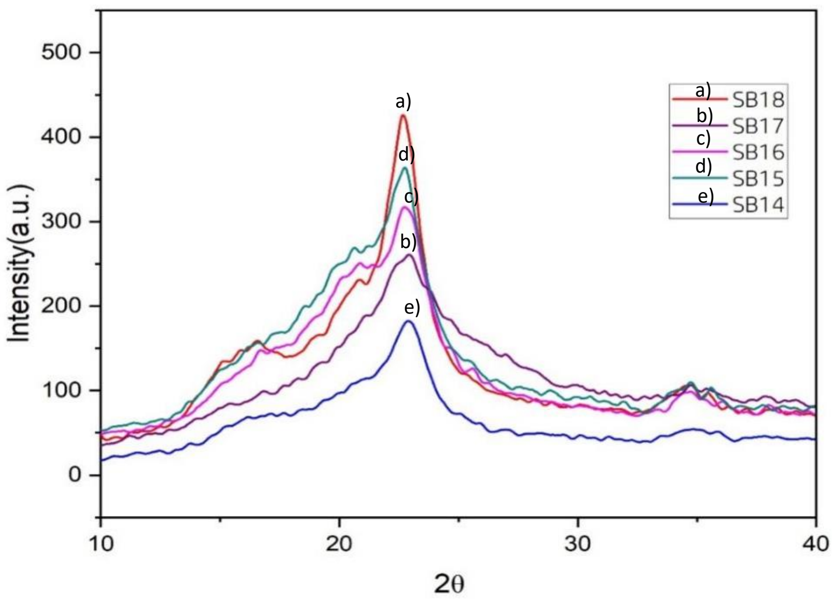


Figure 3 X-ray diffractogram of modified NFC a) SB18, b) SB17, c) SB16, d) SB15, and e) SB14

Table 3 Crystallinity values of modified NFC at different time parameter

NFC	Crystallinity value (%)
SB18	68
SB17	62
SB16	55
SB15	58
SB14	69

Modified NFC began to degrade at 247 °C, which could be attributed to the low thermal stability of pure decylamine, which starts to degrade at 207 °C. Other reasons that may cause the main degradation of cellulose at 247 °C include depolymerization, dehydration, or the formation of char residue. At 370 °C, the entire organic compound in the fibers degraded. Based on the results, it shows that the thermal stability of the modified NFC was not affected upon modification to much extent compared to pristine NFC.

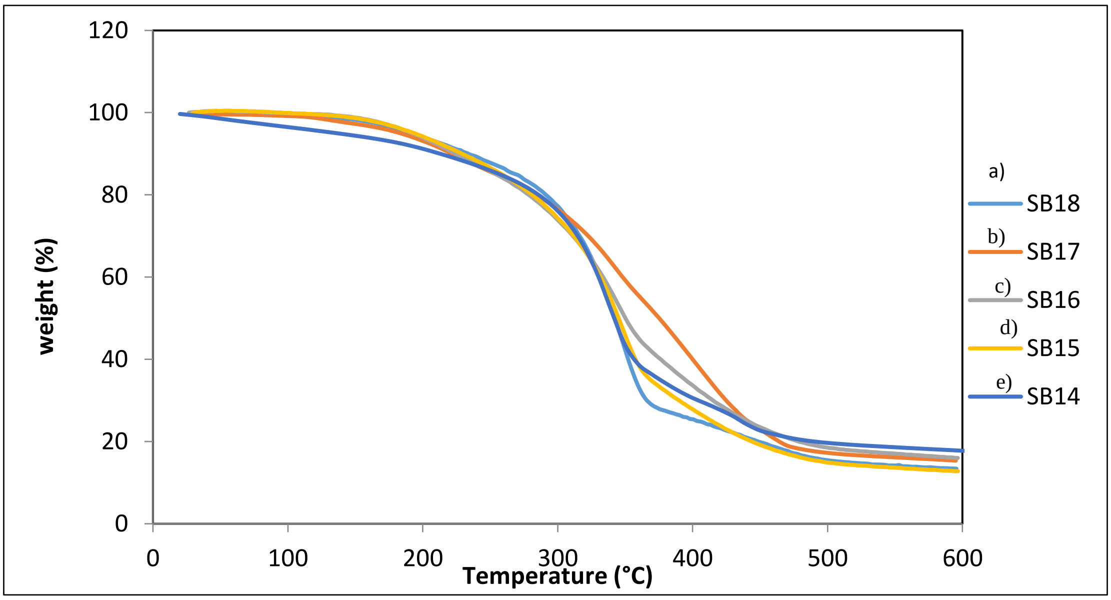


Figure 4 Thermal gravimetric graft for modified NFC a) SB18, b) SB17, c) SB16, d) SB15, and e) SB14

The surface hydrophobicity of modified NFC was evaluated by contact angle measurement (Table 4). Comparing the extent of modification obtained for the modified NFC with other reported studies (Foo et al., 2019), the contact angle obtained for all Schiff base modified samples here is relatively high. The exceptionally high contact angle obtained in this study is most likely attributed to the high degree of substitution. A value greater than 90^{o} denotes a highly hydrophobic material.

Table 4 Contact angle results for modified NFC

NFC	Contact angle (°)
SB18	129.30
SB17	128.30
SB16	130.70
SB15	126.20
SB14	128.50

CONCLUSION

Surface modification via Schiff base method using a combination of tannic acid and decylamine at a molar ratio of 1:2 (tannic acid: decylamine), reaction time of 12 hours yielded a modified NFC with the highest degree of substitution (0.95), moderate crystallinity (55%) and high hydrophobicity (131°). Based on these findings, hydrophobic nanocellulose has the potential to be widely used as a reinforcement material, film, and emulsion stabiliser in many different industries, like polymer composites, drug delivery, energy storage, cosmetics, pharmaceuticals, and many more.

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ECOSYSTEM SERVICES VALUATION OF KEDAH PERMANENT RESERVED FOREST

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ABSTRACT

Kedah permanent reserved forest (PRF) plays an essential role as water catchments intended for domestic used in Kedah, Perlis and Penang. Kedah itself always known as ‘rice-bowl’ of Malaysia serves local rice and the most important rice-growing state in Malaysia. The supply of water from Kedah PRF also crucial to domestic, agricultural as such industrial sectors. Disruption of the permanent reserved forest in which acts as water catchment disrupt the supply of water sources to these sectors. Economic evaluation on cost-based approach is one of the evaluation methods that take into account the environmental costs involved, due to the damage or loss of forest ecosystem services. This study aim to identify the importance of PRF as provisioning and regulating services. There are four scopes that support the overall objective of the study. Mainly the benefits of PRF for agricultural, domestic resources and as preventing erosion and flooding. Study also includes the evaluation of quality and quantity of raw water with management regimes by forestry department. The information from the study can help particular agencies, Forestry Department in documenting as much information related to the contribution of PRF that can lead to the planning and implementation of a good management system.

Keywords: permanent reserved forest, ecosystem valuation, cost-based approach

INTRODUCTION

Kedah has approximately 344,945 hectares of forested area according to statistics released by the Forestry Department of Peninsular Malaysia in 2019. This amount shows that the state of Kedah still has forested areas that cover 36.6% of the state's area. Of that total, 341,976 hectares or equivalent to 99% of the entire forested area has the status of Permanent Reserved Forest (PRF). PRF in Kedah plays an essential role as water catchments intended for domestic used in Kedah, Perlis and Penang, as well as for agriculture and industrial sectors in Kedah, Pulau Pinang and Perlis. Kedah is known as a Malaysian “rice-bowl” state of Malaysia. Although the Kedah is covered by vast areas of rice plantations, aware of the importance of forests to the state's environment, Kedah State Government always strives to implement the concept of sustainable development and from time to time always ensures that the state still has forested areas.

Changing land use from PRF to other land uses for development, housing, agriculture and so on will cause a loss of forest ecosystem services that can be obtained such as carbon stocks, ecotourism, medicinal plants and others. Therefore, the loss of forest ecosystem services needs to be evaluated so that policy makers have a solid basis in making decisions during the land use change process. Christie (2006) states that environmental assessment techniques can be used as useful evidence to support policy by quantifying the economic value associated with it. Economic valuation on cost-based approach is one of the evaluation methods that take into account the environmental costs involved, due to the damage or loss of forest ecosystem services. Among the factors that lead to the increase or increase in the cost are changes in land use for development, agriculture or unplanned development around the forest ecosystem. The need to obtain these costs is to study how much cost the government needs to bear to repair damage or replace existing ecosystem services for the stability and sustainability of the environment for the well-being of the community.

Hence, this project general objective is to carry out a research project focused on the economic evaluation of the environmental services of Kedah's PRF conservation to help organizations and the country towards the development of a good and responsible sustainable environmental management plan. Specific objectives of the project are to identify the importance or justification of the need of the forest as a provider of regulatory control services and estimating the value of natural resources (PRF) on cost-based approach due to loss of services.

This study consists of four scopes that support the overall objective of the study. The components involved are:

i. Scope 1: Valuing the economic benefit of PRF conservation as a function for agricultural sources
ii. Scope 2: Valuing the economic benefit of PRF for domestic water supply sources
iii. Scope 3: Evaluation of the forest management regime taking into account the forest canopy density, quantity and quality of water as a measuring indicator
iv. Scope 4: Valuing the economic benefits of selected PRF conservation as a function of preventing erosion and flooding [Economic Assessment the Impact of "kepala air" at Gunung Jerai and Baling Forest reserved]

MATERIALS AND METHODS

In valuing the economic benefit of PRF conservation as a function for agricultural sources (scope 1), the residual valuation approach is often used by researchers in determining the shadow price of agricultural water (Young & Loomis, 2014). This residual evaluation technique uses market-based information such as the value of total revenue and total cost obtained directly from farmers through focus group discussion (FGD) sessions. Other required inputs that also collected are capital and labour. Three FGD were held in three territories of MADA with total of 25 farmers prior to the study to identify the inputs for questionnaire. A total of 405 respondents (farmers) were surveyed facilitated by MADA's officer.

In valuing the economic benefit of PRF for domestic water supply (scope 2), several data were gathered from several agencies including data on treatment plants and forest land uses. Information on 36 water treatment plants for Kedah was collected from Syarikat Air Darul Aman (SADA) such as intakes coordinates, raw water sources, volume of treated water produced, operational cost including chemicals and electricity consumption for the year 2021.

Water purification services assessment is constructed by study of economic model in Perak (Vincent et al., 2015) using benefit transfer approach.

Evaluation of the forest management regime taking into account the forest canopy density, quantity, and quality of water as a measuring indicator. Most impacts on water quality are often associated with forest management operations. The physical and chemical study of water quality in the catchment area can be referred to Yusop & Anhar (1994) in Berembun Negeri Sembilan; Yusop et al. (2006) in Kerling, Selangor and Malmer (1996) in Sipitang, Sabah. Hydrological assessment involved water sampling at three different points to assess the different of water quality by different forest regime, protected and logged. Water samples were collected based on grab sampling for several parameters such as total suspended solid, biochemical oxygen demand (BOD), chemical oxygen demand (COD) dissolved oxygen (DO) and ammonical nitrogen (AN) for laboratory analysis. Water quality index (WQI) were based on index classified by Department of Environment.

Saiful Iskandar et al. (2013) showed that the opening of the canopy as a result of logging activities and the reduction of forest density had a significant impact on the deterioration of river water quality. A micro-monitoring approach was also implemented involved several logging licenses in several production forest. A remote sensing method is used which involves the processing of Landsat-8 OLI satellite data observed on several different dates, before, during and after the logging activities are implemented. An indicator of forest canopy density (Forest Canopy Density - FCD) has been produced and the FCD index is used as an indicator to determine the opening of the canopy that occurs as a result of logging activities.

In valuing the economic benefits of selected PRF conservation as a function of preventing erosion and flooding in a case of “kepala air” event (scope 4), survey of victims were carried out. A total of 202 respondents (100 and 102 respondents respectively for Jerai and Baling) were interviewed. The questionnaire is divided into 4 main parts which are part A (Socio-demographic information), part B (Loss due to the disaster), part C (Trauma faced by respondents) and part D (Willingness to pay respondents for a trust fund to install a warning system (EWS) in the involved areas to reduce the impact of disasters in the future). The incident has resulted in changes in the structure of the earth's surface - causing several recreational areas to be temporarily closed because they are no longer safe to visit. Therefore, an economic assessment study was conducted to assess the public's willingness in an effort to continue recreational activities in the Gunung Jerai area in the future with total of 302 publics were interviewed. Analysis used Contingent Valuation Method approach.

RESULTS AND DISCUSSION

Results from scope 1 is estimated that the use of water from catchment to irrigate 1 ‘relung’ of paddy fields is 415.80m3 (M2/2020) and this value increases to 699.22m3 (M1/2021). The MADA agricultural area practices rice cultivation twice a year, so the residual value and aggregate value of PRF annual agricultural water, the average of season M2/2020 and M1/2021 is RM423.8 million per year. Meanwhile, the current tax collected from farmers is RM1.9 million. But after taking into account the additional value of the hypothetical water line tax that farmers are willing to pay, the value is RM3.56 million. There is an additional tax value of 1.66 million per year.

Result from scope 2 estimated the value of water purification services by Kedah PRF is RM49,140,171/year. The total gazetted watershed forest area covers around 37% of the entire watershed area by treatment plants. This value can be used to consider the operational cost implications of treatment plants operators to pay for water purification services by PRF.

Results from scope 3 showed that the current status of WQI decreased from Class I to II with the parameter experiencing the most significant impact is TSS. However, the increase in TSS occurred due to the opening of the log road for harvesting operations and not due to the complete opening of the canopy. It shows that the impact of logging activities on water quality is minimal for current water conditions, that is, without seeing the impact during rain. However, the average value of FCD for the compartment in the study area is 0.48. To scientifically prove the proportion between FCD and water quality, the simulation found that logging operations contributed a total of 0.242 tons/ha of TSS at one time.

Results for scope 4 of mudflood disaster victims indicated that 44% of Jerai and 50% of Baling respondents having symptoms of trauma (including anxiety and depression). In addition, more than 75% of respondents in both areas, are willing to pay for the installation of an early warning system (EWS) to reduce the impact of disasters in the future regardless of whether they have a trauma problem or no. The mean WTP of respondents in the Jerai area is RM33.54 and Baling is RM37.35. The aggregate value of WTP obtained for Yan District is RM2.64 million and for Baling District is RM5.84 million. In public perspective, a total of 93.4% of the visitors who were interviewed agreed to contribute towards the preservation and conservation of the Permanent Forest Reserve, especially in Gunung Jerai forest reserve (FR) through the Gunung Jerai Conservation Fund. The results of the analysis also show that the average WTP is RM16.80 for entrance fee. Taking into account the number of visitors to the recreation area around Gunung Jerai FR amounting to 20,000 people per year, the economic value is RM 430,000 (single bounded) & RM 420,800 (double bounded).

CONCLUSION

Kedah PRF play an important role for water catchments that supply water for domestic used for Kedah and Pulau Pinang also agriculture irrigation especially for paddy fields. Forest in PRF also function of preventing erosion and flooding despite it generating income to the state through forest log. This project assessed the benefits of Kedah PRF in several sectors including agricultural of paddy, water for domestic consumptions as well as the benefits for protecting from erosion and flood. Project also evaluated current management regimes by scientifically measured the status and relationships of forest canopy density by forest logged to water quality.

The concept of the economic evaluation that carried out were to provide an estimate of the cost of environmental damage in the Kedah PRF. Although there are limitations in setting the actual cost in financial form or 'monetary' for environmental degradation, it is hoped that the estimate can be an effective and significant way to increase awareness of environmental issues, especially the environment of PRF. The value obtained can be used as a guide for the department to make new choices or alternatives for the use of PRF's environmental ecosystem services in line with the national aspirations of "Forest beyond Timbers". In addition, it can also be used as an instrument to integrate considerations from environmental aspects in economic and social development at the state level.

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DIFFERENTIAL GENE EXPRESSION OF *MITRAGYNA SPECIOSA* IN RESPONSE TO HIGH-TEMPERATURE

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ABSTRACT

Mitragyna speciosa is a unique psychotropic tropical plant of the coffee family, Rubiaceae. Its major alkaloid is mitragynine, an important pharmacologically active compound besides 7-hyroxymitragynine. Locally known as ketum, its leaf has been used as a traditional remedy to treat various ailments and when boiled, the drink is consumed to boost energy. Its medicinal properties have been supported by empirical studies. It is reported that abiotic stress increases the level of secondary metabolites in some plants. Therefore, we investigated the effect of high temperature on the mitragynine content of *M. speciosa* leaf by conducting high-temperature treatment. Through HPLC analysis, it was found that the amount of mitragynine content in the high-temperature treated ketum leaf extracts was higher than the control samples. Subsequently the RNA samples from the control and treated leaves were subjected to RNASeq. The aim of this study was to compare the gene expression before and after high-temperature treatment, for the purpose of identifying differentially expressed genes involved in mitragynine biosynthesis.

Keywords: Kratom, transcriptome, gene expression, mitragynine, high-temperatue stress

INTRODUCTION

Mitragyna speciosa, locally known as ketum, is a tropical medicinal plant from the coffee family (Rubiaceae). Ketum leaf has been used as a traditional remedy in Southeast Asia for treating fever, cough, diarrhea, and pain. Studies showed that it has many medicinal properties, with opioid-like, analgesic, and antitussive effects. In Malaysia, the use of ketum is prohibited under Section 30(3) of the Poisons Act 1952 that allows for a maximum fine of RM10,000 or imprisonment of up to four years. Mitragynine (MG), the major alkaloid in ketum leaf is a psychotropic substance and is regulated under the Poison Act 1952.

Plants have primary and secondary metabolisms. Primary metabolism produces metabolites essential for plant growth and development while secondary metabolism produces compounds involved in plant defense system. In *M. speciosa*, the primary and most important active alkaloid is mitragynine (9-methoxy-corynantheidine) (Chittrakarn et al., 2012). Environmental factors such as temperature, drought and salinity can influence plant growth and secondary metabolite production. Studies have shown that high temperature increases the production of some plant secondary metabolites (Zu et al., 2003; Guo et al., 2007; Yang et al., 2018).

With the availability of Next Generation Sequencing, comparative transcriptome analysis of abiotic stress-treated leaf samples can unravel the differentially expressed genes (DEGs) in the biosynthesis pathway of plant secondary metabolites (Kliebenstein, 2004; Roychoudhury & Banerjee, 2015). The global gene expression analysis provides better understanding into the biochemical and molecular mechanisms underlying the acclimation to abiotic stresses (Miao et al., 2015; Shankar et al., 2016). The objective of this study was to study the differential gene expression of *M. speciosa* in response to high-temperature treatment.

MATERIALS AND METHODS

Mitragyna speciosa was vegetatively propagated via stem cutting technique. Branches from naturally grown adult *M. speciosa* individuals were collected from FRIM Research Station Jengka, Pahang for propagation at FRIM nursery. We encountered various types of pests attacking the propagated *M. speciosa* plantlets in the nursery (Figure 1), such as caterpillars of several moth species (Ong et al., 2022), white flies, scale insect, etc. Therefore, periodical pest control was carried out to maintain the propagated plants.

Figure 1 *Mitragyna speciosa* plants infested by insect pests

At the height of about 1m, *M. speciosa* clonal plants were subjected to high-temperature treatment in a growth chamber. MG content was determined using a validated HPLC-PDA method (Norliana et al., 2024) modified from Mudge & Brown (2017). Based on the HPLC results, leaf samples of two time points of the lowest (control, Day 0) and highest (high temperature treated, Day 4) mitragynine contents were selected for RNA extraction and transcriptome sequencing. The transcriptome sequence data was generated using llumina platform, followed by reference-guided assembly (Brose et al., 2021) and identification of differentially expressed genes. Figure 2 shows the work flow of the bioinformatic analysis outsourced.

RESULTS

Herein we very briefly report on the preliminary results of the comparative transcriptome analysis of control vs treated *M. speciosa* leaf samples. From the heat map generated based on the cluster analysis, more genes are downregulated compared to the upregulated genes after the high-temperature treatment. A total of 16,003 DEGs were identified, with 43.08% of genes (6,894) up-regulated, and 56.92% (9,109) down-regulated. Enrichment analysis was performed to assign the biological functions or pathway of the DEGs. Gene Ontology (GO) enrichment analysis grouped them into Biological Process (BP), Cellular component (CC) and Molecular function (MF).

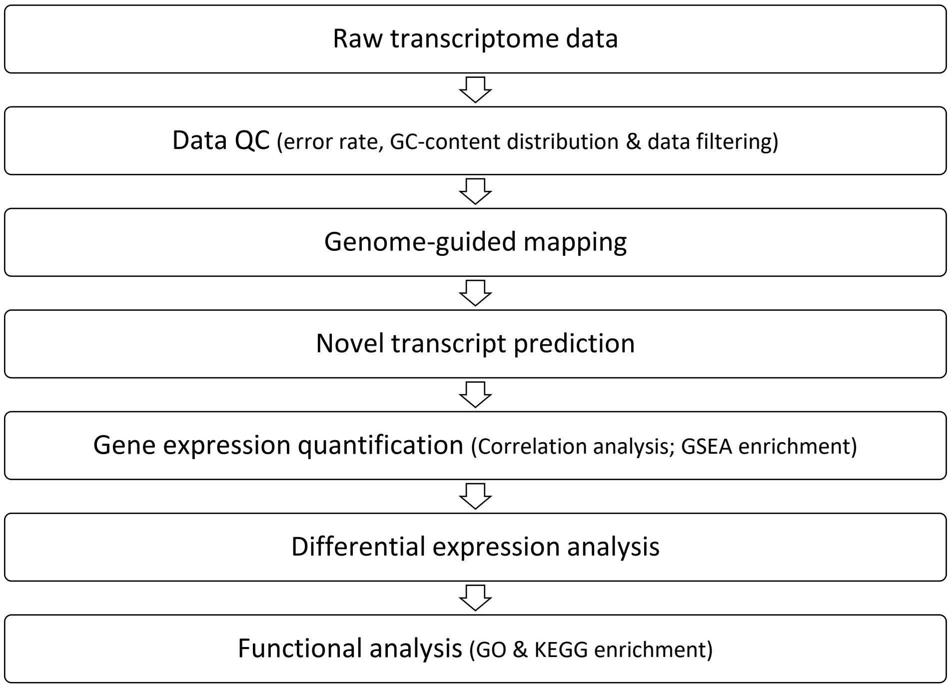

Figure 2 Bioinformatics analysis work flow

CONCLUSION

High temperature increases mitragynine production in *M. speciosa* leaf. Mitragynine biosynthesis pathway is related to the monoterpene indole alkaloid (MIA) pathway. Further down-stream analyses will be carried out to identify candidate genes involved in the MG biosynthesis pathway. Selected DEGs related to that pathway will be validated through RT-qPCR with Polyubiquitin (UBIQ) as the reference gene.

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SOIL WATER CONTENT AND EVAPOTRANSPIRATION IN LOWLAND TROPICAL FORESTS

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ABSTRACT

The El-Niño Southern Oscillation (ENSO) caused severe drought in Malaysia. According to research, tropical forests face a high evapotranspiration demand during the dry season. The dryness of a region is determined by variables such as the vapour pressure deficit (VPD) and rainfall quantity. This investigation focused on the variability of soil water content and evapotranspiration in response to dry circumstances. Pasoh Forest Reserve (FR) was chosen as our test location because it is located in Jelebu, which has the lowest annual rainfall in this region. As a result, this region is ideal for studying the possible impact of climate change on the forest habitat. By monitoring soil water content and evapotranspiration and comparing them to the quantity of rainfall and VPD during the research period, this study generally tries to understand the reaction of the forest ecosystem to severely dry circumstances. According to the findings, a considerable portion of the rain in Pasoh contributes to the evapotranspiration of the forest. Less than 10% of the rainfall helps to restore the water content of the soil. Due to the high intensity and brief duration of the rainfall, between 20 and 45% of it contributes to surface runoff. New data and conclusions published in scientific journals have considerably improved and updated forest hydrology information in this region, which is important for improving research ideas and might be utilized for climate change mitigation plans. In conclusion, the response of soil moisture and evapotranspiration to dry conditions in tropical forests is an important area of research because it can provide insights into these ecosystems' vulnerability to climate change and inform adaptive management strategies to ensure their long-term resilience and sustainability.

Keywords: Tropical forest, climate change, water use, vapour pressure deficit

INTRODUCTION

Evapotranspiration is a vital process in the hydrological cycle of tropical forests. It involves the movement of water from the soil surface to the atmosphere with the help of plants and soils and has a significant impact on the local climate. However, droughts can severely impact evapotranspiration in forests. During a drought, soil moisture is limited, and trees have to work harder to obtain water, causing evapotranspiration to decrease. This can lead to water-stressed plants and low soil moisture, which can cause forests to lose their cooling effect and contribute to higher regional temperatures. The IPCC describes droughts as periods of low precipitation that can cause water scarcity and other negative impacts on ecosystems and human society. A meteorological drought is characterized by a prolonged period of exceptionally dry weather that can cause significant hydrological imbalances (IPCC, 2012). This type of drought can impact both soil moisture and groundwater storage, as well as lead to increased evapotranspiration and decreased precipitation.

Malaysia's monsoon season is notorious for its heavy rainfall, which can occasionally give way to droughts, particularly during El-Niño events. The Pasoh region, in particular, has been known to experience dry conditions and receive less than 2,000 mm of precipitation annually. It is imperative to conduct research on the correlation between soil water content and evapotranspiration in the lowland tropical forest of Pasoh Forest Reserve, Negeri Sembilan during periods of dryness. Our research aims to gain a more thorough understanding of the impact of meteorological droughts on forest ecosystems by developing spectral indices that can accurately estimate soil water content. We also strive to investigate the relationship between soil water content and evapotranspiration. By doing so, we hope to gain insights into how forest ecosystems adapt to and recover from droughts, as well as how we can better manage and protect these vital ecosystems amidst changing climatic conditions. Such an investigation would provide invaluable insights into the dynamics of this ecosystem and could inform future management strategies.

The Vapour Pressure Deficit (VPD) is a critical factor in determining the rate of evapotranspiration. VPD refers to the difference between the saturation vapour pressure and the actual vapour pressure present in the air, and it also indicates the air's ability to retain moisture, which is influenced by temperature and humidity. When VPD values are high, it increases the rate of evapotranspiration. Consequently, more water is lost from the soil and plants, leading to a reduction in soil moisture over time. Plants can experience negative effects when exposed to high VPD levels. This is due to the exponential decrease in stomatal conductance (*gs*) as VPD increases. While this response helps to prevent dehydration, it can also lead to a reduction in carbon assimilation when stomata are only partially open. As a result, VPD is considered a significant climatic factor that can impact tree mortality and crop productivity. Extensive research, including studies by Schulze et al. (1972), Jones (1992), Oren et al. (1999), McDowell et al. (2013), and Eamus et al. (2013), have all confirmed the negative effects that high VPD can have on plants. Conversely, when VPD values are low, generally in cooler and more humid conditions, the rate of evapotranspiration decreases, which can help maintain soil moisture. The threshold for evapotranspiration varies based on the specific vegetation type and climate conditions. Typically, when VPD values exceed 2.0 kPa (kilopascals), it suggests hot and dry conditions, causing higher evapotranspiration rates and greater water loss from soil and plants. Conversely, VPD values ranging from 1.0 to 2.0 kPa are moderate, while values below 1.0 kPa are low. Insufficient soil moisture levels can have detrimental effects on the growth and survival of plants in forested areas. Additionally, these dry conditions can exacerbate the frequency and intensity of wildfires, as parched soil and vegetation provide ideal fuel for ignition and propagation. As a result, managing and conserving these forests becomes even more challenging due to decreased water availability caused by low soil moisture and high evapotranspiration rates. Such challenges can have serious consequences for both wildlife and human communities who depend on these forests as a vital source of water. It's worth noting that the threshold values for soil moisture and VPD can differ based on environmental factors, vegetation type, and region. Ecological studies have demonstrated that it's vital to maintain healthy soil moisture levels and avoid high VPD values for the sustainable management of tropical forests.

MATERIALS AND METHODS

A study was conducted in the Pasoh Forest Reserve in Negeri Sembilan from 2019 to 2020. The study utilized both field and satellite remote sensing data to monitor meteorological variables such as rainfall, soil temperature, Volumetric Soil Water Content (VSWC), vapour pressure difference, and solar radiation. Sensors such as Photosynthetically Active Radiation sensor (PAR), Open-path gas analyzer (Li7550), 3D Ultrasonic anemometer (Wind speed and direction) and Solar Radiation were placed at the 52-m flux tower for this purpose. In addition, remote sensing data obtained from a high-resolution Worldview-2 satellite image with eight multispectral bands provided a better understanding of drought effects on forest ecosystems. The Normalized Difference Vegetation Index (NDVI) and Soil Moisture Index (SMI) were used to calculate soil moisture levels and improve knowledge of drought effects.

To better understand the impact of dryness on soil water content and evapotranspiration in Pasoh FR, we analyzed rainfall amount and vapour pressure deficit (VPD). Rainfall is a crucial factor in replenishing soil water content, and we classified rainfall into three categories: wet, dry, and very dry, based on the Schmidt-Ferguson Climate classification. Rainfall above 100 mm/month is considered wet, 60-100 mm/month is dry, and rainfall less than 60 mm/month is very dry. On the other hand, evapotranspiration is a complex process that requires calculation from weather data. Therefore, we used VPD to examine the response of forest ET to dryness conditions. A VPD threshold of 1.5 kPa was selected as the approximate value above which stomata close (Kurjak et al., 2012). This VPD value was used to represent the extremely dry air during the observation period.

Soil moisture is a key variable in controlling the exchange of water and heat energy between the land surface and the atmosphere through evaporation and plant transpiration. As a result, soil moisture plays an important role in the development of weather patterns and the production of precipitation. The volumetric soil moisture content remaining at field capacity is about 15 to 25% for sandy soils, 35 to 45% for loam soils, and 45 to 55% for clay soils. According to Malaysia Meteorology Department (2007), soil moisture can be categorized into very wet (> 30%), Wet (25 - 30%), Moderate (20 - 25%), Dry (15 - 20%) and Very Dry (<15%). The threshold for soil moisture in tropical forests can vary depending on a range of factors, including soil type, vegetation cover, and climatic conditions. Generally, the soil moisture threshold for healthy growth and functioning of tropical forests is estimated to be around 60% of field capacity. Field capacity refers to the maximum amount of water that soil can hold after saturation and drainage. When soil moisture falls below this threshold, it can limit plant growth and productivity, leading to changes in forest structure and composition.

RESULTS AND DISCUSSION

The Spectral Indices for Estimating Soil Water Content Based on Satellite Remote Sensing

Based on satellite images, two indices have been developed: The Normalized Difference Vegetation Index (NDVI) and the soil moisture index (SMI). The NDVI values for the study site indicate healthy vegetation, ranging from 0.3 to 0.8, despite the lowest value of 0.3, which could be due to natural canopy openings caused by wind. The SMI range was 2 – 4 and more area was covered by moderate to low index values. To make the results more comprehensible, we have created a map that divides the study area into five classes based on the Natural Breaks (Jenks) classification. These classes are very low (1.19 – 1.81), moderate (1.81 – 2.52), intermediate (2.52 – 2.97), high (2.97 – 3.32), and very high (3.32 – 3.84) (Figure 1). We also have analyzed the distribution of soil moisture at various depths, specifically from the surface to one meter below the ground (Figure 2). After conducting a thorough analysis of soil moisture levels, we have discovered some interesting findings. Our observations indicate that soil moisture at depths ranging from the surface to one meter below ground varies from very dry to moderately moist. Specifically, the topmost layer (between 0.1 to 0.3 meters) typically exhibits a soil water content of less than 15% to 25%. Interestingly, we observed that the very surface layer (at 0.1 meters) consistently remained dry, never reaching the very wet classification. We believe this is due to the forest environment, which features hairy roots in the A horizon that act as water suction, and significant evaporation in the surface layer. Although we found very wet conditions at depths of 0.4 to 1.0 meters, we still observed very dry conditions in this profile.

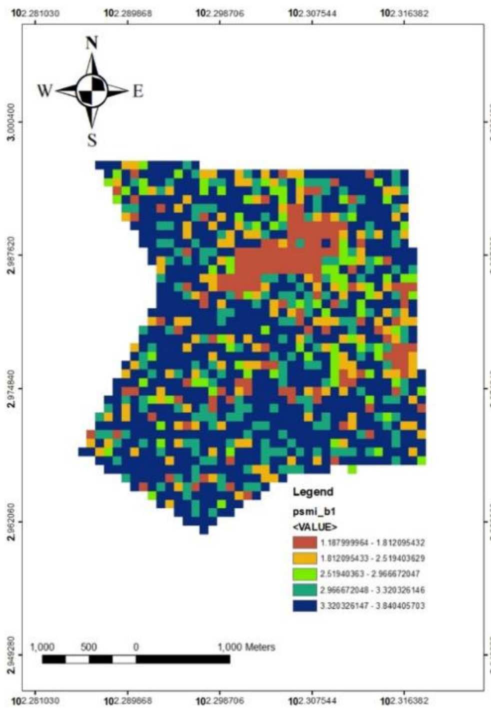

Figure 1 The map depicting the soil moisture index for the designated study area

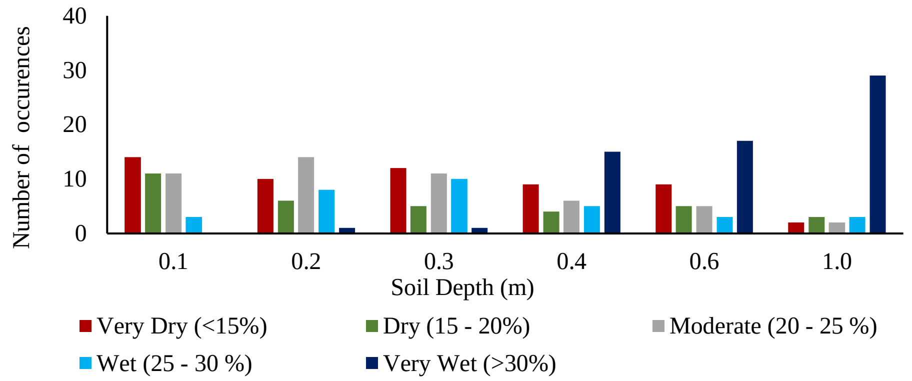

Figure 2 Profile soil moisture based on spatial distribution within the plot

Dryness Condition in Pasoh FR Based on Rainfall Amount Received

Pasoh FR received an average annual rainfall of 2,016.96 mm (±286.15) over the span of 5 years. This is 500 – 1,000 mm less than the average annual rainfall for Peninsular Malaysia, which stands at 2,500 to 3,000 mm per year. The monthly average for those 5 years was 168mm per month. Unfortunately, 2019 was the driest year yet for Pasoh FR with only 1,737 mm of annual rainfall. The Malaysia Meteorological Department (MMD) reported that Malaysia experienced medium El-Niño events in both 2018 and 2019. Table 1 shows that Pasoh FR also faced 6 instances of very dry periods based on the Schmidt-Ferguson Climate classification. The occurrence of very dry months in Pasoh typically happens in January and February.

Table 1 The recorded very dry months in Pasoh FR for 5 years based on the Schmidt-Ferguson climate classification

Year	Month	Rainfall (mm)
2018	February	13.34
2019	January	37.12
	February	3.56
	August	40.17
2020	January	30.25
2021	February	45.56

Daily Evapotranspiration Pattern Based on Soil Water Content and Vapour Pressure Deficit

Plants derive their water from the soil, and the quantity available affects their stomatal water release (Gentine et al., 2019), which, in turn, impacts evapotranspiration (ET). During the dry season, when the soil water content (VSWC) falls below 0.37, ET remains constant but is concentrated between 2-4 mm per day when the air is dry (between 5-10 kPa). VPD is the drying power of the atmosphere and affects the rate of ET. As the atmosphere becomes drier, the tree reduces water loss by closing its stomata, leading to a drop in ET. At high VPD, ET is highly reactive to shifts in soil water content, resulting in a swift reduction in ET as soil water content declines (Figure 3). However, ET is largely unrelated to soil water content at low VPD. On average, daily soil water content is 0.37% (±0.03), with the range extending from 0.30% to 0.44%. Daily evapotranspiration averages 3.44 mm (±0.71), with a minimum of 0.47 mm and a maximum of 5.44 mm per day.

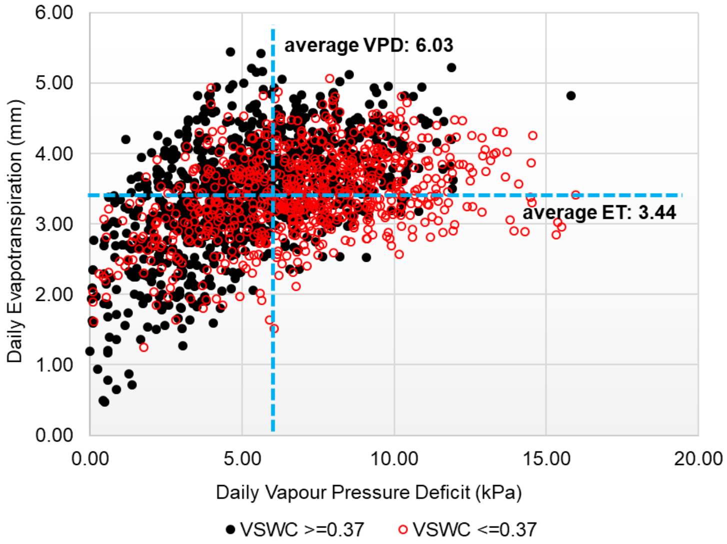

Figure 3 The daily evapotranspiration corresponds to the daily vapour pressure deficit and soil water content

CONCLUSION

The response of soil moisture and ET to very dry conditions in a forest can be likened to a dry sponge. Just as a dry sponge has less moisture and is unable to soak up as much liquid as a wet sponge, the forest soil also has less moisture and is unable to support as much ET under very dry conditions. This could lead to a decreased ability for trees and plants to take up water from the soil and carry out photosynthesis, which could affect the overall health and productivity of the forest ecosystem. Most of the rainwater in Pasoh contributes largely to forest evapotranspiration. Less than 10% contribute to the soil water content replenishment. Approximately 20-45% contribute to the surface runoff due to high intensity and short time rainfall duration. If dryness increases in magnitude in the future, it will impact the forest ecosystem.

Forest ET is crucial for regulating the carbon dioxide (CO_2) balance in the atmosphere. When trees take in CO_2 during photosynthesis, they release oxygen and water vapour. This release of water vapour through ET is an essential part of the water cycle, but it also has a significant impact on the global carbon cycle. In addition, ET causes leaves to cool down, reducing the temperature of the surrounding atmosphere. This cooling effect can increase the uptake of CO_2 by trees' leaves, making them more efficient at photosynthesis and creating a carbon sink in the forest. The increased ET rate can lead to the uptake of more CO_2 from the atmosphere, which can improve the air quality, and mitigate the impacts of climate change. However, this effect depends on the availability of water, and if soil moisture is limited, the transpiration rate may decrease, reducing the carbon uptake potential of the forest canopy.

Recent studies show a positive trend in global terrestrial evapotranspiration, with a varying rate of increase among datasets. There is high confidence that global terrestrial annual evapotranspiration has increased since the early 1980s (Zhang et al., 2015). The rate of increase in datasets varies. An ensemble mean terrestrial average rate of 7.6 ± 1.3 mm per year per decade was observed for 1882-2011 (Zeng et al., 2018a). The study by Miralles et al. (2014b) found that the lack of trend in evapotranspiration post-1998 was partially due to ENSO variability. According to this study, the forest was able to perform evapotranspiration (ET) at an average rate of 3.44 mm per day even when there was a shortage of rainfall. However, if the shortage of rainfall continues to increase, it could lead to water stress in the forest in the future. Therefore, it is important to conduct long-term research on the relationship between water and climate in order to fully understand the potential impact and resilience of the forest.

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ESTABLISHMENT OF THE MULTILOCATIONAL PROGENY TRIAL OF *SHOREA PARVIFOLIA* (MERANTI SARANG PUNAI) FOR THE PRODUCTION OF GENETICALLY IMPROVED PLANTING MATERIALS

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ABSTRACT

Shorea parvifolia Dyer locally known as *meranti sarang punai* or light red meranti as a trade name, belongs to the family of Dipterocarpaceae. This species is native to Peninsular Malaysia, Sabah, Sarawak, Sumatra, and Thailand. This species can be found mainly in lowland to hill forests and seasonally dry tropical rainforests. In Peninsular Malaysia, this species is usually found at 800 m altitude and is considered among the most common dipterocarp in Malaysia. IUCN Red List (2018) and Malaysia Plant Red List (2021) classified *S. parvifolia* as a Least Concern (LC). However, since this species is among the species of interest for timber of light red meranti, it is under threat of selective logging. Harvesting the species without strategic planning to sustain the genetic resources might reduce the population's genetic diversity. Therefore, an improvement program via progeny trial for *S. parvifolia* has been initiated to ensure a sustainable supply of quality planting materials, especially for the reforestation and rehabilitation of degraded forest areas. Planting using genetically improved planting materials has been proven to increase yield by at least 15%. Progeny trial is an important first step in the improvement program of hardwood species. Progeny trial is conducted by selecting plus trees, obtaining seeds produced from the plus trees and planting in replicated field trials. In tree breeding, plus tree refers to the selected tree that has been graded (quantitative and qualitative traits) for the sources of production for further breeding study. Seeds were collected from a total of 17 selected plus trees originating from 11 forest reserve areas in Peninsular Malaysia. In this study, two progeny trial plots have been established at FRIM's research station in Segamat, Johor (December 2022) and Mata Ayer, Perlis (October 2022), with a total of 280 saplings planted in 0.6-hectare areas and a total of 260 saplings planted in 0.31-hectare areas, respectively. Additionally, a germplasm with a common garden concept has been established at SPF Jeli, Kelantan (December 2022), with a total of 350 saplings planted in 0.42-hectare areas. Currently, the collection of growth data is ongoing. At this stage, the growth performances of the different families at different locations are still at the infancy level. Tree breeding programs will take a significant amount of time to obtain reliable results as the maturity of the trees is longer. However, we already have a gene pool of *S. parvifolia* from several states in Peninsular Malaysia. The growth performance data collected from this study will be able to provide important information for future research. In addition, this establishment also contributed to the empowerment of the nation's germplasm bank.

Keywords: selection, plus trees, families, half-sib, genotype by environment (GXE)

INTRODUCTION

The timber industry and wood-related products have become an important pillar of the Malaysian economy. However, current harvesting practices focus on obtaining timber from natural forests. Relying solely on natural forests to meet this demand will lead to rapid deforestation and biodiversity loss. Therefore, an initiative has to be taken to conduct tree improvement studies to develop high-quality planting materials for desirable forest tree species to reduce dependence on natural forests. Tree improvement programmes are an important part of forest plantation industries. The use of genetically improved trees in conjunction with good silvicultural practices will ensure optimal yield. This paper summarizes the improvement program carried out on *Shorea parvifolia* (Meranti Sarang Punai). *S. parvifolia*, locally known as meranti sarang punai or light red meranti as a trade name, belongs to the family of Dipterocarpaceae. This species is native to Peninsular Malaysia, Sabah, Sarawak, Sumatra, and Thailand. This species can be found mainly in lowland to hill forests and seasonally dry tropical rainforests. In Peninsular Malaysia, this species is usually found at 800 m altitude and is considered among the most common dipterocarp in Malaysia. IUCN Red List (2018) and Malaysia Plant Red List (2021) classified *S. parvifolia* as Least Concern (LC) (Chua et al., 2010). This species was selected based on its high-value timber and accessibility. The improvement program was started with the selection of plus trees. Plus, trees are defined as those selected trees classified for production resources for further breeding studies. However, the genetic superiority of the selected plus trees needs to be tested. However, the progeny produced from the selected plus tree is more likely to have a good genotype due to heritability. Seeds collected from selected additional plants were planted and grown in progeny trials. Conceptually, in progeny trials, seedlings are grown in replicated field trials. Growth performance is evaluated periodically (Hettash et al., 2002). Furthermore, the established progeny trial plots can be converted into seedlings seed orchards (SSO) in the near future. SSO is an orchard where only selected trees will remain, and inferior trees will be removed. The establishment of progeny trial plots has also increased the number of field gene banks, thereby strengthening the national germplasm bank.

MATERIALS AND METHODS

Selection of Plus Tree

The selection criteria for a plus tree are as follows: height, diameter at breast height, crown size, straightness, stem form, crown dominancy, angle of the third branch, size of the third branch, the ability of the tree to self-pruning, non-forking and free from pest and diseases (Figure 1). The data were recorded on a standard tree grading form and the assessment was made based on the scoring. The sampling activities were conducted in natural forest areas in eight (8) states in Peninsular Malaysia. The GPS coordinate of the locations was recorded. A total of twenty (20) selected plus trees were identified (Table 1); however, the seeds were only managed to be collected from seventeen (17) plus trees.

Figure 1 Selected plus trees identified in Edutourism Centre Sungai Menyala, Negeri Sembilan (N-MSP2), Forest Reserve Ulu Muda, Baling, Kedah (K-MSP5) and National Park (Forest Reserve Tekai), Pahang (C-MSP19)

Table 1 Sampling locations of the *S. parvifolia*

States	Locations of survey/ observation and seed sampling	No of the selected plus trees with seeds obtained
Pahang	Recreational Forest Terenggun, Kuala Lipis	1
	National Park (Forest Reserve Tekai)	2
	Forest Eco Park Lata Meraung	1
	Forest Eco Park Som	2
Negeri Sembilan	Edutourism Centre Sungai Menyala	0
	Recreational Forest Tanjung Tuan	0
	Recreational Forest Ulu Bendul	0
	Jeram Papan to Kuala Pilah Road	1
	Bukit Putus road	0
Kelantan	Recreational Forest Bukit Bakar, Machang	1
	State Park Gunung Stong	1
	Forest Reserve Nenggiri, Gua Musang	0
Perak	Kg. Cherakoh, Kuala Kangsar	0
	Forest Reserve Kekal Bubu	0
	Recreational Forest Ulu Licin, Beruas	0
	Recreational Forest Ulu Kenas	0
	Forest Reserve Kledang Saiong	1
Kedah	i. Forest Reserve Ulu Muda, Baling	2
	ii. Forest Reserve Kekal Rimba Teloi	0
	iii. Forest Eco Park Lata Mengkuang	2
	iv. Recreational Forest Lata Bayu	0
	v. Forest Eco Park Bukit Wang	0
	vi. Forest Eco Park Lata Bukit Hijau	2
Johor	Kota Tinggi	0
	Recreational Forest Gunung Arong, Mersing	0
	Forest Reserve Ulu Sedili (kaw. Petri Jaya)	1

Terengganu	Forest Reserve Jengai, (Pasir Raja) Terengganu	1
Selangor	Bukit Hari (FRIM)	1
	Waterfall area FRIM	1

Preparation of Planting Materials

Seeds were collected from identified plus trees and were sown in the sand bed (Figure 2).

Figure 2 Seed germination was conducted on the sand bed. Three to four weeks after germination, the seedlings were potted into the polybags and grown until maturity for planting activities at the field trial.

Planting Activities at SPF Mata Ayer, Perlis, SPF Segamat, Johor and SPF Jeli, Kelantan

Upon the saplings' maturity, the progeny trial plots were established at SPF Mata Ayer, Perlis and SPF Segamat, Johor, whereas a common garden plot was established at SPF Jeli, Kelantan (Figure 3). The progeny trial plots and the common garden plot were laid out in a Randomized Completely Block Design (RCBD).

Figure 3 Overview of the planting activities and the establishment of the progeny trial plots at SPF Mata Ayer and SPF Segamat, and the establishment of the common garden at SPF Jeli

RESULTS AND DISCUSSION

Two (2) progeny trial plots have been established at FRIM's research station in Mata Ayer, Perlis (October 2022) (Figure 4) and Segamat, Johor (December 2022) (Figure 5) and with a total of 280 saplings planted in 0.6-hectare areas and a total of 260 saplings planted in 0.3-hectare areas, respectively. Additionally, a germplasm with a common garden concept has been established at SPF Jeli, Kelantan (December 2022) (Figure 6), with a total of 350 saplings planted in 0.4-hectare areas. The three locations were selected based on the different environmental and soil properties. SPF Mata Ayer has sandy loam type of soil and extremely hot weather conditions. Second, SPF Jeli, on the other hand, has a silty clay loam type of soil and higher annual rainfall. Third, SPF Segamat has a sandy clay loam texture of the soil. The planting conditions were also experimented with; in SPF Mata Ayer, the saplings were planted integrated with *Tectona grandis* trees. The same conditions were also applied at SPF Segamat whereby the saplings were planted integrated with *Acacia* hybrid. As for SPF Jeli, the saplings were planted at a location with natural shades of nearby hills and trees.

Figure 4 Progeny trial plot established at SPF Mata Ayer, Perlis

Figure 5 Progeny trial plot established at SPF Segamat, Johor

Figure 6 Common Garden plot established at SPF Jeli, Kelantan

CONCLUSION

Currently, the collection of growth data is ongoing. At this stage, the growth performances of the different families at different locations are still at the infancy level. Tree breeding programs will take a significant amount of time to obtain reliable results as the maturity of the trees is longer. However, we already have a gene pool of *S. parvifolia* from several states in Peninsular Malaysia, and this would provide a wide selection basis for further tree breeding programs. The growth performance data collected from this study will be able to provide important information for future selection.

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INVESTIGATION OF AGARWOOD QUALITY PRODUCTION AND PHYTOCHEMICAL STUDIES FROM INOCULATED *AQUILARIA* SPECIES

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ABSTRACT

Agarwood is an aromatic and valuable natural resin found in plants, especially in the *Aquilaria* species of the Thymelaeaceae family. The sustainability of the raw material in natural resources and a method to ensure the agarwood quality of this plant are important to ensure customer satisfaction. In this study, the effects of agarwood quality are evaluated by various extraction methods along with phytochemical analysis using scientific instruments. Laboratory scale hydrodistillation, large scale hydrodistillation, steam distillation, steam-oil distillation, and supercritical fluid extraction (SFE) were used for the extraction of essential oils. The highest essential oil was obtained by hydrodistillation on a laboratory scale (0.51 %). The lowest essential oil was obtained by steam distillation, 0.02%. The highest yield of essential oil was recorded for *A. malaccensis* at 1.36 %, while the lowest yield of essential oil was recorded for *A. sub-integra* at 0.1 %. The highest resin content was found in *A. microcarpa* at 18.9 %, and the lowest resin content was in *A. sinensis* at 5.2 %. Major chemical compounds found in the agarwood oil of *A. malaccensis* in this study were 4-phenyl-2-butanone (14.5%), 10-epi-g-eudesmol (7.5%), agarospirol (1.2%), a-eudesmol (0.6%) and valerianol (5.0%). This study shows that agarwood extracted from plantations is comparable to agarwood from natural forests.

Keywords: *Aquilaria*, agarwood, extraction, quality, plantation

INTRODUCTION

The *Aquilaria* tree species are well-known as valuable aromatic plants. This species from the family Thymelaeaceae could produce resin from the injured wood cell tissues called agarwood (Sen et al., 2017). Chemical reactions due to infected wood areas are important in the formation of agarwood (Faizal et al., 2017). The agarwood development in the injured *Aquilaria* trees requires a longer duration.

The sustainability of raw materials in natural resources and the method to ensure agarwood quality are essential in guaranteeing customer satisfaction (Desa et al., 2021; Mustapa et al., 2022). The depletion of agarwood supply from the natural forest has encouraged industries to cultivate this species as a plantation crop (Chua, 2008; Mohd Parid et al., 2010; Rahman et al., 2012).

Agarwood quality is the main factor to thrive in this industry. The effects of agarwood quality are evaluated by different extraction methods along with phytochemical analysis using scientific instruments (Ismail et al., 2013). Laboratory scale hydrodistillation, large scale hydrodistillation, steam distillation, steam oil distillation and supercritical fluid extraction (SFE) were used for essential-oil extraction (Nor Azah et al., 2013; Sulaiman et al., 2015; Karlinasari et al., 2017). To determine agarwood quality, resin content was analysed to determine resin percentage, gas chromatography was used to identify chemical components, and an electronic nose was used for odour classification (Lias et al., 2016). Therefore, this study will quantify the agarwood oil quality of inoculated *Aquilaria* sp. trees. The evaluation is conducted through different extraction methods and identifies the agarwood phytochemical content using scientific instrumentation.

MATERIAL AND METHODS

Five *Aquilaria* sp. with five different extraction methods and three quality assessment methods have been conducted in this study. *A. malaccensis* sample was collected from trees planted at FRIM Research Station, Maran Pahang. The rest of the raw materials were bought from a local supplier who owned the plantation. The samples were prepared in grounded form for each batch. The *Aquilaria* sp., extraction techniques and quality assessment methods are shown in Table 1.

Table 1 The *Aquilaria* sp., extraction techniques and quality assessment methods

Aquilaria sp.	Extraction techniques	Quality assessment
A. malaccensis *A. sinensis* *A. subintegra* *A. beccariana* *A. microcarpa*	Laboratory scale hydrodistillation Large scale hydrodistillation Steam distillation Steam oil distillation Supercritical fluid extraction (SFE)	Resin content percentage Gas chromatography

RESULTS AND DISCUSSION

For the extraction optimization process, five extraction processes were carried out on *A. sinensis* (Table 2). The highest yield of agarwood essential oil, 0.51%, was obtained by the laboratory-scale hydrodistillation method. The lowest agarwood yield, 0.02%, was recorded in the steam distillation process. The hydrodistillation process was begun at a low temperature and raised to a water boiling point up to 72 hours of extraction (Sulaiman et al., 2015). This method can optimize the production of essential oils, as many volatile organic compounds in sesquiterpenes can be trapped in the condensation system. The surprising heating to high temperatures during steam distillation leads to a low yield. This is due to the higher heating of the raw material, which destroys certain chemical compounds (Handa, 2008).

Table 2 Essential oils yield determination of *Aquilaria sinensis* using different distillation processes

No.	Distillation Method	Weight	MC (%)	EO (ml)	Yield (%)
1.	Steam oil distillation	25.0 kg	11.16	10.0	0.05
2.	Large scale hydro-distillation	20.0 kg	11.16	30.0	0.16
3.	Steam distillation	20.0 kg	11.16	3.2	0.02
4.	Lab scale hydro-distillation	400 g	11.16	1.8	0.51
5.	Supercritical fluid extraction (SFE)	200 g	11.16	-	0.30

Note: MC= Moisture Content, EO= Essential Oil

The efficiency of the hydrodistillation process was then tested on various *Aquilaria* sp. Each sample was used for this process according to the quality of the wood chips for essential oil production (Table 3). The highest yield of essential oil, 1.36%, was obtained in *A. malaccensis*. The lowest yield, 0.10%, was recorded in *A. subintegra*. On the other hand, *A. microcarpa* had the highest resin content with 18.96%, followed by *A. beccariana* with 17.69%, which can be attributed to the lower moisture content of the samples (9.84% and 9.75%, respectively). Moisture content plays an important role in optimising the extractive resin content.

Table 3 Moisture content percentages, essential oils yield, and resin content percentages determine agarwood content from different species using the hydrodistillation process

No.	Sample	MC (%)	Yield (%)	Resin (%)
1	*A. sinensis*	11.16 ± 0.75	0.51	5.21 ± 0.07
2	*A. subintegra*	13.33 ± 0.42	0.10	10.07 ± 0.34
3	*A. malaccensis*	12.45 ± 0.38	1.36	6.42 ± 0.25
4	*A. microcarpa*	9.84 ± 0.23	0.60	18.96 ± 0.38
5	*A. beccariana*	9.75 ± 0.66	0.35	17.69 ± 0.26

Note: MC= Moisture Content

The quality of agarwood essential oil was then measured using *A. malaccensis* (Table 4). The major chemical compounds in common agarwood were found in the essential oil of *A. malaccensis* in this study, such as 4-phenyl-2-butanone (14.5%), 10-*epi*-g-eudesmol (7.5%), agarospirol (1.2%), a-eudesmol (0.6%), and valerianol (5.0%). All these chemical compounds from t *A. malaccensis* essential oil were also found in other research studies on the chemistry of agarwood oils (Azah et al., 2008; Wang et al., 2018). The result shows that agarwood oil from plantations can be accepted as pure agarwood oil in the market.

Table 4 Identification of the major chemical components' peak areas of inoculated *A. malaccensis* using GC-FID/GCMS

Peak No.	Chemical Components	Kovats Index	Inoculated *A. malaccensis* (%)
1	4-Phenyl-2-butanone	1218	14.47
4	β-Agarofuran	1474	0.93
6	α-Bulnesene	1509	0.55
7	α-Agarofuran	1548	0.78
12	10-*epi*-γ-eudesmol	1622	7.48
14	Agarospirol	1646	1.19
15	α-Eudesmol	1652	0.64
16	Valerianol	1656	4.97
18	Bulnesol	1670	3.75
20	Selina-3,11-dien-9-ol	1692	7.34
24	γ-Costol	1742	3.63
25	Khusimol	1744	0.73
26	β-Costol	1766	1.3
30	Hexadecanal	1794	2.25
33	Dihydrocolumellarin	1900	7.16

CONCLUSION

Hydrodistillation processes are the best technique for the production of essential oil in terms of quality and economy. To optimize the production of agarwood oil and resin content, the moisture content must be taken into account. The results show that the quality of agarwood extracted from *Aquilaria malaccensis* plantations is comparable to that of agarwood from natural forests.

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IN VITRO AND IN VIVO ANTI-ALLERGIC STUDY OF KRATOM LEAF EXTRACT (GREEN VEIN LEAF)

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ABSTRACT

Allergy is a condition caused by hypersensitivity of the human body's immune system to substances that are normally harmless to the human body (allergens). Antihistamines or steroids are commonly used to treat allergies, especially allergic rhinitis and allergic conjunctivitis. However, antihistamines cause many side effects. In this study, Kratom (*Mitragyna speciosa*) was used as research material because *M. speciosa* is used as a traditional medicine for allergies. The *M. speciosa* aqueous extract (KWE) was prepared and subjected to a phytochemical test to measure saponins, proteins, and polysaccharides. In addition, an arginase enzyme inhibition assay was performed to verify the anti-arginase activity of KWE. Then, an in vivo assay was performed in which 36 male rats were injected with 0.2 ml of compound 48/80 to induce an allergic response. The rats were treated with KWE at concentrations of 100, 200, and 400 mg/kg. At the end of the experiment, histamine, histidine decarboxylase, tryptase, and interleukin 6 were determined from rat blood plasma. The results show that KWE contains 44.6% saponins, 16.3% proteins, and 23.06% polysaccharides. Mitragynine as an analytical marker for Kratom was quantified in KWE with 6.13% w/w. Moreover, it was found that at a concentration of 1.25 mg/ml, 50% of arginase enzyme activity could be inhibited. This indicates that KWE requires moderately high concentrations to reduce arginase enzyme activity. In vivo tests have shown that KWE treatment can reduce the 48/80 effect that causes allergic reactions in the rat body. KWE treatment also reduced histamine, histidine decarboxylase, and tryptase. Treatment with 400 mg/kg KWE increased the concentration of interleukin 6, which is related to the antiallergic effect. This demonstrated that KWE can effectively reduce allergic reactions in the 48/80 allergy-induced rat model and it was suggested that the mechanism could be through the reduction of tryptase and IL -6 levels.

Keywords: Kratom (*Mitragyna speciosa*) water extract, allergy, phytochemistry, mitragynine, arginase enzyme

INTRODUCTION

Allergy is a condition caused by hypersensitivity of the immune system to substances that are normally harmless to the human body (allergens) (Güngör et al., 2019). Allergic reactions most commonly occur on the skin as well as in the respiratory tract and mucous membranes (Kimber et al., 2018). Besides that, allergic reactions always occur in the areas of the body that come into direct contact with the allergen (Lazzarini et al., 2018).

Moreover, mast cells are activated during allergic reactions, releasing inflammatory mediators that include tryptase (Caughey, 2006). This process leads to the symptoms of a severe allergic reaction and anaphylaxis (Caughey, 2006; Brändström et al., 2015). Anaphylaxis is a severe, life-threatening, generalized or systemic hypersensitivity reaction (Turner et al., 2019). It is characterised by rapidly developing life-threatening problems involving the airway (pharyngeal or laryngeal oedema), breathing (bronchospasm with tachypnoea), circulation (hypotension or tachycardia), or a combination of these. In most cases, there are associated skin and mucosal changes (Turner et al., 2019).

Nevertheless, antihistamines or steroids are commonly used to treat allergies, especially allergic rhinitis and allergic conjunctivitis (Randall et al., 2018). However, antihistamines cause many side effects such as drowsiness, dry mouth, dry eyes, blurred or double vision, dizziness and headache, low blood pressure, thickening of mucus in the airways, rapid heartbeat, difficulty urinating, and constipation. Also, steroids cause swelling in the lower legs, stomach upset, and weight (Simon et al., 2008).

In this study, kratom was used as a research material. The tree commonly referred to as kratom (*Mitragyna speciosa* Korth., Rubiaceae) is native to Southeast Asia, primarily growing in Thailand, Malaysia, and Indonesia (Hughes et al., 2022). Kratom leaves have been used in local communities to treat pain, cough, fever, and diabetes; to enhance work performance; and are used as substitutes for illicit substances, mainly opioids (Limcharoen et al., 2022). Mitragynine is the major alkaloid of kratom. Since *M. speciosa* contains a large quantity of mitragynine and is exclusive to the species, the HPLC method is performed for the purpose of screening kratom products based on this unique compound as the analytical marker.

MATERIALS AND METHODS

Kratom Water Extract (KWE) Preparation

The method for the preparation of KWE was carried out according to Mohd Kamal *et al*., (2023) with minor modifications. The leaves of *M. speciosa* were washed and dried. Then, the leaves were placed in the oven for the drying process at a temperature of 55°C for 48 hours. After that, the *M. speciosa* leaves were ground into powder. Then, 100 g of *M. speciosa* was weighed using an electronic balance and placed in a beaker. Then the beaker was filled with 1000 ml of distilled water and boiled at 100^{o}C for 1 hour. The solution was filtered and evaporated using a rotary evaporator. The extract was stored in a -20^{o}C freezer until use. The KWE was subjected to a phytochemical test to determine the content of saponins, proteins, and polysaccharides. Additionally, a quantitative HPLC analysis was also carried out to determine the mitragynine content in the extract.

Saponin Measurement in KWE

Total saponin content was determined using the method previously described by Helaly et al., (2001). The KWE (5 g) was dissolved in 0.5 ml 80% methanol and then mixed with 0.5 ml 8% vanillin in ethanol and 5 ml 72% H2SO4 in water. These mixtures were placed in a 60°C water bath for 20 min and then cooled at 0°C for 5 min, after which the absorbance was measured at 544 nm using a VersaMax plate reader. The saponin content was calculated from a calibration curve constructed using a purified saponin standard (Sigma-Aldrich; Merck KGaA).

Quantification of Total Protein Content in KWE

The method used to measure total protein was based on Ravisankar et al., (2022) with minor modification. A 4.5 ml of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1%sodium potassium tartrate + 1ml of 0.5% copper sulphate) was added to the KWE extracts and incubated for 15 min. After this, 0.5 ml of freshly prepared reagent 2 (1 part Folin-Ciocalteau: 1 part water) was mixed with each sample and left for 30 min of dark incubation. After that the absorbance was measured at 660 nm and the amount of protein was expressed as mg BSAE/ g of fresh weight. BSA is used as a standard reagent to prepare the standard curve for estimating the unknown protein concentrations.

Estimation of Total Polysaccharides

The method used for the estimation of polysaccharides in KWE is based on Hussain et al., (2008). The KWE sample (0.2 g) was dissolved in 7 ml of hot ethanol (80%) in a centrifuge tube to remove sugars. After shaking for 2 min, the tube was centrifuged at 2700 rpm for 10 min. The procedure was repeated several times with the residue until the anthrone reagent no longer took colour when washed. The residue was dried in a water bath and extracted with 5 ml of distilled water and 5 ml of 25% HCl at 0°C for 20 minutes. Then the tube was centrifuged at 2700 rpm for 10 min and the supernatant was kept. The supernatant was collected in a 100 ml volumetric flask and made up with distilled water. A 0.1 ml of the supernatant was transferred to a test tube, the volume was brought up to 1 ml with distilled water, and subsequently, 4 ml of anthrone reagent was added. The tube was heated in a boiling water bath for 8 minutes and then cooled rapidly. The intensity of the green colour was measured at 630 nm compared to a blank sample containing all reagents except the sample.

Mitragynine Measurement in KWE

The method used for detecting mitragynine compound in KWE was adopted from Parthasarathy et al., (2013) with minor modification. The sample was analysed using the HPLC system (WATERS 600 quaternary gradient pump, WATERS 717 plus auto sampler, and WATERS 2996 PDA) with the HPLC column KINETEX EVO C18 (5 µm, 250 mm x 4.6 mm) and a gradient system consisting of 2 solvents: A (5 mM ammonium bicarbonate buffer, pH 9.5) and B (acetonitrile). The analysis was conducted over a 20-minute period. Initially, at 2 minutes, using 80% solvent A and 20% solvent B. Over the next 3 minutes, there was a transition to 70% solvent A and 30% solvent B. At 10 minutes, the composition was further modified to 40% solvent A and 60% solvent B. Subsequently, at 15 minutes, the mobile phase contained 20% solvent A and 80% solvent B. The composition returned to its initial state, with 80% solvent A and 20% solvent B, at 20 minutes, and this ratio was maintained for the remaining duration of the analysis. The flow rate was set at 1 mL/min with a sample volume of 10 µL. Retention time data and UV spectra for the distinct and clear peaks were analysed and recorded in the report.

In situ Arginase Inhibitory Activity Assay

The method used in this study was based on Wulansari et al., (2018) with modifications according to the manufacturer's protocols (Sigma Aldrich, USA). In this study, the substrate and enzyme concentration used in the assay were modified for optimisation. 15 µL of arginase 1 U/mL, 20 µL of L-arginine 570 mM and 10 µL of KWE solution were incubated at 37°C for 30 min. After pre-incubation, 100 µL of the arginase kit assay was added and incubated at room temperature for 60 min. Arginase activity was determined using a microplate reader (TECAN, USA).

In vivo

An in vivo test was performed in which 35 male rats were divided into 7 groups (n=5) as listed below:

1) Normal control: rats were not induced and treated
2) Negative control: rats were induced by injecting 0.2 ml of compound 48/80 and were not treated
3) Positive control: rats were induced by injection of 0.2 ml of compound 48/80 and treated with 50 mg/kg desloratadine
4) Kratom control: rats were not induced and treated with 400 mg/kg KWE
5) Kratom treatment 100 mg/kg: rats were induced by injection of 0.2 ml of compound 48/80 and treated with 100 mg/kg KWE
6) Kratom treatment 200 mg/kg: rats were induced by injection of 0.2 ml of compound 48/80 and treated with 200 mg/kg of KWE
7) Kratom treatment 400 mg/kg: rats were induced by injection of 0.2 ml of compound 48/80 and treated with 400 mg/kg of KWE

At the end of the experiment, the blood plasma of the rats was used to determine histamine, histidine decarboxylase, tryptase, and interleukin 6.

RESULTS AND DISCUSSION

Phytochemical test evaluation showed that the KWE was found to have 44.6% saponins, 16.3% protein, and 23.06% polysaccharides. Previous studies revealed that *M. speciosa* contains a diverse group of secondary metabolites, such as indole alkaloids, flavonoids, triterpenoids, saponins, and glycosides (Limcharoen et al., 2022).

Table 1 Saponin, protein and polysaccharide percentage in KWE

Metabolites in KWE	Metabolite content (%)
Saponins	44.6
Protein	16.3
Polysaccharides	23.06

Mitragynine is the major active alkaloid of kratom. Reported, it accounts for 12% (w/w) of the total alkaloid content in Malaysian kratom and 66% (w/w) in Thai kratom on a dry weight basis (Takayama, 2004). Mitragynine is found exclusively in *M. speciosa*. HPLC quantitative analysis showed that KWE contains 6.13% of mitragynine (w/w), which is small compared to the amount of mitragynine in MeOH extract reported by Takayama et al. (2004). The low quantity of mitragynine found in the water extract was mainly due to its poor solubility in water. Figure 1 shows the HLPC Chromatogram of mitragynine as an analytical marker in KWE.

a)

b)

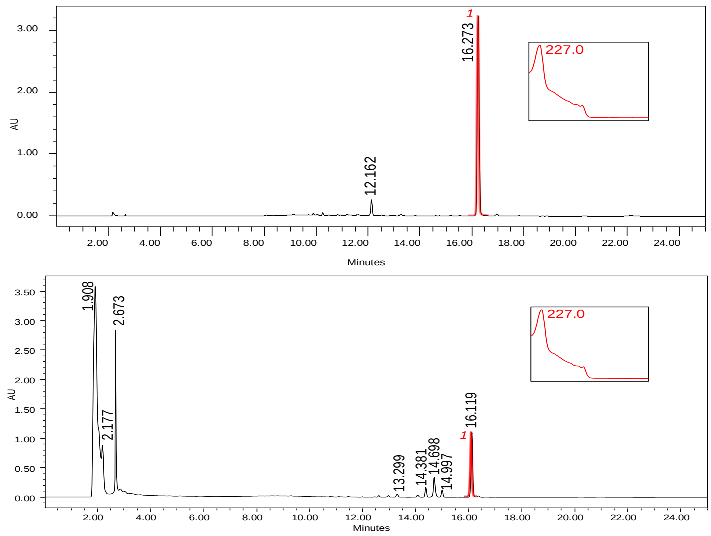

Figure 1 HPLC chromatogram and UV spectra of a) mitragynine (RT 16.273 min) and b) KWE extract (RT 16.113 min), PDA 277 nm

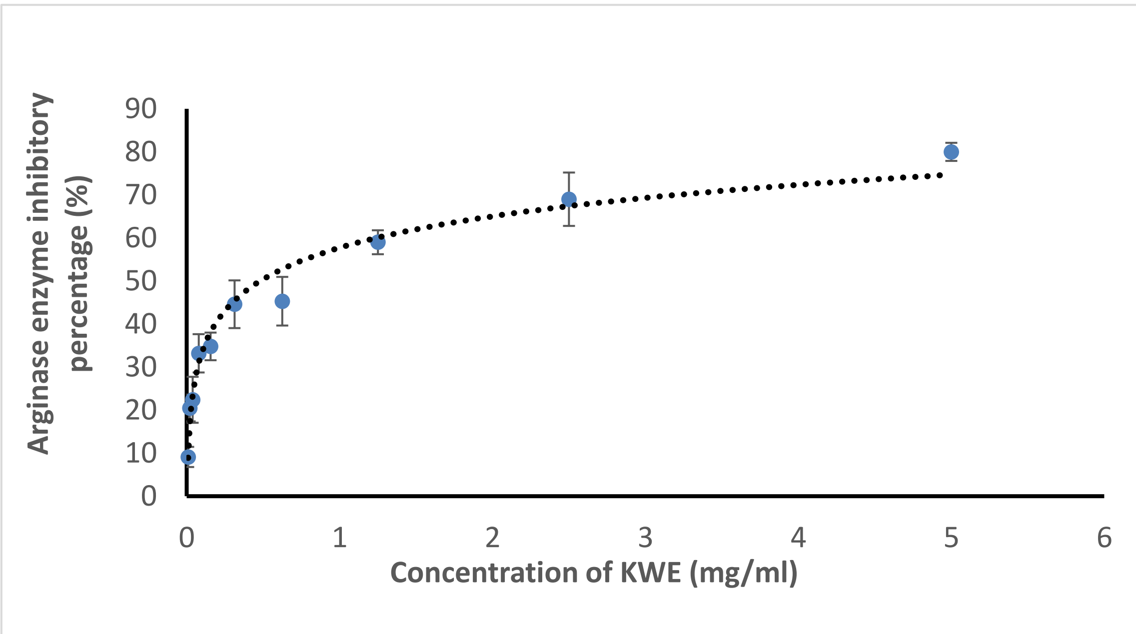

Figure 2 Arginase enzyme inhibitory effect of KWE

In addition, it was found that 50% of the arginase enzyme activity can be inhibited at a concentration of 1.25 mg/ml. This suggested that kratom water extract requires relatively moderate concentrations to decrease arginase enzyme activity. Arginase regulates various processes involved in the regulation of respiratory tract function (Maarsingh et al., 2009). Arginase is involved in the pathophysiology of various respiratory tract diseases, including asthma, and occurs during allergic symptoms (Renée et al., 2011). The ability of an extract to lower the activity of arginase may help in reducing the symptoms of an allergy.

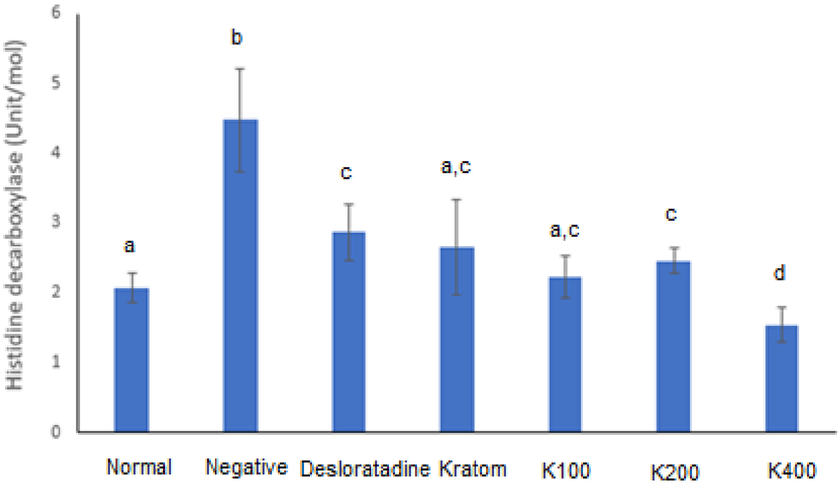

Figure 3 Histidine decarboxylase enzyme concentration after treatment of KWE

The histamine-producing enzyme, histidine decarboxylase (HDC), is commonly induced at inflammatory sites during the late and chronic phases of both allergic and non-allergic inflammation (Hirasawa, 2019). Treatment of 400 mg/kg KWE can effectively reduce the concentration of HDC enzyme compared to negative control. Besides that, desloratadine can decrease HDC and was not significantly different compared to kratom control. Treatment with KWE high-concentration showed a high reduction of HDC level compared to the lower concentration of kratom.

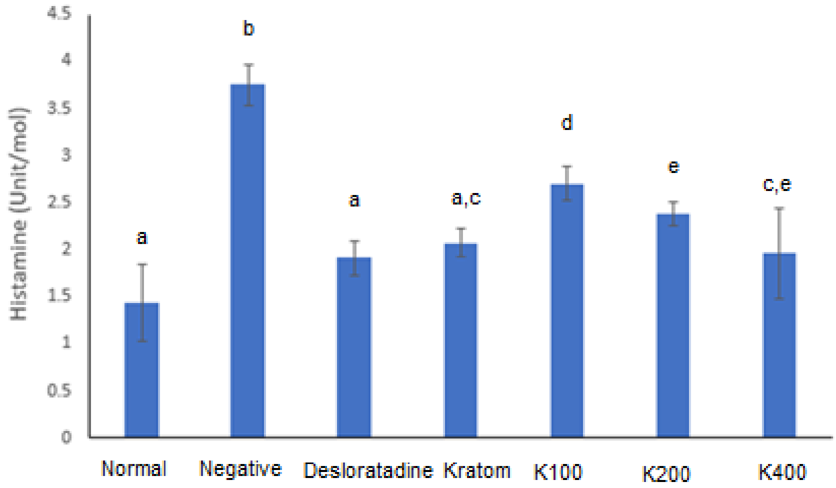

Figure 4 Histamine concentration after treatment of KWE

Histamine is a known mediator of inflammation released by mast cells and basophils (Yamauchi et al., 2019). Histamine is a central messenger released by mast cells during allergic reactions. The negative control had the highest histamine concentration. Desloratadine effectively reduced histamine levels compared to other treatments. In addition, KWE showed a concentration dependence in reducing histamine levels. A higher concentration of KWE reduced histamine levels more than a lower concentration of KWE.

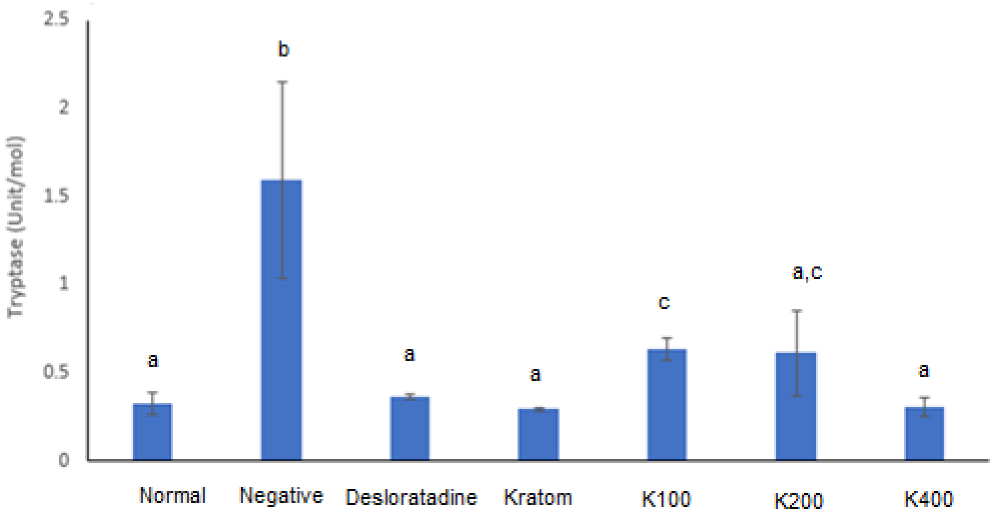

Figure 5 Tryptase enzyme concentration after treatment of KWE

Tryptase is an enzyme found mainly in mast cells. Mast cells are white blood cells that are part of the body's immune system and are activated during allergic reactions. A transient increase in serum total tryptase levels indicates mast cell activation, e.g., an anaphylactic reaction (Braendstroem et al., 2015). KWE can effectively and non-significantly decrease tryptase levels compared to desloratadine. Treatment of the kratom group without inducing compound 48/80 showed lower tryptase levels. This suggests that the KWE effect in reducing allergic reactions in rats may be through lowering the blood tryptase level.

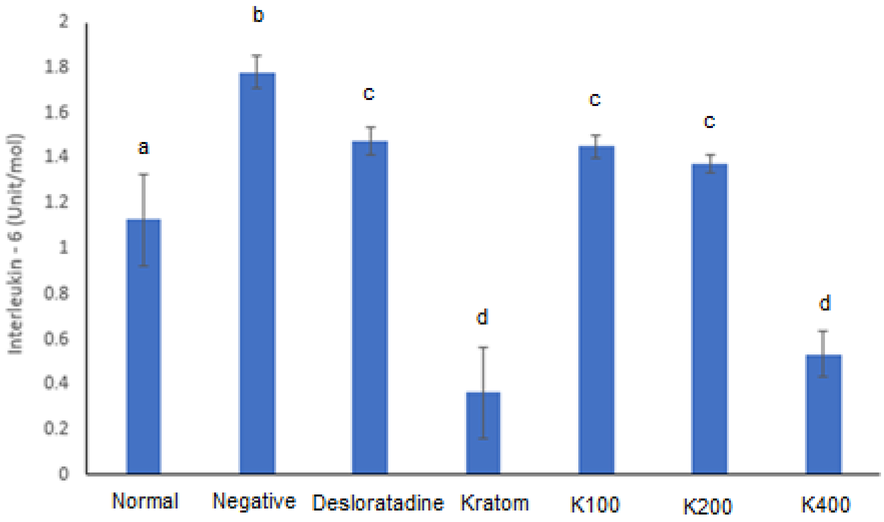

Figure 6 Interleukin 6 concentration after treatment of KWE

IL-6 is a pleiotropic cytokine that, along with TNFα and IL-1β, has traditionally been considered a biomarker of ongoing inflammation rather than a regulatory cytokine with the potential to modulate the immune response. Treatment of KWE with 400 mg/kg effectively reduced IL-6 levels compared with desloratadine. However, desloratadine did not effectively reduce the concentration of IL-6 compared to the negative control. Desloratadine blocks only one type of receptor for histamine (the H1 receptor) (Geha & Meltzer, 2001). The effect of kratom in reducing IL -6 was concentration-dependent, as the higher concentration reduced IL-6 concentration compared to the groups with lower concentrations of kratom extract.

CONCLUSION

This study showed that KWE contains 44.6% saponins, 16.3% proteins, 23.06% polysaccharides, and 6.13% mitragynine. It was found that at a concentration of 1.25 mg/ml, 50% of arginase enzyme activity could be inhibited by KWE. KWE can also effectively reduce allergic reactions in rat models and the mechanism could be through the reduction of tryptase and IL -6 levels.

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FORESTS AND PEOPLE'S LIVELIHOOD: CONTRIBUTION OF FOREST USE TO LOCALS' SOCIO-ECONOMICS IN ULU MUDA FOREST RESERVE

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ABSTRACT

Protected forests are the cornerstones of biological conservation. The forests of the world are one of the most important of these biological conservation. More than 1.6 billion people depend to varying degrees on forests for their livelihoods. About 60 million indigenous people are almost wholly dependent on forests. Some 350 million people who live within or adjacent to dense forests depend on them to a high degree for subsistence and income. The establishment of forests play an important role in the community's livelihood, and this role has created an important relationship or mutual dependence between the forest and the community. However, ignorance of the socio-economic benefits of the forest resources among the adjacent livelihoods is a great limitation to communities' dependency. Although many studies have seen significant dependency of the local people on forest resources, the degree of dependency on forest resources might vary across various localities of rural communities due to dynamic changes in the socio-economic status. The economic and social benefits of forest to local people are clearly seen as critical aspects of forest sustainable development. To meet one of the needs of current forest management, research aimed to examine the role of protected forests and their relation to local people, especially in Ulu Muda Forest Reserve (UMFR), Kedah through a face to face interview. This paper focuses on examining how the forest contributes to the socio-economic status, dependency, and household benefits in the UMFR. The socio-economic importance of UMFR was therefore studied in three districts, namely Sik, Baling, and Pedu. Personal interviews were conducted in the 32 settlements, approximately six to 10 km away from the UMFR. Study showed that forest has contributed to the local income through socio-economic activities for adjacent populations mainly agriculture and other forest-related activities such as fishing, non-timber forest products (NTFPs); collecting rattan, bamboo, honey, handcrafting, and medicinal plant collection. The community's forest dependency showed quite an extensive range of socio-economic backgrounds of the livelihoods, and they have been exposed to a certain degree of development. The strategies through a few series of stakeholders' consultation workshops were also adopted. Such effort could assist an active community involvement towards developing strategies to improve the socio-economic status of the population, as well as contributing to the sustainable conservation of the forest ecosystems of stakeholders that wish to find ways of facilitating local empowerment through better benefit sharing mechanisms.

Keywords: socio-economic, consumption, benefits, local communities, social impacts

INTRODUCTION

Forest is a vital component to the local communities and rural people living in the area. More than 1.6 billion people depend to varying degrees on forests for their livelihoods as The World Bank's Forest Strategy reported. About 60 million indigenous people are almost wholly dependent on forests (World Bank, 2004:16). Consequently, the socio-economic condition of local people strongly influences the sustainable forest management including in Malaysia. In 2019, Permanent Forest Reserve (PFR) PFR in Peninsular Malaysia was recorded as 4,812,326 ha, which is 43.4% of the total forested areas in the same region (Forestry Department of Peninsular Malaysia, 2020). As such, based on 2017 statistics, the forest area in Kedah is 344,4945 ha (Forestry Department Peninsular Malaysia, 2020). Of the total, the PRF status area is 341,976 ha or 36% of the remaining state land area of 455 ha with Government Land status (FDPM, 2020). Socioeconomics refer to the study of relationship between economic activity and social life (Van der Merwe, 2008). A socio-economic study's general objective is to evaluate socio-economic progress, typically in terms of improvements in indicators such as gross domestic product (GDP), life expectancy, literacy and employment rates (Van der Merwe, 2008), in order to enhance the benefits that society receives. Mounting evidence demonstrates that poverty— especially in rural areas—can be reduced only by sustainably managing natural resources both for the income they generate and for the environmental services they provide. The forests of the world are one of the most important of these natural resources. The establishment of forest play an important role in the community's livelihood, and this role has created an important relationship or mutual dependence between the forest and the community. However, ignorance of the socio-economic benefits of the forest resources among the adjacent livelihood is a great limitation to communities' dependency. A lack of awareness of the socio-economic benefits of forest resources among nearby communities poses a significant challenge to their reliance on these resources. Thus, this research in particular is to examine how forest contributes to the socio-economic status, dependency, and household benefits in the Ulu Muda Forest Reserve (UMFR), Kedah.

MATERIALS AND METHODS

Section 10 (1) (b-l), Forestry (Application) Enactment 1985 in the Ulu Muda Forest cluster, which serves as the source of water supply (water catchment area) to the three main lakes, namely Tasik Muda, Tasik Ahning and Tasik Pedu, the Ulu Muda Forest Cluster is a 'forest complex' with an area of 163,000 ha covering eight (8) Permanent Reserve Forests (PFRs) and should be protected from excessive exploitation. The administration and management of UMFR are Central Kedah and South Kedah Forest District Offices. Data collection in this study involves four main techniques, namely rapid rural appraisal (RRA), survey research, field research and stakeholders' consultation workshop. The RRA technique is crucial to enable the research team to have a general overview of the existing environment and the extent of forest resource utilisation by the local community living near UMFR. It was conducted on March 2021 through observation, focus group discussion with key informants, head of villages and gathered socio-economics profile of the adjacent villages. A cross-sectional study was conducted through face to face interviews in April to December 2021 to December 2021 among 1,567 respondents from 32 villages bordering Ulu Muda protected forest. The districts involved were Baling, Sik and Pedu. The study settlements/villages have diversified economic activities, mainly in three domains: agriculture/plantation, forestry-related and business revenue. These villages were selected due to their proximity to the study area, in which these localities were located six to 10 km radius from UMFR (see details in Figure 1). A structured questionnaire served as a main instrument contained both descriptive and causal questions, consisted scaled (Likert-scale), pre-coded and open-ended questions. Stratified sampling technique was used for community survey and enable the sample of member are been chosen. Stratified sampling technique put basis of random samples divide the population into groups or homogeneous subpopulations called strata or stratum (plural) based on specific characteristics (e.g., location). The basis is every member of the population should be in exactly one stratum (Thomas, 2023). This technique enables the researchers to explain and brief the respondents on the objectives and purpose of the survey.

The questionnaire covered respondents' socio-demographic and economic background, the view of forest conservation programs, the household member's information (without name and identification number), the status of employment, and finally the households' sources of monthly income to relate their their dependency towards resources from forests alike. Data were coded and anlysed using Excel and Statistical Package for Social Sciences (SPSS). The survey team collaborate with the Kedah Forestry Department, local authorities and head of villages who arrange the interview schedule with the local communities. Surveys and face to face interview sessions were conducted with 12 trained field assistants.

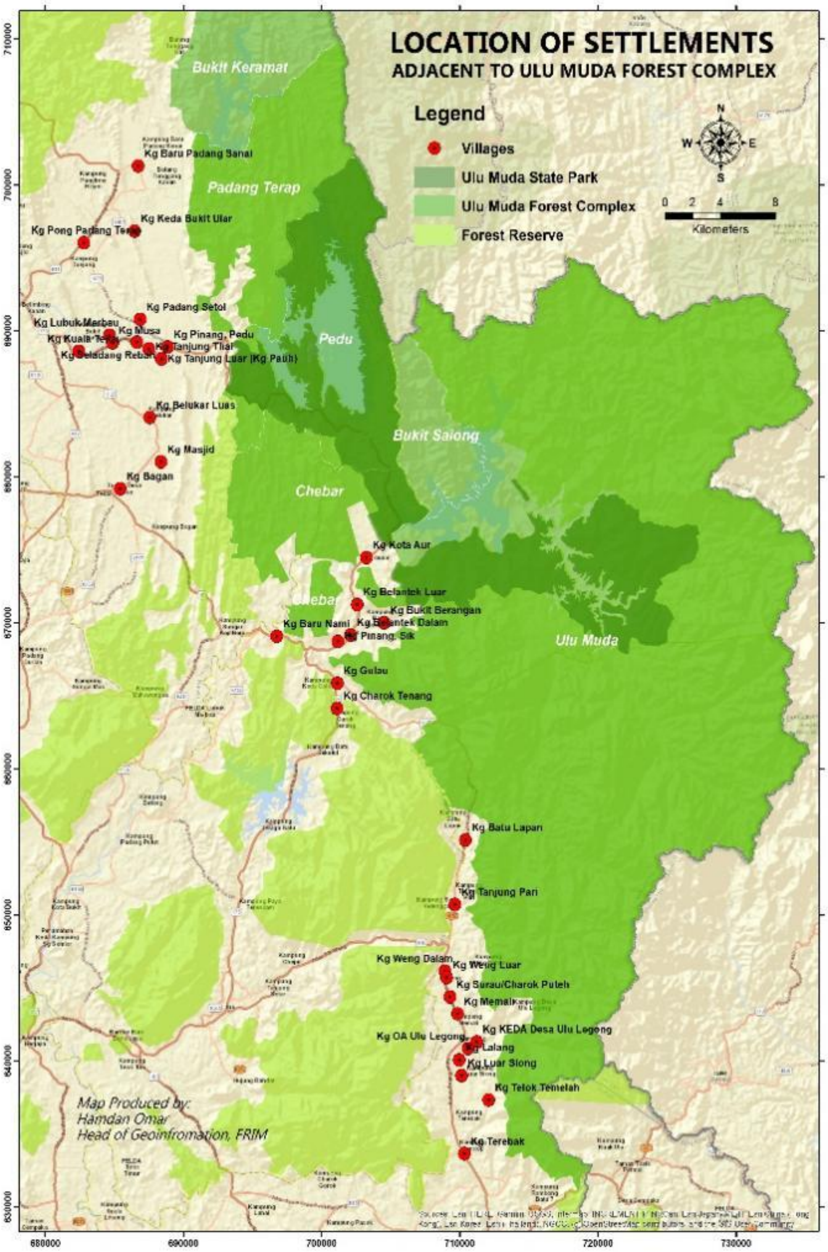

Figure 1 Location of the settlements adjacent to Ulu Muda Forest Reserve
(Credit: Hamdan O, 2023)

RESULTS AND DISCUSSION

Socio-Demographics Profile

Table 1 Socio-demographics profile households head

Socio demographic characteristics	Percentage (%) (n = 1567)		
	Baling	Sik	Pedu
Age (years)	(N = 696)	(N = 425)	(N = 446)
18-29	10.00	7.44	6.00
30-39	14.38	15.98	13.00
40-49	18.59	33.33	18.00
50-59	28.44	23.96	30.60
>60s	28.59	19.29	32.40
Education attainment	Baling	Sik	Pedu
No formal education	4.53	5.79	3.63
Primary school	31.41	46.28	27.44
Secondary school	51.56	43.53	61.68
Upper Secondary (Form 6)	4.84	0.28	2.50
Tertiary education (University)	7.66	4.13	4.76

Source: Field community survey, 2021

Of the total sampled population, most of the household heads were in their senior-age group (M = 50.03, SD =13.78) (see Table 1), except in Sik district, which was in middle-age group (40 to 49 years old group). More than one third of the household heads 51.56% in Baling, and 61.68% in Pedu had attained secondary education as their highest education. However, in Sik district, the household heads attained primary education as their highest education level, while only minority household heads (Baling=7.66%, Sik= 4.13% and Pedu=4.76%) indicated received their tertiary education. The minor group that has not attained any formal education was small and less than 6% (Baling= 4.53%, Sik= 5.79% and Pedu= 3.63%) as indicated in Table 1.

Type of Employment of Local Communities by Sector

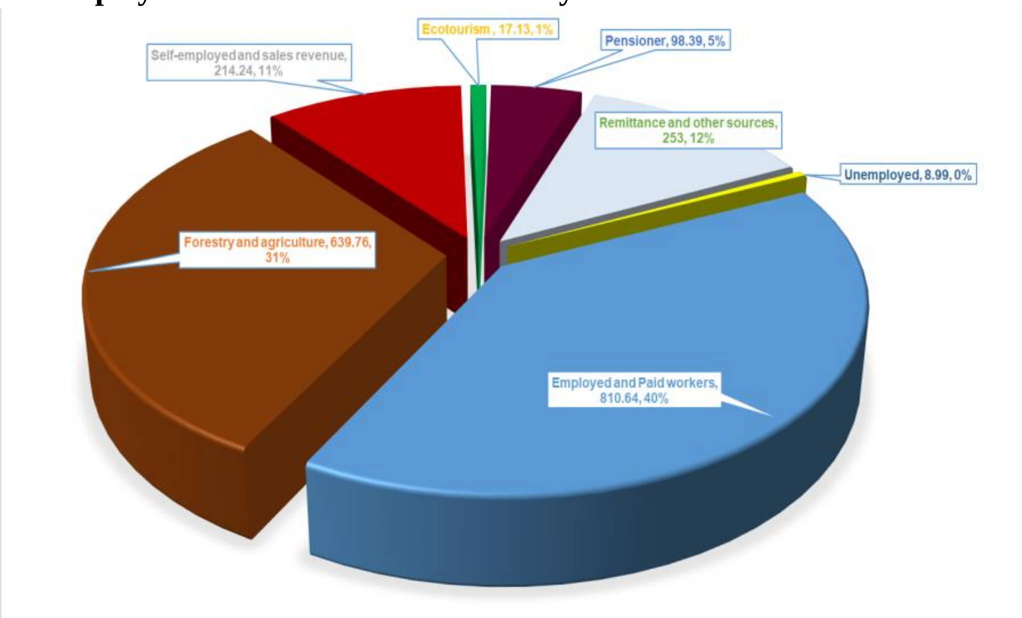

Figure 2 Type of employment of local communities by sector (RM/monthly)

Household income represents the sum of monies accruing to all people in a given household; this includes income from wage and salaried jobs and other sources such as business, remittances, and social grants. However, due to the sensitivity of the variable to most respondents, it was recorded as categorical data to facilitate the respondents to reveal their monthly income expenditures. The level of monthly household income in a destination or site indicates the local economic situation and is an important proxy for a measurement of socio-economic status or standard of living. The contribution is calculated based on the percentage of cash income (direct income) through their employment status including any forest products and other related forest-based activities, ecotourism activities, sales revenue and other sources (see Figure 2) About 40% of households are employed or paid employees in government or private sectors, as contract staff or factory staff in industrial sectors (Figure 2). The forestry and agriculture sectors contributed to 31% (RM 639.76 monthly) of the monthly household income. The nearby community is primarily dependent on socio-economic activities associated with rubber estates, paddy farming, and various forest-related sources such as inland fishermen, gathering rattan, bamboo, honey, vegetables crop, orchard ownership, livestock farming, collecting non-timber forest products (NTFPs) and harvesting medicinal plants. This acknowledge that forest play an important role in maintaining the productivity of agricultural and environmental systems including provisioning for ecosystem services (Ferraro & Hanauer, 2011) and have the significant ability as an effective nature conservation strategy especially in conserving forest habitats.

Type of Household Income Sources

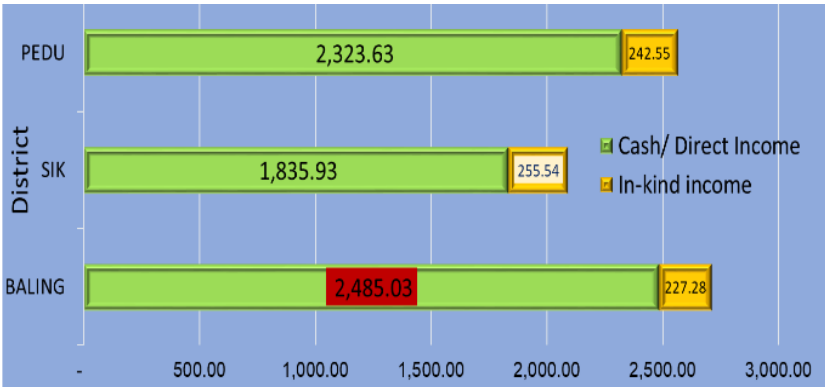

Figure 3 Type of monthly household income sources (RM)

There are two types of household income sources namely cash income and in-kind (non-cash) income. Direct income sources or cash income refer as paid employee or employed, sales revenue, forestry and agricultural resources and other sources (family members giving, dividend, bonus, rental and services). Local communities in adjacent of UMFR still rely on NTFPs for subsistence and cash income. The average declared cash household income in the forest-adjacent community was estimated at RM 2,485.03 monthly in Baling; Pedu district was RM 2,323.63/monthly while in Sik district was the lower with RM 1,835.93/monthly for cash income per household (Figure 3). In-kind income refers to non-cash income (such as properties, house, transports, forest resources consumed by households; food sources and water intake).

Social Benefits Perceived by Local Communities

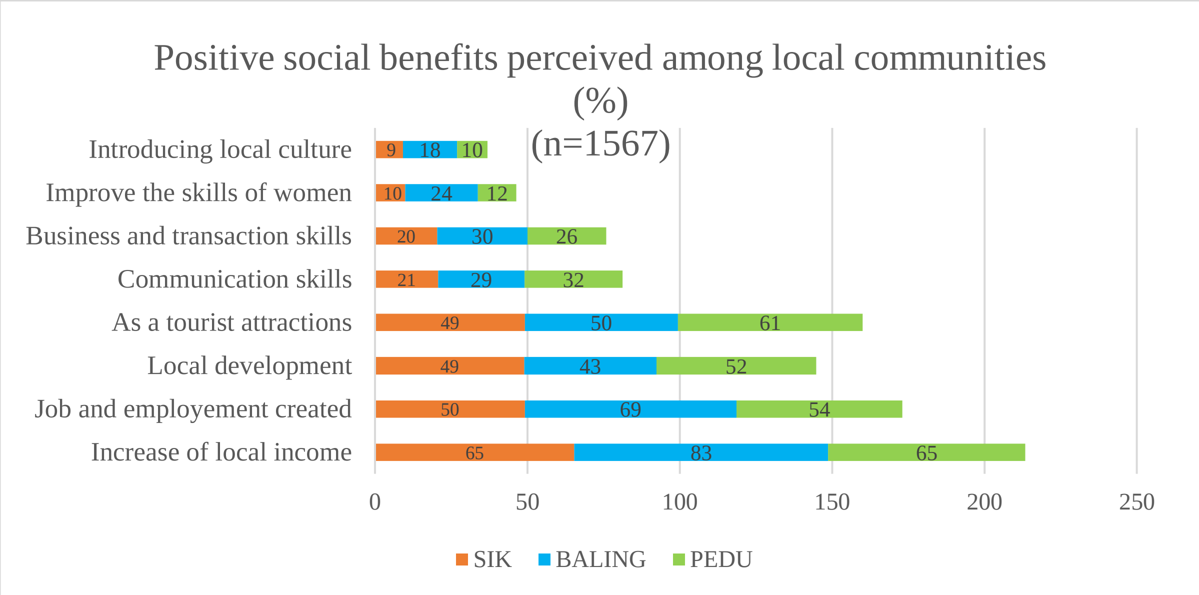

Figure 4 Positive social benefits perceived among local communities (n=1567)

The social dimensions facilitate the individuals' need to live dignified and healthy condition live (Choi & Sirakaya, 2005; Mitchell & Reid, 2001). The household agreed that UMFR establishment had contributed to the increase in income level and (Baling= 83%; Sik=65% and Pedu = 65%) jobs and employment opportunities created (Baling= 69%; Sik 50% and Pedu= 54%) for the local as indicated in Figure 4. The establishment UMFR had brought changes to local communities socio-economic where it had created suitable attractions and popular destinations to the tourist (Baling=49%; Sik =50; Pedu=61%) such as Gubir Lake, Ulu Muda Jetty and Ulu Muda Eco Park. The fact that the local communities' borders on UMFR are living in the community has brought a positive benefit on community perceptions of the protected area included improved local development (e.g; road infrastructures) and increased communication skills.

CONCLUSION AND RECOMMENDATIONS

Considering the importance of forests for both conservation and source of livelihood, existing and planned models through action plans must be strengthened by the local government, which enhanced the livelihood of rural people. The research shows income generated near UMFR enhances the benefits received by society. This findings had a significant contribution to be as an important baseline input to provide a clear picture in support of one of the important principles in the Social Forestry Strategic Plan of Malaysia (2021-2025) which emphasizes to improve the socio-economic conditions of indigenous peoples and local communities. Understanding the level of consumption and pattern of forest dependency will enable researchers, policy makers and practitioners to design empirically informed intervention to diversity household's livelihood portfolio and promote sustainable resource utilisation to foster a balance between forest dependency and nature conservation.

ACKNOWLEDGEMENTS

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SUNGAI KUANTAN MANGROVE FOREST: A JEWEL OF KUANTAN CITY, PAHANG

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ABSTRACT

Malaysia is blessed with an abundance of mangrove forests. Mangrove forests are among the most important types of forest in Malaysia, where Malaysia contributes 3.7% of the 15.62 million hectares of world mangrove forest coverage. Mangroves have enormous ecological significance, both to the functioning of the natural environment and to humans. Mangrove forests provide a wide range of ecosystem goods and services, such as acting as frontiers that protect the coastal land against the destruction of ocean waves, providing habitat for various marine life forms, functioning as natural filters that improve the quality of water, and also play important roles as significant carbon sinks in a coastal environment. Not only have extensive values, mangrove resources also serve great importance to the socio-economics of the country, especially to the local community. With all these goods and services, it is clearly shown that mangrove forests are important and need to be conserved. Hence, this paper will showcase the ecosystem goods and services of Sungai Kuantan Mangrove Forest. Ecosystem services that will be highlighted in this paper are dependent communities.

Keywords: mangrove forest, household, socio-economic, Sungai Kuantan, income

INTRODUCTION

There are various types of ecosystems on the Malaysian coast; one of the ecosystems is the mangroves. Mangrove forests are among the most important forest types in Malaysia, where Malaysia accounts for 3.7% of the world's mangrove forest coverage (Wan Juliana et al., 2018). Meanwhile, Abdul Shukor (2004) stated that Malaysia's coastline is estimated to be 4,810 km along Peninsular Malaysia (west and east coast), Sabah and Sarawak. Mangrove forests act as a boundary that protects the country's coast from strong waves, especially during the monsoon season. In addition, mangrove forests also provide habitat for various forms of marine life, function as natural filters that improve water quality and also play an important role as significant carbon absorbers in the coastal environment (Hamdan & Muhammad Affizul, 2020). Most importantly, the mangrove ecosystem is also a breeding nursery for various shrimp and marine fish. This makes mangroves one of the most fertile and productive areas in nature because they function as nesting places and feed biodiversity (Kamarulzaman et al., 2011).

Mangrove resources also provide great importance to the socioeconomics of the country, especially to the local communities that live bordering this ecosystem. The importance of this resource is derived from the direct goods obtained from the mangrove forest as well as from the services provided. Fishery items obtained from the mangrove ecosystem include mud crabs, various species of shrimp, gastropods and even clams. As such, this ecosystem provides an important source of food and is able to generate income for nearby communities. The preservation and conservation of this mangrove ecosystem are very important not only for the stability and safety of the environment but also for the well-being and livelihood of the local community.

Therefore, the study in 2020 aims to analyse the contribution of mangrove forests to the lives of local communities, especially in the mangrove areas of Sungai Kuantan, Pahang. The objectives of this study are (i) to identify the use of mangrove forests by the local community, (ii) to determine the mangrove resources that are harvested and used by the local community, and (iii) to estimate the financial value of how much mangrove forests contribute to the income of the home.

MATERIALS AND METHODS

Study Area

In general, it is estimated that there are approximately 1.55 million hectares of Permanent Forest Reserve in Pahang, of which 2,416 hectares are made up of mangrove forests with 339 hectares of mangrove forest coverage is in Kuantan, Pahang. This small green pocket area located in the middle of the city of Kuantan provides various goods and services that contribute to the economy and livelihood of the local community but also functions as an ecotourism place, especially for domestic visitors to enjoy and feel the uniqueness that is still preserved. Boating along the Kuantan River by a local operator, recreational fishing (where visitors can hire a boat or hire a local boat), watching fireflies at night, visiting fishing villages and also a boardwalk with a lookout tower in the mangrove area are among the attractions and activities that can be done in Sungai Kuantan. However, the sustainability of this mangrove forest is in a vulnerable state and is also under pressure due to the rapid development around this area. Land use change from forested land to development or agricultural land can also occur due to its strategic location. Therefore, the time has come for a study to be carried out to act as a supporting policy document that proves that this area needs to be protected and preserved so that the sustainability of the mangrove forest can be maintained and the dependence of the local community on the goods and services of the mangrove forest continues to be guaranteed especially for future generations. A total of nine (9) villages were selected to be sampled in this study, namely Kampung Anak Air, Kampung Belukar, Kampung Kempadang, Kampung Peramu, Kampung Permatang Badak, Kampung Sungai Isap, Kampung Tanah Putih, Kampung Tanjung Api and Kampung Tanjung Lumpur. These villages have been sampled together with the Kuantan Land District Office.

Study Approach

There are two main research approaches used in this study, which are the Rapid Rural Appraisal (RRA) and the socio-economic survey of households around Sungai Kuantan. The RRA technique is widely used globally. Studies by Ganesh (2010), Jarrett & Lucas (2003), Alayne et al. (1997), Gellardo (1995), and Melville (1993) also use this approach in their study. The

RRA technique is a tool that enables rapid assessment of the existing environment and the possible impact of the use of other environmental resources and services on local socio-economic life (Liswanti et al., 2012). Techniques used in RRA include group interviews, methods of cross-checking information from different sources, direct observation at the study site level, and the use of secondary data (Crawford, 1997). This technique provides useful information to be implicit in the design of the questionnaire. In this study, RRA was applied in the early stages of the study to gather basic information and understand the role of mangrove forests and their contribution to local communities living near the study area. Meanwhile, the household survey is a formal face-to-face interview based on a structured questionnaire. The questionnaire was constructed into several sections that cover household income sources, local residents' perceptions of mangrove forests and finally, household demographic characteristics. Trained enumerators conducted household socio-economic surveys. During data collection, respondents were given a brief explanation of the objectives and purpose of the study. The time taken for each interview was approximately 30 minutes per interview.

Sampling

The estimated sample size is based on the number of households around the study area, where the Kuantan District and Land Office provided information about the households. This study uses a simplified sampling formula from Yamane (1985) and takes 5% as the level of accuracy. A total of 384 households were successfully interviewed during the survey (Table 1).

Table 1 Sampled Information

Villages	Number of Household	Number of Samples	Percentage (%)
Anak Air	150	21	5.5
Belukar	400	70	18.2
Kempadang	150	44	11.5
Peramu	900	80	20.8
Permatang Badak	60	20	5.2
Sungai Isap	120	43	11.2
Tanah Putih	150	41	10.7
Tanjung Api	180	35	9.1
Tanjung Lumpur	80	30	7.8

RESULTS AND DISCUSSION

Resource Usage

Mangrove forests provide various functions and uses that not only maintain the balance of the ecosystem but also benefit the local community. The mangrove forest serves as a breeding habitat for various types of marine life, which is a source of daily food for local residents and also income for local fishermen. Meanwhile, amenity services provided by mangrove forests also create employment and income opportunities for local boatmen, tour guides and small-scale ecotourism operators. The results of the study found that 36% of respondents use mangrove areas in Sungai Kuantan for various purposes. The purpose of the respondents to use the mangrove area can be categorised into three: either for their use, to generate income for fishermen, and also just recreational fishing in the mangrove forest as a hobby in their leisure time.

Table 2 shows the use of mangrove forests by the local community. The results of the study show that the highest percentage is the use of mangrove trees as a recreational place for fishing in free time, followed by personal use and to generate income. Wherein 25% of respondents use the mangrove forest for recreational fishing, especially in their leisure time. There are also 13% of respondents who use the mangrove forest to obtain food sources such as mud crabs, fish and shellfish for their daily meals. Finally, there are 10% of respondents directly dependent on mangrove goods and services to generate their monthly income. Most of these respondents work either as fishermen or Fishmongers. The study found that mangrove forests make a great contribution to the surrounding community. This proves that mangrove forests in Sungai Kuantan not only provide goods (in terms of fisheries) but also provide facilities and services that give great value to the local community. This discovery is also proof that it is important to conserve and maintain the mangroves in Sungai Kuantan because the area provides enormous benefits to the people, either to the communities living nearby or to the people of Kuantan as a whole.

Table 2 The use of mangrove forests by the local community

Usage	**N**	Number of Households Involved	Percentage (%)
Own Consumption	384	51	13%
Income Generation	384	38	10%
Recreational Fishing	384	94	25%

Mangrove Forest Resources

One of the direct benefits of mangroves is their function as a food source. To further support this function, the slogan "No Mangrove, No Seafood" was established to help protect the mangrove ecosystem as a breeding ground and habitat for many marine lives. Goods from the Kuantan River mangrove forest that the villagers often harvest are *Macrobrachium rosenbergii* (Shrimp), *Scylla serrata* (Nipah crab), *Polymesoda expansa* (Lokan), *Cerithidea obtusa* (Snail/Belitung) and also various types of fish such as grouper, sea bass, silver, white croaker or *gelama* and more. Table 3 shows the mangrove forest resources that local residents obtain. Among the catch in Sungai Kuantan is "Udang Galah", where the price is between RM 40-RM 90 per kilogram. It is estimated that the average catch is 4 kg per trip in the season during which this study was conducted. In addition, various types of fish are caught in this area, such as grouper, sea bream and thorn fish. Most of this catch will be sold to fishmongers or at the market. However, most respondents do not sell shellfish such as lokan and conch. Usually, local people only take it to meet their subsistence needs or give it away. Looking at these results, it seems that mangrove resources have been supplying the local community continuously and, at the same time, generating income for them.

Table 1 Mangrove Forest products harvested by the community

Mangroves Goods	N	% of Households Involved	Usage
Udang Galah	384	41%	Sale and own consumption
Mud crabs	384	36%	Sale and own consumption
Fishes	384	11%	Sale and own consumption
Shells	384	8%	Own consumption
Others	384	3%	Sale and own consumption

The Contribution of Mangrove Forests to Household Income

The results found that the average monthly household income was RM 2,882 per month (Table 4). There are two types of income, either in the form of cash or non-cash (in-kind income). Cash income refers to income gains from wages, salaries, or business. Meanwhile, income in the form of goods can come in the form of provisions, such as free food, which is mangrove resources used for daily food sources by households, or it can come in the form of property or exchange of services. For this study, 92% of the household income of this community is cash income, and only 8% is from goods income, which is equivalent to RM 2,653/month and RM 230/month, respectively.

Table 4 Household monthly income

Type of income	RM/ month	Percentage (%)
Cash	2,652	92
Non-cash	230	8
Average household monthly income	2,882	100

The estimated contribution of mangrove forests to household income can be calculated based on the type of income earned by the household. The ability of Sungai Kuantan's mangroves to generate income for local residents can be measured through household income sources, which is to ascertain if mangroves are one of the sources of income and how much they contribute to monthly income. Contributions can be from cash income and goods. For example, sales or business related to mangrove goods and products, whether direct produce such as fresh fish, crab and other seafood or processed indirect produce food such as fish balls, dried fish, fish crackers and shrimp paste / belacan. Studies show that 31% of household income is generated from mangroves and related resources, which averages RM 862/month. This discovery is proof that mangroves contribute to the life of this nearby community.

Table 5 Income generated from mangrove forests

The average monthly household income	RM 862
Revenue generation percentage	31%

CONCLUSION

In conclusion, the mangrove forest in Sungai Kuantan, Pahang, not only provides goods but also amenities and services that provide high value. Where 36% of the nearby community uses and visits the Kuantan River mangrove forest for various purposes, some of these communities also depend directly on mangrove resources to sustain their lives. Therefore, it is important to conserve and maintain the mangroves in Sungai Kuantan because the area provides enormous benefits to the people. The results of this study act as evidence that Sungai Kuantan mangroves contribute to the lives of the local community either directly or indirectly.

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ULU MUDA FOREST RESERVE'S CONTRIBUTION TO LOCAL ECONOMY: AN ECOSYSTEM VALUE CHAIN PERSPECTIVE

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ABSTRACT

This study undertakes a comprehensive analysis of the ecosystem value chain linked with the Ulu Muda Forest Reserve (UMFR), a pivotal repository of natural resources. The study discusses the dynamics underpinning UMFR's abundant forest resources, delineating their profound impacts on the local community and various industrial sectors. Employing a functional analysis methodology, we systematically quantified the economic benefits derived from UMFR's ecosystem services by all value chain players. Spanning across two states, namely Kedah and Perak, the exploratory survey encompasses a noteworthy 2,810 economic activities located within a 15-kilometer radius of UMFR. Our study also incorporates economic activities originating from UMFR-affiliated sources in Penang and Perlis. To facilitate a systematic analysis, we employed the Malaysia Standard Industrial Classification (MSIC) to categorize these activities into industrial and sub-industrial sectors. Empirical findings of the study divulge that a substantial majority of these economies are concentrated within the services/retailing sector (87%), followed by manufacturing (6%) and agriculture/forestry/fisheries (4%). Notably, 75% of these activities operate within a 10 km radius of the forest. Through an exacting verification survey of ecosystem services, we identified a total of 203 economic activities fundamentally reliant on UMFR's invaluable resources. The investigation of the study comprehensively assessed 150 of these economic activities. This systematic framework not only elucidates the network linkages interconnecting participants within the value chain but also unveils the creation of economic value across eleven industry chains. The total output generated was RM 17,163,912. These encompass a diverse spectrum of sectors, including non-wood forest products, fishing, water resources, tourism, beekeeping, honey production, freshwater aquaculture, fruit plantations, mushroom cultivation, sand and limestone mining or quarrying, and timber production. These empirically grounded findings underscore the profound reliance of proximate industries on UMFR, thereby affirming its status as a protected forest region of paramount significance. Furthermore, they underscore the irreplaceable value intrinsic to UMFR's natural resources, accentuating the critical exigency of implementing sustainable conservation and management practices to safeguard this indispensable ecosystem.

Keywords: value chain, natural resources dependency, economic activities, forest ecosystem services, sustainable conservation

INTRODUCTION

The Ulu Muda Forest Reserve (UMFR) emerges as a sanctuary of ecological richness, nestled within the verdant embrace of Kedah and Perak states in Northern Malaysia. Spanning over an expanse of pristine wilderness, this sanctuary stands as a testament to nature's abundance, harbouring a diverse array of flora and fauna (Rajoo et al., 2021). Beyond its fundamental ecological value, UMFR embodies a wellspring of economic success associated with a multifaceted value chain. As the foundation of an extensive economic value chain, UMFR supports numerous livelihoods and industries. From indigenous communities that have forged their existence in harmonious coexistence with the forest to modern enterprises that rely on its resources for their vitality, UMFR's economic reach is both wide and profound. The value chain emanating from this forest encompasses a mosaic of activities, from traditional practices like beekeeping and honey production to contemporary sectors like tourism and manufacturing (Samdin et al., 2023).

Additionally, the UMFR serves as the primary water catchment area for Sungai Muda, which supplies approximately 96% of Kedah's water and 80% of Penang's water. Consequently, the preservation of this forest is crucial in guaranteeing water security for these two states in the northern region. In 2005, the UMFR catchment area contributed RM157 million to Kedah and RM139 million to Penang in terms of yearly water supply for both domestic and industrial purposes (Perbadanan Bekalan Air Pulau Pinang, 2023).

Acknowledging the crucial function of the UMFR in supporting both the environment and the economy, it is essential to thoroughly examine and assess the complexities of this interconnected system. By comprehending the various connections that tie UMFR to regional economies, we can acquire valuable knowledge about the genuine economic value of this forest reserve. Furthermore, such an examination enables the development of strategies that harmonize the needs of conservation with those of economic progress, guaranteeing the enduring significance of UMFR's contributions to both the natural world and human society (Mensah, 2019).

This paper identifies economic activities within a 15 km radius of the UMFR and assesses the value of downstream industry within the study area. The study highlights the complex dynamics of UMFR's rich forest resources and their significant effects on the local community and various industrial sectors.

MATERIALS AND METHODS

The three main stages of the data gathering approach for this study were an ecosystem verification survey for economic activity related to forests, an exploratory survey on economic activity within a 15 km radius, and a comprehensive survey on the value chain that has been identified.

The exploratory survey started with the observation of the location and all roads around 15 km from the UMFR with Google Maps. The research team was divided into two teams, which covered the north and the south of UMFR's surrounding area. A simple survey form consists of the information of the company name, address, district, forest juridical area, distance from forest, type of activity, contact number, number of workers and input source. Through this survey, all of the economic activities within the area are captured and marked with GPS coordinates. All of the study team are extensively trained in GPS marking and fieldwork surveys.

All economic activities that use forest resources or engage in forest-related activities were questioned for the ecosystem verification survey. The respondents also included the industry players recognized from the focus group discussion conducted by other scopes in the main project, which consists of Scope 1: Economic valuation of ecosystem services and biodiversity and development of a centralized database and website of the ecosystem services and biodiversity, and Scope 2: The dependence and level of awareness of the local communities adjacent to the protected forest area towards the sustainable use of resources and through snowball method. Detailed surveys on the identified value chain players were conducted face-to-face. To facilitate a systematic analysis, all of the economic activities identified were categorized with reference to the Malaysia Standard Industrial Classification (MSIC), 2000. The type of activities are classified by industrial and sub-industrial sectors, whereby, the distribution and their distance from the forest are briefly discussed. Functional analysis is the first stage in value chain analysis. The role of the chain participants was identified in this stage. Among the participants identified in a value chain are collectors, manufacturers, wholesalers, agents, middlemen, and retailers. The total number of players for each value chain and the total sales generated from their activities are concisely discussed.

RESULTS AND DISCUSSION

Figure 1 shows the distribution of economic activities in a 15 km radius from UMFR. A total of 2,810 economic activities were found near UMFR (Figure 2). All of them were identified and classified into the industrial sector according to MSIC, which are 2000 categories. The findings disclose that a substantial majority of these economies are concentrated within the services/retailing sector (2444), followed by manufacturing (175), agriculture/forestry/fisheries (123), construction (10), and mining/quarrying (8). There were economic activities under more than one industrial sector (49) (Figure 2). This category can be any combination of the aforementioned list.

Through a verification survey of ecosystem services, a total of 203 economic activities or 7% of total economic activities, have been identified as fundamentally reliant on UMFR's invaluable resources. Out of 203 economic activities, the agriculture/forestry/fisheries industry sectors seem to rely the most on UMFR forest resources (107), followed by the services/retailing sectors (56) and economic activities that fall under more than one sector (24). The sectors that rely on the UMFR forest resources are manufacturing (13), mining/quarrying (2), and construction (1).

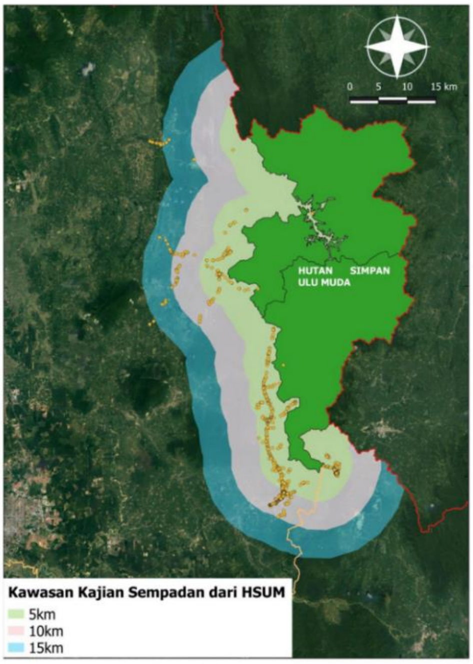
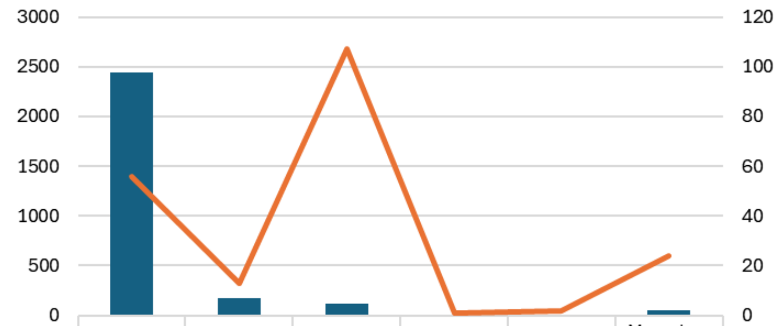


Figure 1 Distribution of economic activities in a 15 km radius of UMFR

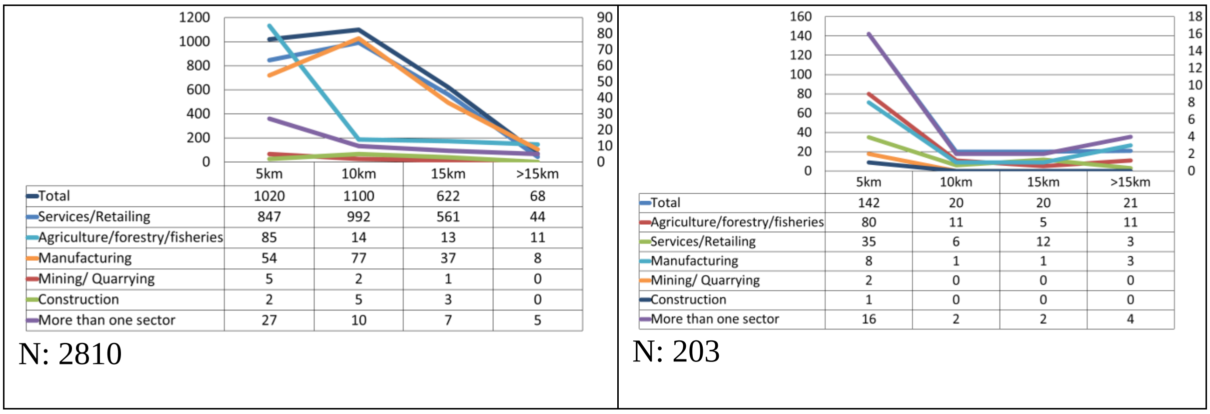


	Services/ retailing sector	Manufacturing	Agriculture/ forestry/ fisheries	Construction	Mining/ quarrying	More than one industrial sector
Economic activities identified	2444	176	123	10	8	49
Reliant on UMFR's invaluable resources	56	13	107	1	2	24

Economic activities identified — Reliant on UMFR's invaluable resources

Figure 2 Economic activities by the industrial sectors

The findings from the exploratory survey indicated that the majority (75%) of the 2810 economic activities operated within a 10 km distance from the forest, which consists of 1,020 economic activities in a 5 km radius and 1,100 economic activities in a 10 km radius (Figure 3A). Similarly, the ecosystem verification survey highlighted that almost 80% of economic activities that use forest ecosystem services are located within a 10 km radius of the forest, 142 economic activities in a 5 km radius and 20 economic activities in a 10 km radius (Figure 3B).



(A) (B)

Figure 3 Number of total economic activities around UMFR (A) and forest-dependent economic activities (B) by distance from forest

The investigation comprehensively assessed 150 of these 203 economic activities that use forest ecosystem services. The roles of these participants along the chain were identified as collectors, manufacturers, wholesalers, agents, middlemen, and retailers. Some participants are involved in more than one value chain and have more than one role (Table 1). The largest number of participants is shown by collectors (120), manufacturers (71), and wholesalers (13). The largest number of participants serving as collectors is seen in the non-wood forest products chain (73) and the fishing chain (40). As for manufacturers, which are the second-highest number of participants in the downstream industry, the largest manufacturers are in domestic tourism (12), followed by non-wood forest products (12) and the fishing chain (13).

Table 1 Total chain participants in the downstream industry involved in the forest ecosystem services by roles

Value Chain	Collector	Manufacturer	Wholesaler	Agent	Middleman	Retailer
1- Beekeeping and production of honey	2	6	2	0	0	2
2- Fruit plantation	1	4	0	0	0	0
3- Mushroom cultivation	0	2	0	0	0	1
4- Fishing	40	13	6	0	0	2
5- Freshwater aquaculture	0	3	1	0	0	0
6- Sand and limestone mining or quarrying	0	1	0	0	0	0
7- Tourism (Domestic)	2	12	1	0	0	1
8- Tourism (International)	2	8	1	0	0	1
9- Timber	0	2	0	0	0	0
10- Non-wood forest products	73	12	2	1	2	1
11- Water	0	8	0	0	0	0
	120	71	13	1	2	8

N:150

The value chain that has been discovered comprises a variety of industries, including non-wood forest products, fishing, water resources, tourism, beekeeping, honey production, freshwater aquaculture, fruit plantations, mushroom cultivation, sand and limestone mining or quarrying, and timber production. A total output of RM17,163,912 from these chains was produced. The largest output value is produced by the participants in the non-wood forest products value chain (RM5,293,275), followed by fishing (RM4,889,280) and timber (RM 1,800,000). Quarrying and mining for sand and limestone produced the lowest value chain (RM 84,000) (Table 2). The entire output value produced by this downstream industry will be utilized to calculate the natural asset values in the primary study, "Economic Assessment of Ecosystem Services and Biodiversity of Selected Protected Forest Area and Development of Database for Natural Asset Values".

Table 2 Total output value generated by downstream industries

Value chain	Output (RM)
Non-wood forest products	5,293,275
Fishing	4,889,280
Timber	1,800,000
Tourism (Domestic)	1,366,595
Water	989,605
Tourism (International)	983,885
Beekeeping and production of honey	951,425
Freshwater aquaculture	413,520
Fruit plantation	269,636
Mushroom cultivation	122,692
Sand and limestone mining or quarrying	84,000
Total	17,163,912

N:150

CONCLUSION

In total, 2,810 economic activities were identified within a 15 km radius of UMFR, highlighting its crucial role in sustaining local economies. The systematic framework for functional analysis revealed complex linkages among participants in the value chain, resulting in an output of RM 17,163,912. Notably, non-wood forest products, fishing, and timber emerged as the primary contributors to this value chain. These findings illustrate the significant dependence of nearby industries on UMFR, thus upholding its status as a vital protected forest region. This underscores the urgent need for sustainable conservation and management practices to safeguard this essential ecosystem.

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ASSESSING DYNAMICS OF CARBON STOCK AT ULU MUDA FOREST COMPLEX USING FIELD SAMPLING AND REMOTE SENSING DATA

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ABSTRACT

Forest carbon stock is a key climate variable for the global carbon cycle and has attracted the attention of both scientists and policy makers. However, forest carbon stock is one of the forest attributes that is very difficult to estimate. The study was conducted at Ulu Muda Forest Complex (UMFC) that has an extent of about 163,791 ha. Lowland and hill dipterocarps forests dominate the area, with a composition of about 87% of the entire study UMFR. It is also consisting of some production areas, where logging operation occurred since the last 30 – 40 years, under sustainable forest management (SFM) practices. Since the timber production is still operational, the carbon stock within UMFC is changing from time to time. This study was conducted to quantify the carbon stocks stored in this area as well as the changes that have been occurred from year 2000 to 2021 with 5-year intervals. Landsat satellite images were used as 'activity data' for this study, which were acquired over the year 2000, 2005, 2010, 2015, and 2021. Field sampling was conducted in year 2021 at several locations to measure the forest carbon stock. Since there were no historical ground inventory data available, forest canopy density index (FCD), generated from the satellite images was used as predictor to estimate the historical conditions of carbon stock. The study found that the aboveground carbon stock within UMFC decreased from about 16.28 million Mg C in year 2000 to 15.28 million Mg C in year 2021. Although there were some fluctuations in carbon stock throughout the period, the accumulated number indicated a net loss.

Keywords: carbon stock, ecosystem services, production and protection forests, Ulu Muda

INTRODUCTION

Tropical forests are crucial for reducing climate change, yet people are nonetheless clearing vast amounts of them for other purposes. To encourage more sustainable forest use, forest carbon must be measured and monitored with high geographical and temporal precision. Given that they are known to store significant amounts of carbon, tropical forests are among the most crucial ecosystems for addressing the difficulties posed by climate change (Brendan et al., 2020). Retrieving carbon from tropical forests across large areas has been challenging for decades due to a lack of data, accessibility issues, and numerous technical difficulties. For the past three decades, remote sensing has been used successfully to calculate the amount of carbon stored in forests (Hamdan, 2022).

Deforestation and deforestation, which accounted for nearly 10% of the world's annual total carbon emissions, are tropical countries' main sources of carbon emissions (Pearson et al., 2017). In this regard, Malaysia outlines a clear strategy for delivering on its commitments made at the United Nations (UN) Climate Change Conference 2021 (COP-26) and thoughtfully takes into account global trends. In addition, Malaysia offers an updated Nationally Determined Contribution (NDC) or climate target that calls for a 45% reduction in the economy's carbon intensity (as a percentage of GDP) by 2030. Malaysia similarly established a target of becoming carbon neutral by 2050 (UNFCCC, 2020).

Lowering emissions from deforestation and forest degradation, preserving forests (REDD+), and carbon offsetting are just a few of the national and worldwide actions being taken to combat the effects of global warming. Each nation's carbon emissions from deforestation and forest degradation must be estimated and monitored over time to achieve this aim. At such large geographic scales, a precise, economical, and high-resolution technique of monitoring changes in above-ground carbon stocks is needed. This study examines the efficacy of satellite imagery in measuring dynamics in Malaysia's forest cover and carbon stock.

MATERIALS AND METHODS

The study was conducted at Ulu Muda Forest Complex (UMFC) that has an extent of about 163,791 ha. The study area comprises of four types of forests, which are lowland dipterocarp, hill dipterocarp, upper hill dipterocarp, and montane forests. However, lowland and hill dipterocarps forests dominate the area, with a composition of about 87% of the entire study UMFC. It is also consisting of both protection and production forests, where most of the logging operation occurred since the last 30 – 40 years, under sustainable forest management (SFM) practices. Since the timber production is still active until now – with a certain annual allowable coupe (AAC) at 30-year cutting cycle – the carbon stock within UMFC is dynamic and keep on changing from time to time. Therefore, this study was conducted to measure the carbon stocks contained in this area as well as the changes that have been occurred from year 2000 to 2021 with 5-year intervals.

Landsat satellite images were used as ‘activity data’ for this study, which were acquired over the year 2000, 2005, 2010, 2015, and 2021. These images were used to observe the changes of canopy structure due to logging activities that have been occurred within these periods. The information on canopy structure was used as the main indicator for the aboveground carbon density (ACD) estimation. Variations of the canopy structure is classified into several classes, also called forest strata, to differentiate the level of carbon stock intensity within the entire study area. The stratification was performed by using forest canopy density (FCD) index, which is ranging from 1.0 (very high canopy) to 0.0 (no canopy). This index was further divided into several categories, which are < 0.3, 0.3 – 0.5, 0.5 – 0.7, and > 0.7. Ground sampling was conducted in year 2021 at pre-determined locations based on the FCD distribution to measure the carbon stock. Since there were no historical ground inventory data available for the previous years, the FCD was generated for each series of the satellite images.

Landsat images has a spatial resolution of 30-m, which is suitable for carbon stock estimation over a large area coverage at accuracy of about 60 – 80%. To increase the accuracy of the estimation, higher spatial resolution satellite images were also used in study. Images from Sentinel-2 and Satellite Pour l’Observation de la Terre (SPOT-6) were acquired for the year 2021. Both are having spatial resolutions of 10-m and 1.5-m, respectively. The integration of high-resolution images has increased the estimation accuracy up to 95%.

Fieldwork has been conducted from August 2021 to February 2022. Altogether, 20 sampling plots were sampled within the UMFC, which include all strata types, i.e., the protection forest and production forests, which were divided into four strata; logged 1–10 years, logged 11–20 years, logged 21–30 years, and logged > 30 years. The surveys covered Ulu Muda Forest Reserve (mainly within the production forest) and Pedu Forest Reserve (for protection forest). In additional to the sampling plots, there are 10 validation plots were established at different locations within UMFC that was used for validation and accuracy assessment purposes. This fieldwork has been conducted in July 2022.

RESULTS AND DISCUSSION

The estimated ACD from the field sampling data was correlated with the FCD computed on the satellite images. Images of year 2021 was used for this exercise since this is the only data that are closest to the date of field data collection. The derived empirical correlation between ACD and FCD is expressed as

$$ACD = 11.844e^{3.5572*FCD} \quad \text{eq. 1}$$

where ACD is the aboveground carbon density (MgC ha^{-1}) and FCD is the index values.

This equation was then applied to all FCD series over the years 2000, 2005, 2010, 2015, and 2021 as depicted in Figures 1 and 2. Once the information on ACD were acquired for all series, the changes can be measured as summarized in Table 1.

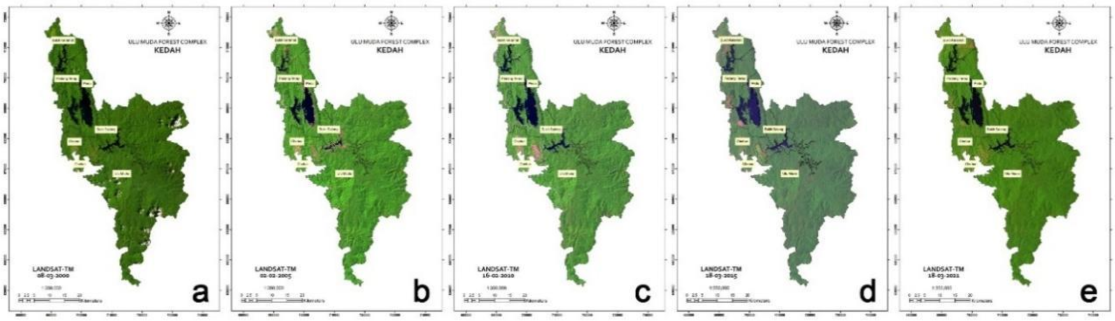

Figure 1 Landsat images over the years 2000 (a), 2005 (b), 2010 (c), 2015 (d), and 2021 (e)

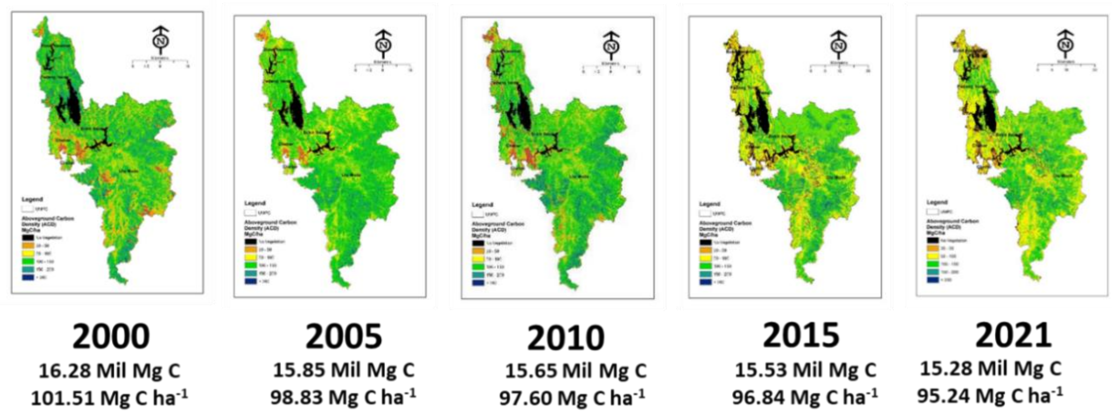

Figure 2 The estimated ACD over the years 2000 (a), 2005 (b), 2010 (c), 2015 (d), and 2021 (e)

Table 1 Summary of CO_2e emission calculation from the total carbon stock between intervals

Year	**2000**	**2005**	**2010**	**2015**	**2021**
Total ACD (Mg C)	16,281,623	15,851,886	15,654,511	15,532,687	15,276,642
Total CO_2 ($Mg\ CO_2$)	59,704,710	58,128,867	57,405,093	56,958,364	56,019,445
Year interval		**2000-2005**	**2005-2010**	**2010-2015**	**2015-2021**
Changes of carbon stock (Mg C)		- 429,736	-197,375	-121,824	-256,046
Changes of CO_2e ($Mg\ CO_2$)		-1,575,714	-723,715	-446,693	- 938,842

Note: Negative (-) sign denotes total emission of CO_2.

Overall, the FCD agrees with the strata or the real condition of the forest within the study area. Figure4 highlights that the logged < 10 years forest strata have the canopy cover of just over 30% (or FCD = 0.3), which means that the area is just sufficient to be defined as forest (Figure 3). In Malaysia, a forest is defined as land spanning more than 0.5 hectares with trees higher than 5 meters and a canopy cover of more than 30 percent, or trees able to reach these thresholds in situ. It does not include land that is predominantly under agricultural include rubber plantation or urban land use.

Based on the developed function expressed in eq.1 earlier, the ACD was estimated for the entire study area. Analysis was also conducted at the strata level as presented in Figure 3. Like FCD, ACD also tend to have direct proportion to the forest strata. It is also notable that the Protected Forest contains a large variation in term of ACD as it contains various stand conditions. However, the maximum value of ACD within this forest is the highest among the other strata.

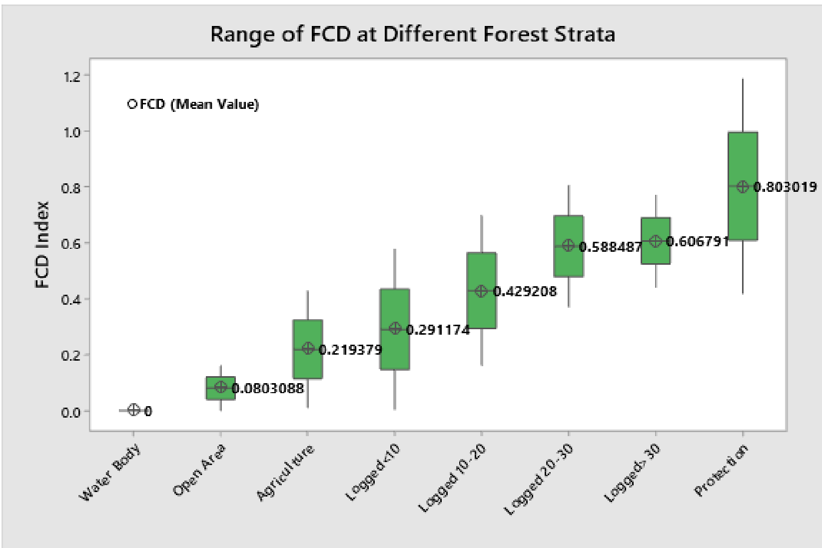

Figure 3 The range of FCD at different forest strata

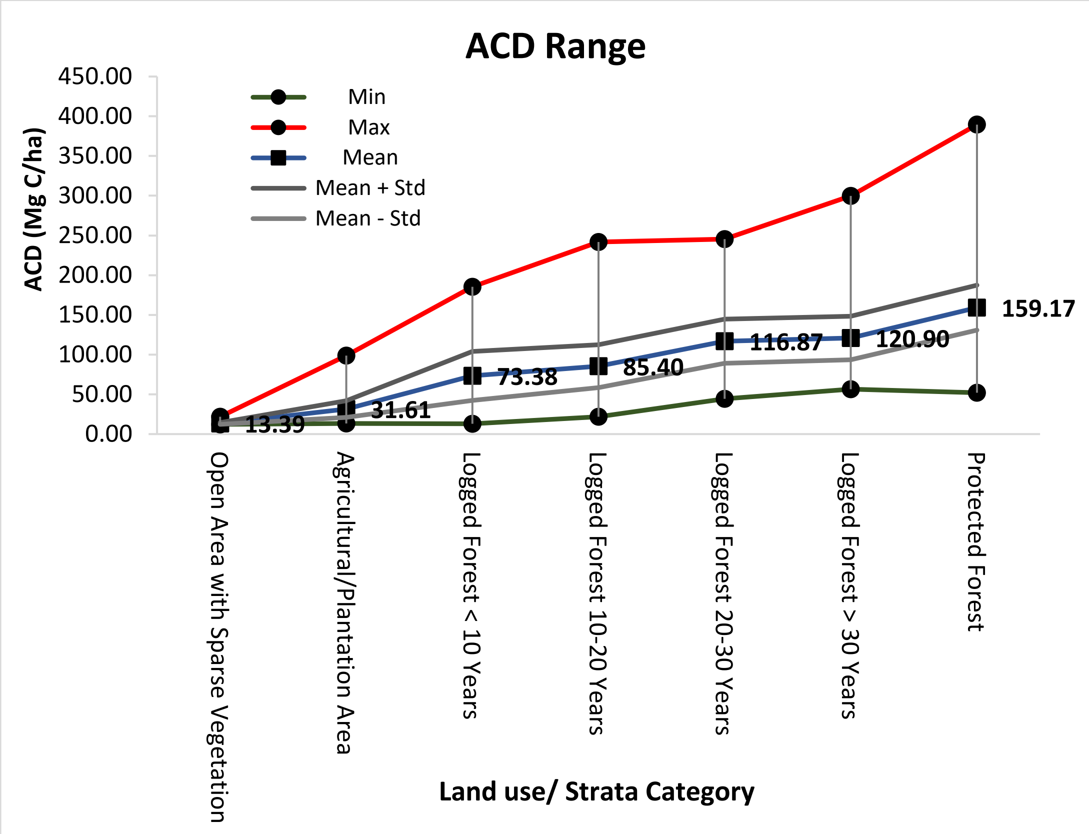

Figure 4 The range of ACD at different forest strata

Other carbon pools, which are belowground, deadwood, litter and soil are also assessed in this study. In terms of composition, ACD dominates the carbon pools by about 62%, followed by belowground, soil, deadwood, and litters (Figure 5). Deadwood, litter, and soil organic carbon were resulted from clip-plot sampling that were collected during the field sampling work. Each forest strata indicates different values in all carbon pools, which reflects the conditions of the forests. Protected/virgin forest has the highest carbon stock in all carbon pools among other strata. Even the logged areas of > 30 years does not have the ability to reach the carbon stock level as that of contained by unlogged forest. This demonstrates that once the forest is logged, it must take longer time (more than 30 years) to recover and getting back to its natural condition.

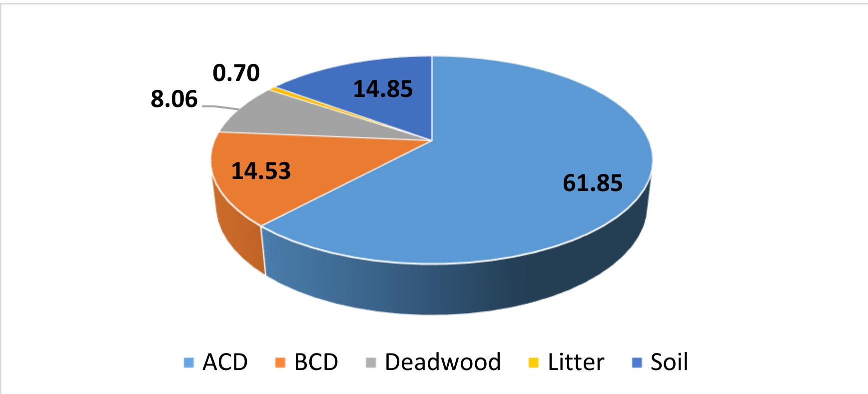

Figure 5 Composition of carbon stock within each carbon pool

The validation process was conducted to assess the accuracy of the estimates. To complete this assessment, the validation sample plots were used, where nine (9) points were surveyed at the field within UMFC. The measured ACD (at field) and the estimated ACD (from the estimation model) was compared by using Mean Absolute Percentage Error (MAPE). The calculation demonstrated that the MAPE was 16.1%, which means that the estimation accuracy was assessed at 83.9%. The model was found to has over-estimated the ACD at lower range (≤150 Mg C ha^{-1}) and under-estimated above this point.

CONCLUSION

This study showed that the combination remotely sensed, and ground sampling data help assess changes of forest carbon stocks and emissions in UMFC. Landsat satellite images collected at 5-year intervals between 2005 and 2021 were processed to derive FCD and with this indicator, the ACD over the entire study area were estimated. The study revealed that the ACD within the UMFC was declined from 16,281,623 Mg C in year 2000 to 15,276,642 Mg C in year 2021, with a total reduction of about 1 million Mg C. this is equals to about -47,856 Mg C yr^{-1}. The study also found that multispectral satellite imagery is appropriate for assessing variability in term of spatial and temporal changes of forest carbon stock within a large landscape like UMFC.

ACKNOWLEDGEMENTS

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DISTRIBUTION AND STATUS OF *MELALEUCA* FOREST IN SABAH AND SARAWAK

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ABSTRACT

The Melaleuca Forest, a distinctive forest type in Malaysia, has received relatively little research attention when compared to other forest ecosystems in the country. This is primarily due to its smaller size and limited distribution. Moreover, the majority of previous studies have concentrated their efforts on the Melaleuca forests located in Peninsular Malaysia, which houses the largest expanse of this forest type in the country. A comprehensive study was undertaken to address this research gap and gain up-to-date insights into the distribution and current status of Melaleuca forests in Sabah and Sarawak. The study involved mapping the distribution of these forests in these regions using imagery from the Sentinel-2 satellite. Valuable on-ground data on the forest stands was collected through field inventory efforts. The findings of this study revealed that Melaleuca forests are primarily confined to Sarawak, covering an estimated total area of approximately 7.41 hectares. Specifically, these forests were identified in the Lundu/Sematan area, while no Melaleuca forests were observed in Sabah. As for the structural characteristics of these forests, the study reported an average stand density of 1,122 trees per hectare, a basal area of 26.6 square meters per hectare, and an above-ground carbon content of approximately 105.5 tonnes of carbon per hectare. The valuable insights obtained from this study can serve as a fundamental resource for the future management, protection, and conservation efforts directed at *Melaleuca* forests in Sarawak. Such initiatives are critical for safeguarding this unique ecosystem from the risk of extinction.

Keywords: Melaleuca forests, mapping, inventory, stand structure, carbon

INTRODUCTION

The Melaleuca Forest, referred to locally as the "Gelam" forest, is a type of freshwater marsh forest characterized by the predominance of the *Melaleuca cajuputi* tree species. This forest derives its name from the *Melaleuca cajuputi* tree, which constitutes nearly the entire forest composition. Melaleuca cajuputi belongs to the Myrtaceae family and the Melaleuca genus. While various species belong to this genus, M. cajuputi is particularly common in Malaysia. It goes by several colloquial names, including Cajaput, Cajaput-tree, Cajeput, Gelam Bark, Paper Bark Tree, White-wood (in English), Gelam, Kayu Puteh, Kayu Putih, and Gelam Tikus (in Malay) (MyBIS, 2021). Melaleuca forests are indigenous to Southeast Asia, New Guinea, and Australia, and they are not found in other parts of the world (Tran, 2015). Unfortunately, there is no available report on the total extent of these forests in the past years. However, as of 2019, the Melaleuca Forest in Peninsular Malaysia covered approximately 22,879.33 hectares, with Terengganu having the largest expanse (Hamdan et al., 2020).

In the past, research has delved into various aspects of M. cajuputi, encompassing its ecology, habitat, as well as biophysical and biochemical characteristics (Masitah et al., 2015; Kasawani and Kamaruzaman, 2009; Jamilah et al., 2014; Jamilah et al., 2017). However, it is worth noting that there has been a noticeable scarcity of studies pertaining to the mapping of Melaleuca forests. This paucity can be attributed, in part, to the perception that these forests are abandoned and possess less commercial value compared to other forest types in the country, such as inland, peat swamp, and mangrove forests.

Previous studies have made use of remote sensing technology, with a focus on techniques for identifying Melaleuca forests, particularly in specific states like Terengganu (Saberioon et al., 2010; Noor Afira, 2007; Kasawani & Kamaruzaman, 2009). Furthermore, the overall status of Melaleuca forests has remained elusive, as comprehensive mapping studies have not been conducted for Malaysia as a whole. Previous research by Hamdan et al. (2020) primarily concentrated on Peninsular Malaysia. Consequently, the distribution and status of this forest type in Sabah and Sarawak have remained unknown. Given these research limitations, the primary objective of this study was to gather current and comprehensive information about Melaleuca forests in Sabah and Sarawak in order to complete the information on the distribution and extent of this forest for Malaysia. This research seeks to fill critical knowledge gaps and make pertinent data available for future studies and forest management initiatives related to Melaleuca forests. Importantly, this study is not solely focused on mapping the distribution of Melaleuca forests; it also aims to provide fundamental insights into the characteristics of these forest stands.

MATERIALS AND METHODS

The primary data source for identifying and classifying Melaleuca forests in Sabah and Sarawak is Sentinel-2 satellite imagery. Ground truth data played a crucial role in both confirming the presence of forests in the satellite images and evaluating the accuracy of the classification results. In addition to ground truth data, field inventory activities were conducted to gather essential information about the forest stands. Satellite images from Sentinel-2 for the year 2021/2022 were acquired to facilitate the identification and mapping of Melaleuca forests in Sabah and Sarawak.

The utilization of satellite imagery for classification purposes is a widely adopted approach in contemporary remote sensing studies. This method offers several advantages, including the ability to cover extensive areas, cost-effectiveness, and rapid data acquisition. Furthermore, each satellite image possesses unique spatial and spectral characteristics, enabling users to select the most suitable images for their specific research purposes. Researchers can also harness multiple spectral bands within a single image to enhance their understanding of the area (see Figure 1) and improve the accuracy of the classification process during mapping activities.

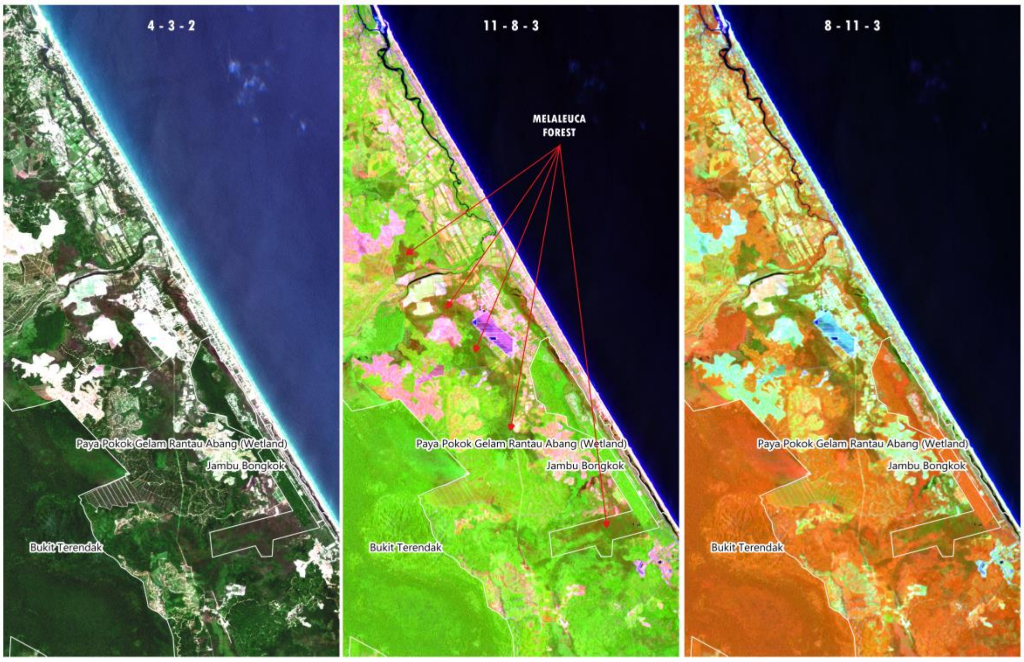

Figure 1 Sentinel-2 images with different color combinations for the identification of the Melaleuca Forest

Sentinel-2 imagery was selected for the identification and mapping of Melaleuca forests due to its accessibility as it can be downloaded from https://scihub.copernicus.eu/dhus/. In cases where cloud cover obscured certain areas, multiple images of the same scene from different dates were acquired. To enhance the image quality, a series of image processing techniques were applied to each image. These processes included the conversion of digital numbers (DN) to top-of-atmospheric (TOA) reflectance, cloud masking, and geometric correction. Subsequently, a comprehensive classification process was conducted on all the images to distinguish Melaleuca forests from other land uses. The Random Forest classification method was employed for this purpose. To enhance classification accuracy, various refinements were implemented. Misclassified areas were corrected, and adjustments were made to the boundaries of Melaleuca forests. The ground truth data collected during this study played a pivotal role as a reference for improving the classification outcomes and assessing their accuracy. As a culmination of these efforts, a distribution map of Melaleuca forests for Sabah and Sarawak was generated, providing a visual representation of their presence and extent in the region. The total area covered by Melaleuca forests was also calculated.

Field inventory activities were a vital component of this study, aimed at gathering fundamental information about the forest stands. The selection of field inventory locations was based on the distribution of Melaleuca forests derived from the classification process. For the field inventory, a plot design originally developed for mangrove forests, as outlined by Kauffman and Donato (2012) and depicted in Figure 2, was adopted. This design consists of one cluster comprising six plots, each with a radius of 7 meters. The distance between adjacent plots is set at 25 meters. During the inventory, all trees with a minimum diameter at breast height (DBH) of 5 centimeters were meticulously measured and recorded. Additionally, the species of each tree encountered was documented.

All the collected field data were centralized into a single database to facilitate efficient management. Subsequently, advanced data analysis was conducted using the R software (R, 2013). This analysis aimed to derive essential forest parameters, including stand density, basal area, biomass estimation, and the percentage of Melaleuca trees within the sampled areas. These parameters are crucial for a comprehensive understanding of the forest ecosystem.

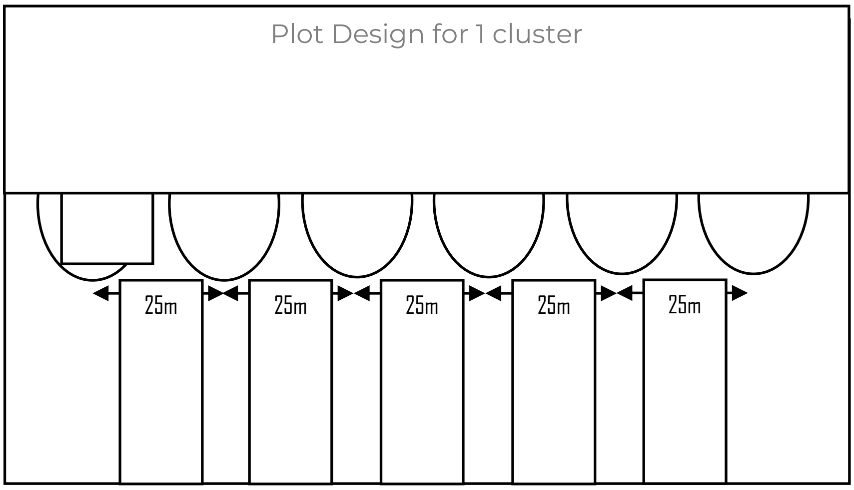

Figure 2 Plot design that was used to collect field data

RESULTS AND DISCUSSION

Based on the findings of this study, Melaleuca forests are exclusively located in Sarawak, covering a total area of approximately 7.41 hectares. Specifically, these forests are concentrated in the Lundu/Sematan area of Sarawak. Notably, there is no presence of Melaleuca forests in any other district within Sarawak. Despite extensive field visits to nearly 50 potentially suitable locations during ground truth activities in Sabah, no Melaleuca forests were identified in Sabah. This result aligns with the observations made by Rimbawan and Susanto (2004), which indicated that the distribution of Melaleuca forests in Borneo is limited to certain regions within Sarawak. The distribution of Melaleuca Forest in Sarawak is shown in Figure 3.

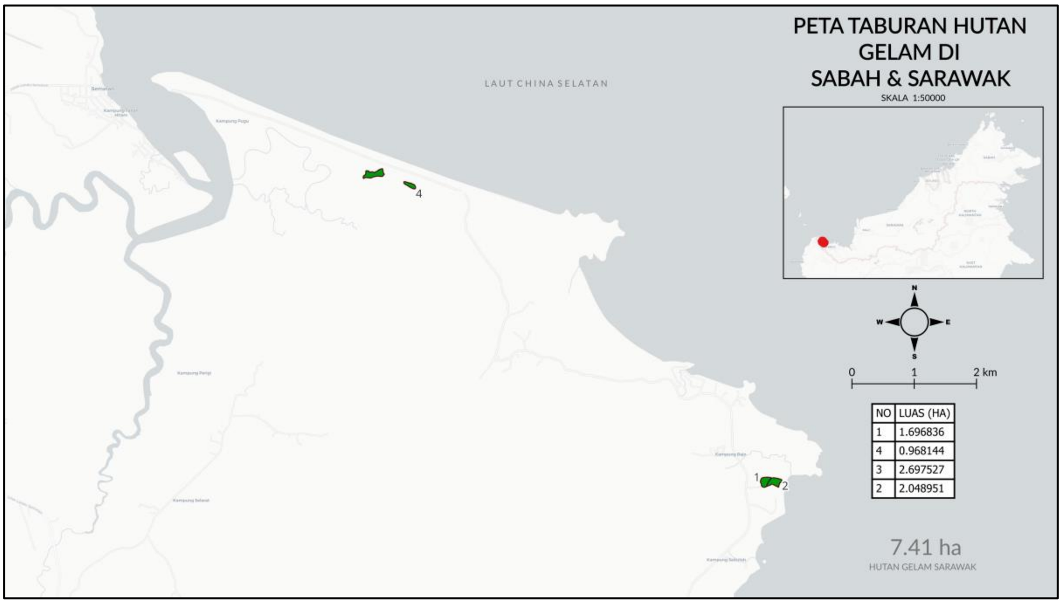

Figure 3 Distribution of Melaleuca Forest in Sarawak based on classification of Sentinel-2 imagery

Combining the results of this study with the research conducted by Hamdan et al. (2020) offers a comprehensive mapping of Melaleuca forests across Malaysia. Although the timeframes of these two studies differ, their outcomes can be collectively used to provide an estimate of the total extent of Melaleuca forests in Malaysia. The total estimated extent of Melaleuca forests in Malaysia is approximately 22,886.74 hectares. Among the states, Terengganu holds the largest portion of Melaleuca forests, while Sarawak has the smallest proportion. Notably, Sabah, alongside Perlis, Kedah, Perak, Pahang, Wilayah Persekutuan Kuala Lumpur, and Labuan, is among the states without Melaleuca forests.

In this study, a total of three clusters, comprising 18 plots, were successfully established to estimate the stand structure of Melaleuca forests in Sarawak. The average stand density, basal area, and above-ground carbon for these forests are approximately 1,122 trees per hectare, 26.6 square meters per hectare, and 105.5 tonnes of carbon per hectare, respectively. When compared to the stand structure of Melaleuca forests in Peninsular Malaysia, those in Sarawak exhibit slightly lower stand density, basal area, and above-ground carbon content.

CONCLUSION

This study has achieved a comprehensive mapping of Melaleuca forests in Borneo through the utilization of Sentinel-2 imagery. The research estimates that the total extent of this forest covers approximately 7.41 hectares, with its presence exclusively confined to Sarawak and not extending into Sabah. Furthermore, the study successfully gathered essential forest stand information based on meticulous field inventory activities. These efforts have yielded valuable insights into the forest's characteristics, with the average stand density calculated at approximately 1,122 trees per hectare, a basal area of about 26.6 square meters per hectare, and an above-ground carbon of around 105.5 tonnes per hectare.

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DIRT MATTERS: SOIL INSIGHTS FOR SUSTAINABLE URBAN TREE CARE AT FRIM

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ABSTRACT

Urban soils play a crucial role in sustaining tree growth and health by providing essential resources. However, urban soils often suffer from degradation due to human activities. While previous research has explored soil composition at FRIM, this study focuses on soil quality surrounding trees planted by Very Important People (VIPs) within FRIM's campus. A short study was conducted on 57 trees from 29 species which was planted from 1973 to 2020. Visual Tree Assessment (VTA) protocols were employed to evaluate tree health. Soil samples were collected within the drip line of each tree, and various soil parameters were measured, including temperature, electrical conductivity, pH, moisture, penetration resistance, soil structure, soil texture and colour. Trees were classified into CAT1, CAT2 and CAT3 management classes. This study demonstrates the importance of soil analysis in urban tree management. It highlights the potential for soil treatment interventions to enhance tree health. This research advocates for the integration of soil assessment as a standard practice in urban tree management, offering potential for sustainable practices in environments like FRIM.

Keywords: urban trees, soil quality assessment, tree health, sustainable urban tree management

INTRODUCTION

Soil plays a pivotal role in supporting the growth of trees by providing anchorage for their root systems and serving as a reservoir for vital resources, including water, oxygen, and essential nutrients. These elements are fundamental for the sustenance and development of trees. However, the quality of urban soils has been a growing concern, primarily due to human activities and suboptimal management practices. Numerous studies have documented the degradation of urban soils, highlighting common issues such as elevated bulk densities, reduced porosity, compromised soil structure, alteration in water dynamics, redoximorphic changes, increased pH and salinity levels, the presence of environmental contaminants, declining organic matter contents, and shifts in microbial populations (Short et al., 1986; Craul, 1991; Craul, 1999; Scharenbroch et al., 2005; Scheyer & Hipple 2005; Pouyat et al., 2007).

A significant portion of the trees inhabiting FRIM's ground was originally planted during the colonial era and has since thrived in a self-sustaining ecosystem. In addition, some of the trees in FRIM's campus serve as living tributes to esteemed guests who have visited over the years. These commemorative trees represent over 100 individual trees, all of which were planted by people of great significance, often referred to as Very Important People (VIPs), since the year 1973 (Azaruddin et al., 2013).

While previous research, such as the comprehensive soil survey conducted by Jeyanny et al. 2019, has contributed valuable insights into the soil composition of FRIM's grounds, a critical aspects remain underexplored. Specifically, our study delves into the soil quality surrounding trees planted by VIPs within the FRIM campus, with a particular emphasis on addressing the implication for tree management. The importance of soil health in sustaining urban trees cannot be overstated, yet a dearth of specific information persists in this domain (Keeren et al., 2021; Sreetheran, 2022). An understanding of soil quality within this unique urban setting at FRIM holds paramount importance, serving as a cornerstone for the development of best practices and optimal growth conditions to promote the health and vitality of its urban trees.

MATERIALS AND METHODS

To assess the health and condition of the trees, a total of 57 representing 29 types of species from 16 families were examined during the period from October to December 2022. These trees were planted from 1973 to 2020. Tree health inspection was conducted using a modified version of the Visual Tree Assessment (VTA) protocol developed by the International Society of Arboriculture (ISA). This assessment method has been employed by certified arborist in FRIM since 2007. Tree height was determined using TruPulse 200 Laser Rangefinder and diameter at breast height (DBH) measurements were taken at 1.4 meter above ground level using a diameter tape.

Soil samples were collected randomly from under the drip line of each tree. Various soil parameters measured, including temperature and electrical conductivity (EC) using Groline H198331, soil pH and moisture using the Takemura DM-13, soil penetration resistance profiles were recorded using a soil compaction tester Spectrum Technologies, Inc., and soil color (hue, value and chroma) were determined using Munsell soil colour charts. Soil structure type was described based on the following criteria: platy-massive = 0, angular blocky =1, subangular blocky = 2 and granular =3. Soil structure grade was categorized as: structureless = 0, weak = 1, moderate = 2 and strong = 3. Soil texture was determined using the "feel method" as described by Ritchey, McGrath & Gehring (2015).

Each tree was assigned a colour code based on the readings obtained from the eight variables. The colour codes were as follows: Red means readings falling above acceptable values, Yellow is intermediate values and Green mean readings falling within acceptable values. Finally, the trees were categorised based on the combined colour codes of the eight variables. In this categorisation, a tree was placed in one of three categories: **Category 1**: If a tree received a majority of red codes, indicating readings that fell above acceptable value across multiple variables. **Category 2**: If a tree received a mixture colour codes, including a combination of red, yellow and green, indicating varying conditions across the different variables and **Category 3**: If a tree received all green codes across these variables, it was placed in this category, signifying favourable conditions in all aspects.

RESULTS AND DISCUSSION

In order to effectively manage the trees within FRIM campus, a categorisation system was devised based on the combined scores of eight factors. These trees were placed into one of three management classes: "CAT1," necessitating immediate attention; "CAT2", requiring intervention within three months; and "CAT3", demanding no immediate action but scheduled re-evaluation within six to ten months. The latter category, CAT 3, involves periodic inspections to ensure sustained tree health and vitality. The assessment resulted in the classification of 13 trees as CAT1, 24 trees as CAT2, and 20 trees as CAT3, based on the scoring system.

Remarkable, all 20 trees categorised as CAT3 were found to exhibit an exceptional condition of soil quality. Their parameter values consistently fell within the recommended range for typical urban trees, reflecting a favourable environment for their growth and sustenance. Further analysis of the soil conditions revealed compelling insights. Among the 20 trees placed in CAT3, it was observed that 15 trees of *Hopea subalata*, planted in 2017 along Jalan FRIM, demonstrated remarkable favourable soil conditions. These trees thrived under the sheltering canopy of surrounding trees, benefiting from the natural leaf litter that covered their drip lines. In stark contrast, the analysis unveiled a less favourable scenario for the four trees of *Schoutenia accrescens* planted in 1986 near D3 building. These trees were designated as CAT1, reflecting an urgent need for management intervention. The extremity of their soil degradation may be attributed to high pedestrian traffic and the exposed top surface of the soil, leading to compaction and detrimental changes in soil quality.

CONCLUSION

Soil analysis has proven to be an invaluable tool for devising effective remedial and mitigation strategies for urban trees. Informed by VTA protocols, certified arborists at FRIM have traditionally recommended actions such as tree pruning, removal, or replacement. However, this study highlights the potential for soil treatment interventions, including vertical mulching and air or mechanical tiling. By incorporating rapid soil assessment of tree soil quality, urban tree managers can make more precise recommendations. This approach not only enhances the efficacy of urban trees management actions but also contributes to the overall health and vitality of FRIM's VIP trees.

This study has demonstrated the utility of quick and efficient assessment in urban tree management. By providing valuable information about soil quality and its impact on tree health, such studies offer a solid foundation for decision-making. This brief project not only advances our understanding of urban forestry but also advocates for the integration of soil assessment as a standard practice in urban tree management. The results demonstrate the potential for improved and sustainable urban tree management practices within FRIM and similar urban environments.

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MANGROVE GERMPLASM HERITAGE OF BAGAN DATUK

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ABSTRACT

The Mangrove Germplasm Heritage of Bagan Datuk project is an initiative to conserve mangrove forests in the district of Bagan Datuk. This project also involves collecting mangrove species in various parts of Malaysia and conserving them in the germplasm. Early assessments of the current status of mangrove forests in the 78-hectare study area were conducted by components. These components include a mapping of the study area, inventory of flora and fauna, as well as local community socioeconomic survey. Through this study, 13 mangrove species were recorded, 12 species were introduced in the germplasm, and 1,065 trees were planted in the study area. Meanwhile, 160 species of birds, 25 species of mammals, 7 species of herpetofauna, and 4 species of fireflies were also recorded in the study area. Apart from that, surveys on local socioeconomic conditions and perceptions were conducted to assess the socioeconomic status and perception on the importance of mangroves among the local community. Through this survey, information on the locals' perception and their dependence on mangrove forest as a source of income was obtained. Preliminary results found that the local community is dependent on mangrove marine products as one of the sources of livelihood and source of income. Overall, the establishment of mangrove germplasm in Bagan Datuk District is a promising initiative that has the potential to make a significant contribution to mangrove conservation in the region.

Keywords: mangrove, germplasm, flora, fauna, socioeconomic

INTRODUCTION

Bagan Datuk, Perak is a district on the west coast of Peninsular Malaysia with a mangrove forest that has yet to be fully explored. This 78-hectare study site is located between Beting Beras Basah, a historical site related to the Perak Sultanate, and Bagan Datuk town center. The main objectives of this project are: i) to establish mangrove germplasm and ii) to conduct research activities related to the conservation and sustainability of Bagan Datuk mangrove forests. Early assessments of the current status of mangroves in the study area were conducted by components. In general, this project can be divided into four general focuses: flora, fauna, socioeconomics and the germplasm. Bagan Datuk mangrove forest is a valuable natural asset that has the potential to play an important role in the sustainable development of the district. This project also aims to balance conservation activities with rapid development in the Bagan Datuk District.

MATERIALS AND METHODS

A total of 1,398 plots (20 m × 20 m) were established, with 76 transects covering approximately 56 ha of mangrove forest for flora inventory. The diameter at breast height (DBH) and height of the trees were measured. The data recorded was then analyzed to identify the structure of mangrove forest stands in the study area. A list of species present in the area was compiled and guided the species collection for mangrove germplasm establishment purposes. The establishment of mangrove germplasm in Bagan Datuk focuses on collecting species from various parts of Peninsular Malaysia. For this purpose, a mangrove nursery was established in Bagan Datuk where propagules and saplings of mangrove species were raised in polybags before being planted in the field. The planting site located in Block A (Figure 1) was previously cleared and used to place a pipeline for a nearby construction project. There are also traces of mangrove harvesting activities in the area. The study area is considered to be in the landward zone of the mangrove ecosystem. The survival and growth data were recorded one year after planting.

Figure 1 Map of mangrove germplasm plot in Bagan Datuk

On the other hand, fauna inventory was conducted using a variety of methods, including camera trapping, direct observation, and mist-netting. Six sampling sessions were carried out, with five transect lines in each sampling. Besides that, specific surveys of migratory birds and water birds were also conducted through direct observation using binoculars. Meanwhile, a survey of fireflies was conducted by establishing 50-meter transects in both Block A and B of the mangrove germplasm to record their presence. As for the socioeconomic survey, seven villages in Bagan Datuk were selected based on their proximity to the study site within a 10-kilometer radius. Questionnaires were developed through the Rapid Rural Appraisal (RRA) method. The RRA method is a participatory research approach that is used to gather information from local communities. It involves using a variety of data collection methods, such as interviews, focus groups, and direct observation. This approach also involves collecting secondary data from printed materials such as annual reports, books, journals, and other related sources. Information collected during this process is important for preparing the content of a questionnaire. This information can help to ensure that the questionnaire is relevant to the needs of the target population and that it covers all the important topics.

RESULTS AND DISCUSSION

In total, 39,077 trees were recorded in the study area, consisting of 17 mangrove species. The most dominant species recorded is *Bruguiera parviflora* (Lenggadai) with 14,729 trees followed by *Bruguiera sexangula* (Tumu puteh) with 11,889 trees. Other mangrove species recorded are *Rhizophora mucronata, Excoecaria agallocha, Avicennia alba, Rhizophora apiculata, Avicennia marina, Nypa fruticans, Avicennia officinalis, Bruguiera gymnorrhiza, Avicennia lanata, Sonneratia caseolaris, Hibiscus tiliaceus, Cerbera odollam, Terminalia catappa, Peltophorum pterocarpum,* and *Calophyllum inophyllum.* On top of that, a total of 1,065 trees consisting of 14 species were planted in the study area. Out of that number, 11 species were introduced species collected in the form of saplings and seedlings from Peninsular Malaysia and Sabah. After 12 months of planting, the overall survival rate of the planted saplings was 80%. *Sonneratia ovata* contributed to the highest survival rate (89%) and highest height increment, while *Xylocarpus moluccensis* exhibited the lowest survival rate (72%) and the lowest height increment.

Generally, mangrove species are able to grow in a wide range of environmental conditions despite having their own range of tolerance to different attributes in their natural habitats (Choong et al., 2019). In most cases, the seedling grows better where the mother trees are found compared to when introduced to a new mangrove area (Khoo et al., 2019). In this study, some of the saplings were planted in a mangrove area where no mother trees of their species were available, yet the survival rate and growth performance are promising. This is also shown in other study findings that species such as *S. ovata are* able to adapt well when introduced to new mangrove areas (Choong et al., 2019). In terms of height increment, some mangrove species do show different growth rates in response to topography (Macintosh et al., 2002).

Despite the fact that the planting activities were carried out in the right zonation of the species, further study on different levels of topography is needed to conclude which species has the best growth performance. Other than that, a longer time frame for data collection would probably give a better conclusion on the growth rate of the introduced species in the area. For example, there are studies that recorded a high survival rate at the early stage of the planting, but the rate decreased rapidly after more than a year (Mohammad Shahfiz, 2019). While the effort of conserving and restoring mangrove forests in Malaysia has been immense, especially post 2004 tsunami, common species such as *R. apiculata* and *R. mucronata* are mostly used for planting activities due to easily obtained seedlings. However, in terms of in situ conservation and mangrove restoration, species diversity needs to be considered. Mixed-species planting should be considered in mangrove restoration rather than only focusing on common species such as Rhizophora sp., especially in less diverse areas (Mukrimah et al., 2019). For example, even though *Bruguiera cylindrica* has been recorded to have a limited distribution and is only found on the west coast of Peninsular Malaysia (Onrizal et al, 2017), it was not found in the study site, which was also located on the west coast of Peninsular Malaysia, thus reflecting the low distribution of species in the area. In establishing mangrove germplasm through in situ conservation, species diversity would be the main concern. However, while the effort to collect seedlings and saplings from all over Malaysia can be continuous, some of the key points to consider are that some species of mangroves are protected, while others are endemic. There will be some conflicting issues in this effort that need to be tackled.

On the other hand, the survey of fauna has led to a few important findings. In general, there are 25 species of mammals, 160 species of birds and seven species of herpetofauna recorded in this study (Onrizal et al., 2017). *Macroglossus minimus* was the most captured mammal, with a total of 40 individuals. Apart from that, surveys on water birds and migratory birds found that Bagan Datuk also has the potential to be listed as one of the Important Bird Areas (IBA) in Malaysia (Pillai et al, 2018). This area also has the potential to be a bird sanctuary, especially for migratory birds and water birds, based on the increasing numbers of birds recorded during the study. This also suggests that new bird species may still be discovered in the area. Meanwhile, survey of fireflies recorded four different species (Rabinowitz, 1978). Three of the species are congregating, while one species is a solitary firefly. A unique finding from the firefly survey is that fireflies can be seen from the bund, where tourists typically need to take a boat to see them. This would make them more accessible to tourists and locals alike. Fireflies are fascinating creatures, and they play an important role in the ecosystem. They are also a popular tourist attraction in many parts of Malaysia. The fact that fireflies can be seen by walking on the bund is a unique and valuable feature of this area and will encourage more people to visit this area and experience the beauty of fireflies. For this purpose, it would be great to learn more about the fireflies and factors that are contributing to their abundance. Through survey of fauna and fireflies, there are a few hotspots that can be recommended for ecotourism activities in the future. These findings are important as part of the establishment of mangrove germplasm as well as a future plan for conservation education.

Community engagement is also a crucial part of any conservation effort. Surveys were conducted to assess their socioeconomic status and understanding of the importance of mangroves. A total of 361 respondents were interviewed throughout this study. According to the survey, the mangrove forest of Bagan Datuk's coastline contributes an average of 22.6% (RM429.48) of the local community revenue (Yoshiaki et al., 2002). The highest revenue recorded was from the residents of Bagan Sungai Burong, with a total of RM1,698.26 per month, while the lowest revenue was from the residents of Kampung Sungai Betul, with a total of RM39.40 per month. Furthermore, this survey also showed that the majority of the respondents were aware of the mangrove forest's role in protecting the coastline from coastal hazards and agreed that the mangrove forest needs to be protected sustainably. The preliminary results of the study show that the local community relies on mangrove and coastal products for food and income. Conserving and developing mangrove germplasm will improve the mangrove ecosystem, which is a critical breeding ground for marine life. This will also benefit the local community's socioeconomic well-being.

CONCLUSION

Mangrove germplasm is important for conservation, education, and socioeconomic development, and the establishment of a mangrove germplasm in Bagan Datuk is a small step in this direction. While this study only provides preliminary results on the flora, fauna, and socioeconomic status of the Bagan Datuk mangrove forest, further data collection and investigation on other ecological attributes is highly recommended. Moreover, the Perak Forestry Department has designated about 139.21 hectares of mangrove area in Bagan Datuk, including 78 hectares of the germplasm plot, as a Permanent Reserve Forest, which is a significant step forward for mangrove forest conservation and preservation in the Bagan Datuk district.

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PROCEEDINGS OF THE 26TH PROJECT EVALUATION AND MONITORING (PEM) SEMINAR 2023

This compilation highlights the outcomes of innovative forestry research undertaken in 2023, showcasing selected projects presented at the 26th PEM Seminar. The proceedings reflect a balanced representation of upstream and downstream forestry research, covering a diverse range of themes including forestry and the environment, forest biodiversity, forestry biotechnology, economic and social forestry, as well as forest and natural products. This proceedings volume includes 25 articles that were chosen to illustrate cutting-edge findings and advancements, underscoring the continuous efforts toward sustainable forestry and environmental stewardship. This volume serves as a valuable resource for researchers, policymakers, and practitioners in the field. The collection reflects the dynamic progress in forestry science, aiming to inspire further advancements in sustainable forest management and conservation strategies.

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