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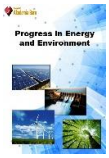

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Original Article

Fueling the future with fungi: Efficient lipid extraction from ganoderma mycelium biomass

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Merashini Tangaraju[1,2], Zul Ilham[1,2*], Muhammad Adlim[3], Wan Abd Al Qadr Imad Wan-Mohtar[1,2], Adi Ainurzaman Jamaludin[1,2]

[1] Biomass Energy Lab, Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603, Kuala Lumpur, Malaysia
[2] Bioresources and Bioprocessing Research Group, Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603, Kuala Lumpur, Malaysia
[3] Chemistry Department, FKIP, Universitas Syiah Kuala, Darussalam Banda Aceh, 23111, Indonesia
* **Correspondence email:** ilham@um.edu.my

Abstract

The extraction of lipids from *Ganoderma lucidum* mycelial biomass presents a potential cost-effective solution for biodiesel production. This study aimed to evaluate the efficacy of three different lipid extraction methods: Soxhlet extraction (SXE), solvent extraction (SVE), and ultrasonic-assisted extraction (UAE). The biomass (5 g) was subjected to varying conditions of hexane solvent (50-300 ml) at a constant temperature of 60°C for 1-9 hours. The research methodology involved a systematic comparison of lipid yields obtained under different extraction conditions. The results demonstrated that lipid yield was significantly influenced by extraction time and solvent quantity, with SVE yielding the highest lipid content of 20.36% (at 2 hours and 100 ml hexane), followed by SXE at 18.8% and UAE at 7.50%. These findings indicate that SVE is the most effective method for lipid extraction from *G. lucidum* mycelial biomass. The implication of this study is that *G. lucidum* mycelial biomass can be considered a viable raw material for biodiesel production. Future research should focus on exploring novel extraction techniques and optimizing parameters to further enhance lipid yields, underscoring the significance of this study in advancing sustainable biodiesel production.

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1 Introduction

Ever since industrial revolution, the consumption of food, water, energy and electricity have risen dramatically due to the rapid population growth and their improvements in lifestyle [1]. Currently, fossil fuels dominate the world's energy demand and economy, especially the demand for transport fuel, which is highly affecting the environment [2]. In fact, fossil fuels are non-renewable, and not sustainable in the long run. Therefore, bio-based fuels, such as biodiesel, have been anticipated to be a viable substitute for fossil fuels, as they are sustainable, cost effective, free of toxic chemicals such as sulphur, and have greater lubricity for automobiles [3].

Biodiesel production also contributes disadvantages, for instance it needs a high production rate of material, and this issue leads to the rising of oil rates demand in the market [2,4]. Consequently, it has been estimated that using crops such as sunflower seed or rapeseed, required a large-scales and duration in order to achieve the existing biodiesel goals. For these reasons, it is important to find new novel raw materials, which can reduce the production price of biodiesel without competing with food security and so on. In recent years, research has been conducted on the production of biodiesel from various feedstock including plant-based oils, animal fats, waste oils, algal oils or even biomass [4]. Nevertheless, the research on using fungi-based feedstock remained limited. As fungi are fast growing and can be utilized easily under favorable conditions, thus the production of biodiesel from this species could serve as a potential resource for energy [5,6,7].

Ganoderma lucidum is a wood decaying fungus that has been used extensively as traditional Asian medicine for more than 2,000 years [8,9]. This mushroom is edible and has many health benefits such as to treat various diseases, most commonly cancer [8-10]. *G. lucidum* undergoes four stages of life cycle: (1) spores, (2) spore germination, (3) mycelium and (4) fruiting body. *G. lucidum* can easily be cultivated from its mycelium in short period of time with the help of biotechnological practises and nutrients. Through fruiting bodies and spores, it requires long period of time [10,11]. According to recent research. *G. lucidum* mycelium was cultivated by submerged-liquid fermentation process which took 3 to 6 months [8,12,13]. By day 10 of the fermentation process, high biomass yield could be obtained which reduces the time needed to produce byproducts and completely decreases contamination possibilities. Moreover, the active ingredients of *G. lucidum* is usually extracted for medicinal purposes. Apart from medicinal benefits, *G. lucidum* also assumed as a good source of biomass [6,14].

In extracting lipid from *G. lucidum* for biodiesel production it is important to know proper extraction techniques and factors that can affect the lipid yield. Extraction methods can be divided into mechanical, physical, and chemical. These include soxhlet extraction, supercritical fluid extraction, pressurized liquid extraction, solvent extraction, and aqueous enzyme extraction [15]. Lipid extraction consumes around 90 % of the energy and is costly by making the process difficult [16]. Thus, the implementation of extraction techniques that are able to extract lipid with less duration, energy and solvent consumption without losing lipid quality are the key factors for cost-effective and environmentally sustainable processes. Earlier studies have shown that the oil yielded from extraction methods are influenced by the sample size, temperature, solvent volume, solvent types and time [17,18].

Recent advances in lipid extraction techniques have demonstrated varied performances, each with distinct advantages and limitations. Soxhlet extraction (SXE) remains a widely used method due to its ability to continuously extract lipids over extended periods, though it is time-consuming and solvent intensive. Recent studies have reported lipid yields up to 18.8% using SXE for fungal biomass, indicating its effectiveness despite operational drawbacks [19]. Solvent extraction (SVE) has gained attention for its simplicity and efficiency, achieving higher lipid yields in shorter times compared to SXE. A recent investigation reported a lipid yield of 20.36% from Ganoderma lucidum mycelial biomass using SVE with optimized conditions [20]. Ultrasonic-assisted extraction (UAE) utilizes ultrasonic waves to enhance cell disruption and lipid release, significantly reducing extraction time and solvent usage. However, its performance can be variable, with lipid yields from fungal biomass such as G. lucidum reported at 7.50%, highlighting a need for further optimization [21]. Collectively, these techniques offer valuable insights into efficient lipid extraction, with recent studies underscoring the potential for optimizing conditions to improve yield and sustainability [22,23,24].

This study focuses on the comparison of three different extraction techniques (Soxhlet extraction, solvent extraction and ultrasonic-assisted extraction) on extraction of *G. lucidum* mycelial biomass lipid (GMBL) cultured through submerged-liquid fermentation. The lipid yield of the GMBL will be identified and compared to determine the most effective extraction technique for GMBL. The study's novelty lies in its comprehensive evaluation and comparison of three distinct lipid extraction methods Soxhlet extraction (SXE), solvent extraction (SVE), and ultrasonic-assisted extraction (UAE) on *Ganoderma lucidum* mycelial biomass. By systematically varying extraction conditions such as solvent volume and time, and analysing their impact on lipid yield, the study provides a detailed assessment of each method's

effectiveness. The application of these techniques to *G. lucidum* mycelial biomass for biodiesel production is a relatively underexplored area, offering new insights into optimizing extraction processes for this specific biomass.

2 Methodology

2.1 Preparation of material

In this research, *G. lucidum* mycelial biomass was prepared in the Omics Laboratory at the Institute of Biological Sciences, University of Malaya. Mycelium of *G. lucidum* was cultivated by submerged-liquid fermentation in which the pellets were formed within 30 days of cultivation. Biomass of the mycelium was mass produced by using a bioreactor in the laboratory [14]. *G. lucidum* mycelium was dried at 60°C for three consecutive days in a drying oven. Next, the biomass obtained was ground into fine powder as shown in Fig. 1.

Fig. 1 Phases from A (mycelium pallets of *G. lucidum* at 30th day of submerged-liquid fermentation), to B (dried G.lucidum mycelial biomass after 3 days) followed by C (finely powdered *G.lucidum* mycelial biomass by using mortar and pestle).

2.2 Extraction of lipid

The lipid extraction of *G. lucidum* mycelial biomass was carried out using soxhlet, ultrasonic and solvent extraction. Hexane was used as a solvent for 5 g of *G. lucidum* mycelial biomass at 60 °C for all three extraction techniques. After extraction, the lipid of *G. lucidum* was extracted from the sample by filtering and the lipid mixture of the solvent was evaporated using a rotary evaporator [15].

2.3 Soxhlet extraction (SXE)

In soxhlet extraction (SXE), the extraction was carried out using a soxhlet equipment from the Biomass Energy Laboratory at the Institute of Biological Sciences, University of Malaya. SXE conducted using 50, 100 and 150 ml of hexane at 3, 6 and 9 h.

2.4 Solvent extraction (SVE)

In solvent extraction (SVE), the biomass powder was mixed with hexane in a 500 ml sample bottle. The sample bottle was then put on a hot plate at 60 ° C with a magnetic stirring of 200 rpm. SVE was conducted using 100, 150 and 200 ml of hexane at 1, 2 and 3 h. After the extraction period, the mixture was subjected to filtration to separate the liquid phase containing the dissolved lipids from the solid biomass residue. The filtrate was then concentrated to isolate the lipid content. Filtration was conducted using a vacuum filtration setup to ensure efficient separation of the solvent and residual biomass [19].

2.5 Ultrasonic-assisted extraction (UAE)

In ultrasonic-assisted extraction (UAE) procedure, an ultrasonic water bath was used to extract lipids. The biomass powder was mixed with hexane in a 500 ml sample bottle. The sample bottle was then put in ultrasonic water bath equipment. The experiment was carried out using 100, 200 and 300 ml of hexane at 1, 3 and 5 h.

2.6 Determination of lipid yield

The lipid remained dissolved in the hexane solvent after the extraction process. A rotary evaporator was used to eliminate the excess solvent through the application of heat and pressure [15,17]. Mass of recovered lipid after the removal of solvent was weighed. The extracted lipid yield (%) is estimated by using equation below [17].

$$\text{Lipid yield (\%)} = \frac{m_i}{m_s} \times 100 \quad (1)$$

where the coefficient m_i (g/l) is the mass of recovered lipid while m_s (g/l) is the mass of dried material (5 g of *G. lucidum* mycelial biomass) used for the extraction of SXE, SVE and UAE.

2.7 Statistical analysis

The statistical analysis was carried out by Minitab version 18 software. All three extractions techniques were repeated three times. The mean values and standard deviations of the results were calculated by analysis of variance (ANOVA).

3 Results and Discussion

3.1 Lipid yield

Extraction of GMBL using SXE, SVE and UAE was evaluated. It was observed that all three techniques were able to extract GMBL with different lipid yield obtained. The amount of the lipid yield was not as high as expected. Fig. 2 depicts the highest lipid yield obtained by each technique with different solvent volume and extraction time. From the result, the highest GMBL of 20.36% were achieved when using SVE (60 min/100 ml), followed by 18.8% by SXE (6 h/150 ml) and 7.5% from UAE (5 h/200 ml). Based on previous research, 1 g of mycelium of *G. lucidum* has lipid content of approximately around 1.67% [25].

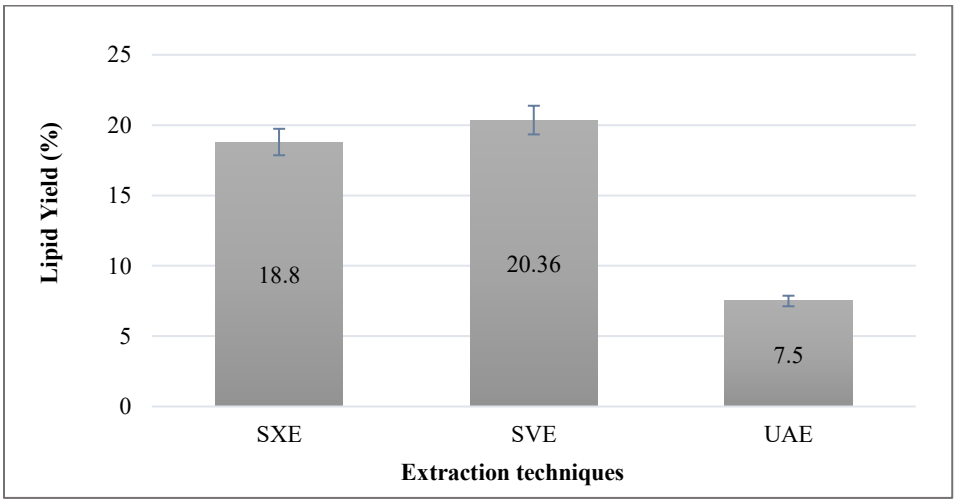

Fig. 2 Bar graph represents the comparison of *G. lucidum* mycelial biomass lipid yield (%) using SXE (6 h/150 ml), SVE (3 h/200 ml), UAE (5 h/200 ml).

In the statistical analysis done, two factors have been tested upon the yield of GMBL which were the solvent volume and extraction time as well as the interaction between both independent variables. The fitness model analysed were 0.9945 for SXE, 0.9927 for SVE and 0.9775 for UAE. These indicate that the lipid yield was strongly affected by the solvent volume used and the extraction time. Table 1, 2 and 3 show that there were significant effects and interactions between the factors for all three extraction techniques (p-value < 0.05). Previous findings have also suggested that the yield of lipid is influenced by several conditions such as solvent type, solvent volume, temperature, and time taken for the extraction [17,26]. On the other hand, Table 4 summarizes the comparison between the three different techniques used on the extraction of GMBL. SXE and SVE have been recorded to be effective in extracting lipid and widely used in previous research [27,28]. In extracting GMBL, SVE (14.57 - 20.36 %) and SXE (9.44 - 18.80 %) was observed to have a higher yield compared with by using UAE (4.30 - 7.50 %).

Table 1 Analysis of variance (ANOVA) for the experimental results of SXE

Source	DF	Sum of sq	Mean sq	F-value	P-value
Solvent volume	2	14.560	7.2800	67.55	0.000
Extraction time	2	139.253	69.6267	646.05	0.000
Solvent volume*Extraction time	4	22.897	5.7242	53.11	0.000
Error	9	0.970	0.1078		

*mean—12.12, variance—10.45, standard deviation—3.03, $\mathbf{R^2}$—0.9945, adjusted $\mathbf{R^2}$—0.987, predicted $\mathbf{R^2}$—0.9782

Table 2 Analysis of variance (ANOVA) for the experimental results of SVE

Source	DF	Sum of sq	Mean sq	F-value	P-value
Solvent volume	2	9.906	4.9532	31.60	0.000
Extraction time	2	137.224	68.6118	437.73	0.000
Solvent volume*Extraction time	4	43.766	10.9415	69.80	0.000
Error	9	1.411	0.1567		

*mean—13.44, variance—11.31, standard deviation—3.36, $\mathbf{R^2}$—0.9927, adjusted $\mathbf{R^2}$—0.9261, predicted $\mathbf{R^2}$—0.9707

Table 3 Analysis of variance (ANOVA) for the experimental results of UAE

Source	DF	Sum of sq	Mean sq	F-value	P-value
Solvent volume	2	1.0844	0.54222	9.76	0.006
Extraction time	2	19.1878	9.59389	172.69	0.000
Solvent volume*Extraction time	4	1.4056	0.35139	6.33	0.000
Error	9	0.5000	0.05556		

*mean—9.24, variance—5.34, standard deviation—2.31, $\mathbf{R^2}$—0.9775, adjusted $\mathbf{R^2}$—0.9574, predicted $\mathbf{R^2}$—0.9098

In SXE and SVE, the transfer of heat between solid and liquid occurs from the outside to the inside of the sample membrane, while the transfer of mass occurs vice versa (from the inside to the outside) [15,27,28]. On the contrary, the UAE utilizes the frequency of electrostatic interactions arising from the development of high-intensity wave propagation. The UAE process involves a physical mechanism whereby the penetration of the cell walls and washing out of the cell material takes place before it becomes disrupted [29,30]. The lower lipid yield in UAE may be due to non-consistent transmission and exposure to sonic power which affected biomass cell wall.

Table 4 Comparison between three lipid extraction techniques on GMBL yield [15,22-25].

Aspects	Extraction techniques		
	SXE	SVE	UAE
Procedure	An appropriate size of cellulose thimble chosen for the sample before place in soxhlet extractor. Solvent in a round bottom flask heated with a mantle	Sample soaked in solvent with a magnetic stirrer in a flat bottom flask and then heated by a hot plate.	Sample soaked in solvent, and placed in an ultrasonic water bath equipment
Solvent	hexane	hexane	hexane
Sample size	1-5 g	1-5 g	1-5 g
Temperature	60 °C	60 °C	60 °C
Solvent volume	50-150 ml	100-200 ml	100-300 ml
Extraction time	3-9 h	1-3 h	1-5 h
Advantages	No filtration needed, easy to use	Short extraction duration	Moderate extraction duration, easy to use
Disadvantages	Long extraction duration	Filtration needed	High solvent amount, filtration needed
Lipid yield	9.44 % - 18.80 %	14.57 % - 20.36 %	4.30 % - 7.50 %
Quantity	Moderate	High	Low

3.2 Effect of solvent volume and extraction time

Fig. 3 depicts the effect of altered hexane volume and extraction time on GMBL yield by SXE (a), SVE (b) and UAE (c). As shown in the results above, the lipid yield increased as the solvent volume and the extraction time increased. A high solvent volume will help to accelerate the chemical reaction resulting in enhanced lipid production. Indeed, one previous research stated that the use of high amount of solvent volume affects the inclination of extraction recovery [31]. Additionally, the author reported that the solvent volume must be sufficient to ensure that the particles of samples are immersed in solvent throughout the extraction process. Hexane was selected for this analysis because it can be quickly recovered, non-polar, low latent vaporization heat and high solvent selectivity [18,31,32]. Alternatively, an optimal solvent volume and extraction time could be determined to enhance the lipid yield of GMBL.

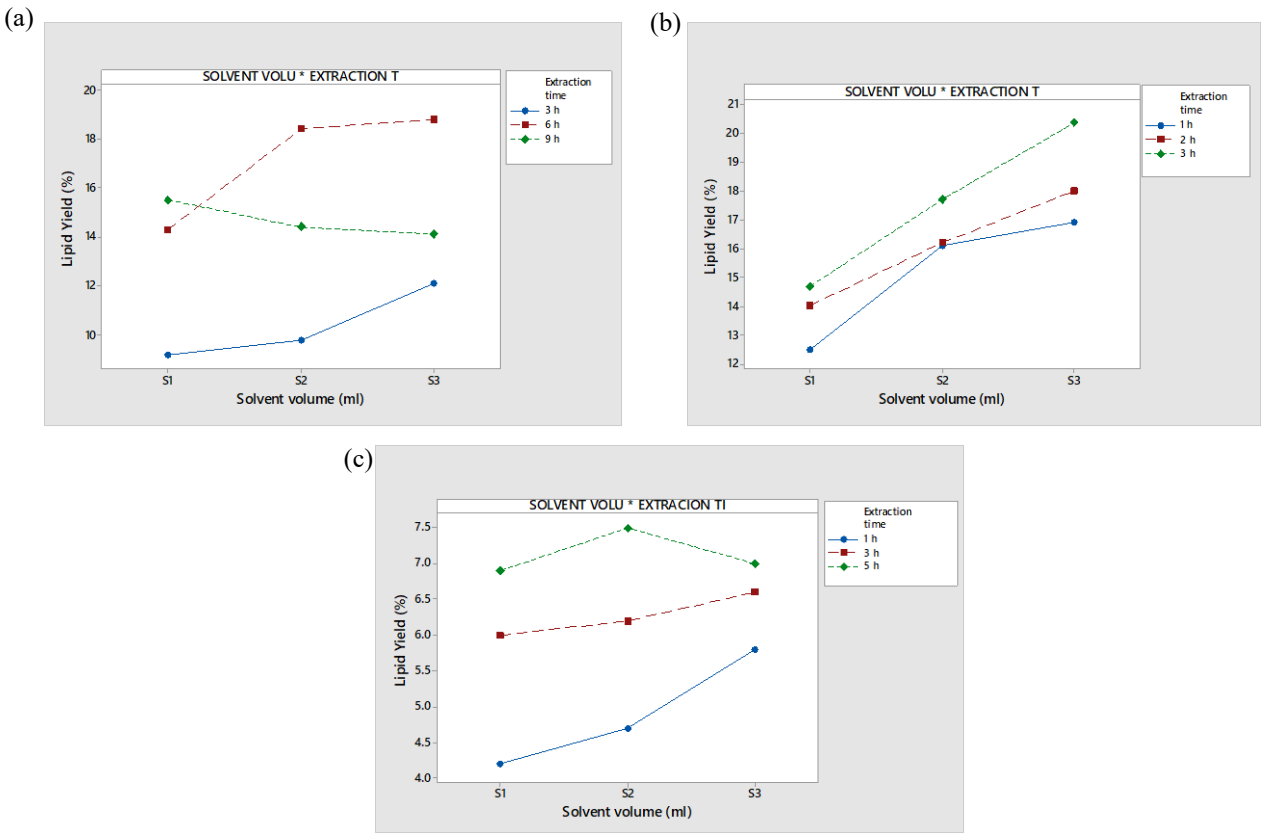

Fig. 3 Effect of solvent volume and extraction time on extracted *G. lucidum* mycelial biomass lipid yield (%): SXE where S1=50 ml, S2=100ml, S3=150 ml (a), SVE where S1=100 ml, S2=150 ml, S3=200 ml (b) and UAE where S1=100 ml, S2= 200 ml, S3= 300 ml (c).

Solvent extraction (SVE) outperformed Soxhlet extraction (SXE) and ultrasonic-assisted extraction (UAE) in lipid yield from *Ganoderma lucidum* mycelial biomass due to its more efficient and adaptable extraction conditions [19]. SVE allows for precise optimization of solvent volume and extraction time, leading to a higher lipid yield of 20.36% with minimal solvent use (100 ml hexane over 2 hours). This direct contact method facilitates effective lipid dissolution, enhancing extraction efficiency. In contrast, SXE, while thorough, requires longer extraction times and larger solvent volumes, resulting in a lipid yield of 18.8%, which may be less efficient due to prolonged processing and greater solvent use. UAE, which utilizes ultrasonic waves to disrupt cell structures and improve solvent penetration, achieved a lower lipid yield of 7.50%. This lower yield likely stems from suboptimal ultrasonic parameters, which may not have fully optimized solvent interaction and extraction efficiency [21,24]. Thus, while SXE

and UAE offer valuable extraction mechanisms, SVE's ability to fine-tune extraction conditions contributes to its superior performance in this study.

In SXE (Fig. 3) the lipid yield decreased when the time was set up to the highest at 9 h (50 ml to 150 ml), while, when the time set up from 3 h to 6 h the lipid yields increased. This is because SXE reached optimal time (3 h-6 h) that after 6 h there was not much lipid obtained. Therefore, the lower the duration is more efficient compared to the higher duration [32,33]. In addition, SVE had used the shortest time duration (between 1 h to 3 h) and the smallest volume of solvents (100 ml to 200 ml) to achieve the highest lipid yield compared to SXE and UAE. The higher yield of GLBM in SVE could be due to the use of a magnetic stirrer that continuously mixed biomass sample with hexane solvent throughout the extraction process, while in SXE and UAE the sample was in static manner [23,34,35].

4 Conclusion

Overall, a reasonable amount of GMBL could be obtained from all three extraction methods in this study. The SVE (20.36%) and SXE (18.8%) techniques extracted higher *G. lucidum* mycelial biomass lipid compared with the UAE (7.5%). In SVE, the GMBL yield was enhanced as the amount of hexane (100-200 ml) and extraction time (1-3 h) increased. SVE also required the shortest time (3 h) and smallest volume of hexane solvent (200 ml) to achieve the maximum quantity of lipid yield. Among the extraction techniques used, SVE could be recommended as an effective extraction technique to acquire lipid yield as it requires a short extraction period with moderately low solvent volume. SVE is also the most effective method for lipid extraction from *G. lucidum* mycelial biomass, achieving the highest lipid yield of 20.36%. This provides a practical and efficient approach for utilizing this biomass in biodiesel production. In addition, this study demonstrated that *G. lucidum* mycelial biomass can be a viable and cost-effective raw material for biodiesel production, supporting the potential for using underutilized biomass in sustainable energy solutions. Nonetheless, future research work should focus on other novel extraction techniques such as supercritical fluid extraction, microwave-assisted extraction and pulse electric field extraction besides optimizing the extraction parameters in improving the lipid yield from *G. lucidum* mycelial biomass.

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Declaration of Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID

Zul Ilham https://orcid.org/0000-0003-3836-0848
Muhammad Adlim https://orcid.org/0000-0001-6278-7775
Wan Abd Al Qadr Imad Wan-Mohtar https://orcid.org/0000-0002-5384-4325
Adi Ainurzaman Jamaludin https://orcid.org/0000-0003-3022-5027

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