Enhanced Knowledge in Sciences and Technology Vol. 2 No. 2 (2022) 078-088

EKST

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

Antimicrobial Properties and Microbiological Shelf-life of *Aquilaria malaccensis* Spray Dried Leaf Extract Powder

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DOI: https://doi.org/10.30880/ekst. 2022.02.02.009
Received 02 January 2022; Accepted 22 September 2022; Available online 23 November 2022

Abstract: *Aquilaria* is an evergreen woody tree which is well known for its resin-impregnated stem called agarwood. The leaves of *Aquilaria* are found to exhibit antioxidant, antidiabetic, anti-arthritic, antibacterial, antifungal, anti-inflammatory and hepatoprotective activities. *Aquilaria* leaves are potential as the raw ingredients and final products. In order to maintain their microbiological qualities during storage, they were prepared in the form of leaf extract powder. Firstly, they were macerated in ethanol and water separately before filtering out the residue. The filtrate was concentrated by using rotatory evaporator and fume hood. Subsequently, the ethanol and aqueous *Aquilaria* leaf extracts were spray dried into powder. Thus, this study aims to investigate the antimicrobial properties and shelf-life of the spray dried *Aquilaria malaccensis* ethanol and aqueous leaf extract powder. Broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) of the spray dried *A. malaccensis* leaf extract powder against the selected bacteria and fungi. The shelf-life analysis of the spray dried *A. malaccensis* leaf extract powder was conducted by using standard plate count (SPC) method. It has been reported that there is no significant inhibitory effect of the spray dried *A. malaccensis* leaf extract powder against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Aspergillus niger*, *Rhizopus oryzae* and yeast. Slightly higher antimicrobial activities were recorded in the spray dried *A. malaccensis* ethanol leaf extract powder than the aqueous leaf extract powder based on the results of antibacterial assay. The shelf-life study of the spray dried *A. malaccensis* leaf extract powder has shown that no microbial growth after 33 weeks or above. This can be explained by the application of spray drying that destroys the microorganisms and reduces the water activity of *A. malaccensis* leaf extract.

Keywords: *Aquilaria* Leaves, Extraction, Ethanol, Aqueous, Spray Dried, Antimicrobial Properties, Shelf-life

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1. Introduction

Aquilaria is a plant genus which is well-known for its formation of agarwood [1]. It is an evergreen woody tree commonly distributed in tropical forests of China, Thailand, Malaysia and Indonesia. Since the tree takes years to produce the resinous fragrant wood, its leaves are often processed into herbal tea as a commercial product. The agarwood tea is also known as 'teh gaharu' or 'teh karas' in Malaysia [2]. The scientific studies have shown that the *Aquilaria* leaves are beneficial to human health because they exhibit abundant biological activities such as antioxidant, antidiabetic, anti-arthritic, antibacterial, antifungal, anti-inflammatory and hepatoprotective effects [1].

Several researches have recorded that *Aquilaria* leaves are rich in the secondary metabolites such as flavonoids, phenols, terpenoids, tannins and steroids. The interest of extracting these biologically active components to be used as the food preservatives and functional food ingredients is increasing over the years [3]. However, the choice of extraction method and solvent are in obtaining the desired phytochemicals at the maximum level is questionable. Aside from tea, *Aquilaria* leaves and their extract also serve as the raw materials of soup, instant noodle, marinade, seasoning, biscuit and coffee [2]. It is important to maintain the qualities of the leaves and leaf extract for long term storage before they are incorporated in other food ingredients or sold directly to consumers. The leaves and leaf extract of *Aquilaria* are susceptible to spoilage due to higher moisture content that promotes the rapid growth of microorganisms.

There are two types of extraction method which are solvent extraction and soxhlet extraction that can be used to manufacture *Aquilaria* leaf extract. The solvent extraction method is found to yield higher quantity of *Aquilaria* leaf extract which is 26.94% as compared with the soxhlet extraction method which is 17.84%. The solvents that commonly used during solvent extraction are water, ethanol, methanol, hexane and chloroform [4]. The types of extraction solvent greatly affect the effectiveness and amount of extract obtained. Polar solvent has better capability in dissolving the phenolic compounds than the non-polar solvent [5]. The phenolic compounds found in *Aquilaria* leaves are closely related to their antioxidant and antimicrobial actions [6]. Previous study has reported that the ethanol leaf extract of *Aquilaria* contains the most abundant total phenolic content followed by water leaf extract [7]. Besides, the yield of aqueous, ethanol, methanol, hexane and chloroform leaf extracts of *Aquilaria* are 21.73%, 26.94%, 16.82%, 2.44% and 5.95% respectively [4]. Thus, ethanol and water are the suitable solvents used for recovering more polyphenols and crude extract from *Aquilaria* leaves.

This paper aims to compare between solvent and aqueous extraction methods for the spray dried *Aquilaria* leaf extract powder in term of their antimicrobial activities and shelf-life. This research benefits to the other researchers that might be interested in exploring the leaves of *Aquilaria* as the intermediate and final product in food industry. The main advantage of this study is to provide information about the potential of the spray dried *Aquilaria* leaf extract powder as the natural source of food preservative to replace the chemical preservative.

2. Materials and Methods

2.1 Materials

The leaves of *Aquilaria malaccensis* were provided by Synergy One Holding Sdn. Bhd. The materials used in this study were distilled water, ethanol (QRëC, Malaysia), maltodextrin, Plate Count Agar (PCA) powder (HiMedia, India), Potato Dextrose Agar (PDA) powder (HiMedia, India), Mannitol Salt Agar (MSA) powder (HiMedia, India), Eosin Methylene Blue (EMB) powder (HiMedia, India), Mueller Hinton (MH) agar powder (HiMedia, India), Sabouraud Dextrose Agar (SDA) powder (HiMedia, India), *Escherichia coli* (ATCC No. 8739), *Staphylococcus aureus* (ATCC No. 25923), *Bacillus cereus* (ATCC No. 14579), *Aspergillus niger* (ATCC No. 16888), *Rhizopus oryzae* (ATCC No. 1987), yeast (ATCC No. 10231), Mueller Hinton (MH) broth powder (Conda, Spain), Sabouraud Dextrose broth powder (HiMedia, India), 0.5 McFarland standard (Remel, United States), tetracycline

(HiMedia, India), resazurin (Sigma-Aldrich, Germany), miconazole (Zarin, Malaysia) and peptone water powder (HiMedia, India).

2.2 Preparation of Dried *Aquilaria* Leaves

A. malaccensis leaves were cleaned by using tap water and distilled water. Subsequently, the leaves were air dried at room temperature for a week and then dried in the oven (MMM Group, Germany) at 40ºC for 3 days. Later, the leaves were ground into powder by using mortar and pestle [6].

2.3 Solvent Extraction and Spray Drying of *Aquilaria* Leaf Extract

The solvent extraction method was used as described by Abbas, Hashim & Salleh (2018) [8]. About 144g of *A. malaccensis* leaf dried powder was added to 1920ml of 96% ethanol (QRëC, Malaysia). The mixture was stirred in a temperature-controlled incubator shaker (IKA, Malaysia) at 50°C and 100rpm for 18 hours. Next, the mixture was filtered by using Whatman No. 1 filter paper. The macerate was concentrated by using a rotary evaporator device (EYELA, Japan) at 50°C and then placed in a fume hood (ESCO, Singapore) to evaporate the remaining solvent. The spray drying procedure was adopted from Tran *et al.* (2020) [9]. 38g of maltodextrin was added to 190ml of warm distilled water at 40ºC. About 19g of crude extract was mixed with maltodextrin solution. The mixture was homogenized and then spray dried by using a spray dryer (SOLTEQ, Malaysia) at inlet temperature of 170°C and feed flow rate of 10rpm.

2.4 Aqueous Extraction and Spray Drying of *Aquilaria* Leaf Extract

About 144g of *A. malaccensis* leaf dried powder was added to 1920ml of distilled water. The mixture was stirred in a temperature-controlled incubator shaker (IKA, Malaysia) at 50°C and 100rpm for 18 hours. After that, the mixture was filtered by using Whatman No. 1 filter paper [8]. 14g of maltodextrin was added to 6ml of warm distilled water at 40ºC. About 18g of crude extract was mixed with maltodextrin solution. The mixture was homogenized and then spray dried by using a spray dryer (SOLTEQ, Malaysia) at inlet temperature of 120°C and feed flow rate of 280ml/h [10].

2.5 Preparation of Bacterial and Fungal Inoculum

E. coli, *S. aureus* and *B. cereus* were subcultured on MH agar and incubated at 37ºC for 18 to 24 hours. *A. niger*, *R. oryzae* and yeast were subcultured on SDA and incubated at 30ºC for a week. A loopful of colony or spore was transferred from agar into 5ml of saline. The density of suspension was compared visually with 0.5 McFarland standard (Remel, United States). The adjusted bacterial suspension was diluted in a ratio of 1:100 in MH broth. The adjusted fungal suspension was diluted in a ratio of 1:10 in Sabouraud Dextrose broth [11].

2.6 Antimicrobial Properties by Broth Microdilution Assay

The broth microdilution assay was carried out based on a method described by Ohikhena, Wintola & Afolayan (2017) [11]. 100µl of the spray dried *A. malaccensis* leaf extract powder (128mg/ml) and 100µl of tetracycline (HiMedia, India) (128µg/ml) in MH broth were each pipetted into triplicate wells at the first row of 96-well plate. 50µl of MH broth was pipetted into the remaining wells. Two-fold serial dilution was prepared by transferring 50µl of leaf extract powder and tetracycline (HiMedia, Indai) to the next row until the eighth row. For the 12^{th} column, 50µl and 100µl of MH broth were pipetted into the wells at the first to sixth rows (growth control) and the seventh and eighth rows (sterility control), respectively. 50µl of each bacterial suspension was pipetted into all the wells. The 96-well plate was incubated at 37ºC for 18 to 24 hours. After incubation, 20µl of resazurin (Sigma-Aldrich, Germany) was added to all the wells.

This assay was repeated for analyzing the antifungal properties of the spray dried *A. malaccensis* leaf extract powder. The MH broth was replaced by Sabouraud Dextrose broth. Miconazole (Zarin,

Malaysia) (1mg/ml) was prepared in Sabouraud Dextrose broth. 50µl of each fungal suspension was pipetted into all the wells. The 96-well plate was incubated at 30ºC for 2 to 5 days. The Minimum Inhibitory Concentrations (MIC) of the spray dried *A. malaccensis* leaf extract powder against bacterial and fungal strains were determined as the lowest concentrations at which blue color was visible.

2.7 Shelf-life by Standard Plate Count (SPC) Method

The SPC method used in this study was outlined by Aini & Mardiyaningsih (2016) [12]. 1g of spray dried *A. malaccensis* leaf extract powder was mixed with 9ml of peptone water. After that, the sample was vortexed (DLAB, China) to obtain 10^{-1} dilution. The sample was then diluted until 10^{-5} dilution. 25ml of molten PCA, PDA, MSA and EMB agar were poured into the petri plates. 100µl of sample at 10^{-5} dilution was spread aseptically on triplicate solidified agar. PCA, MSA and EMB agar plates were incubated at 37ºC for 24 hours whereas PDA plates were incubated at 30ºC for 2 to 7 days. The microbial population was counted in CFU/g following the colony counting rules. The sample was tested for shelf-life on week 32nd and week 33rd.

3. Results and Discussion

3.1 Yield of Spray Dried *Aquilaria* Leaf Extract Powder

Different solvent used for extraction of *A. malaccensis* leaves yields varying amount of leaf extract. This is because the polarity of extraction solvent affects the effectiveness in extracting specific chemical compounds from the leaves [3]. In this study, the yields of the spray dried *A. malaccensis* ethanol and aqueous leaf extract powder were 10.98% and 10.05% respectively. This result is in accordance to the previous research carried out by Rashid *et al.* (2019) [7] which demonstrated higher yield in *A. malaccensis* ethanol leaf extract (14.7%) than in the aqueous leaf extract (13.5%). Therefore, ethanol is a better solvent than water for extracting secondary metabolites from *A. malaccensis* leaves.

3.2 Determination of Minimum Inhibitory Concentration (MIC)

The results of the broth microdilution assay for evaluating the antibacterial properties of spray dried *A. malaccensis* leaf extract powder are shown in Table 1, Figure 1 and Figure 2. The data has shown that *E. coli*, *S. aureus* and *B. cereus* used in this study were less susceptible to the spray dried leaf extract powder of *A. malaccensis* compared to tetracycline. The MIC values of the spray dried *A. malaccensis* solvent and aqueous leaf extract powder against the bacteria in this study were 64mg/ml and >128mg/ml respectively. These values are higher than the MIC values of tetracycline which ranged from <1 to 4µg/ml. As compared with tetracycline, higher amount of the spray dried *A. malaccensis* leaf extract powder was used to inhibit the growth of bacteria. Meanwhile, *A. malaccensis* rotary evaporated leaf extract reported lower MIC value which is 1.25mg/ml than the spray dried *A. malaccensis* leaf extract powder [13]. Therefore, the spray dried *A. malaccensis* leaf extract powder showed very low antibacterial properties. This could be due to the high temperature of above 120°C employed during spray drying that decomposes the heat sensitive bioactive compounds present in the leaf extract of *A. malaccensis* [11]. The phenolic compounds that function as the antimicrobial agents, are sensitive to heat processing. Besides, the amount of phenolic compound decreases as the storage period increases at the storage temperature of 25ºC with protection from light. This is because the exposure of the phenolic compounds to oxygen during storage could lead to their degradation and loss of their bioactivities [14].

Table 1: MIC ranges of the spray dried *A. malaccensis* leaf extract powder and tetracycline against bacteria

Bacteria	MIC range		
	mg/ml		µg/ml
	Solvent leaf extract	Aqueous leaf extract	Tetracycline
E. coli	64	>128	<1
S. aureus	64	>128	2 – 4
B. cereus	64	>128	2

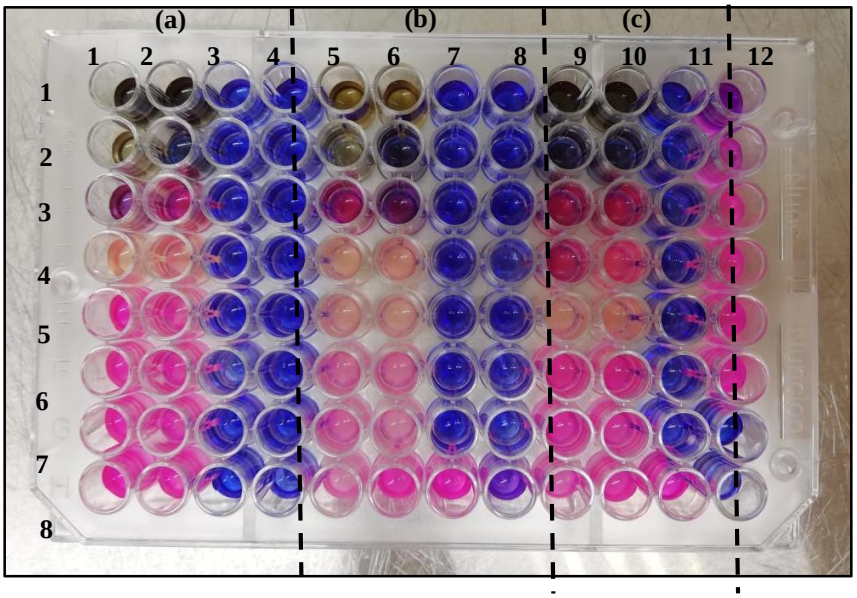

Figure 1: Antibacterial properties of the spray dried *A. malaccensis* solvent leaf extract powder against (a) *E. coli* (b) *S. aureus* and (c) *B. cereus*. The spray dried *A. malaccensis* solvent leaf extract powder was located in the 1st, 2nd, 5th, 6th, 9th and 10th columns whereas tetracycline was located in the 3rd, 4th, 7th, 8th and 11th columns. From the 1st to 8th rows, the concentrations of the spray dried *A. malaccensis* solvent leaf extract powder are 128, 64, 32, 16, 8, 4, 2 and 1mg/ml whereas the concentrations of tetracycline are 128, 64, 32, 16, 8, 4, 2 and 1µg/ml. For the 12th column, the 1st to 6th rows served as the growth control while the 7th and 8th rows served as the sterility control. Blue color indicates inhibition of bacterial growth while pink color indicates growth of bacteria.

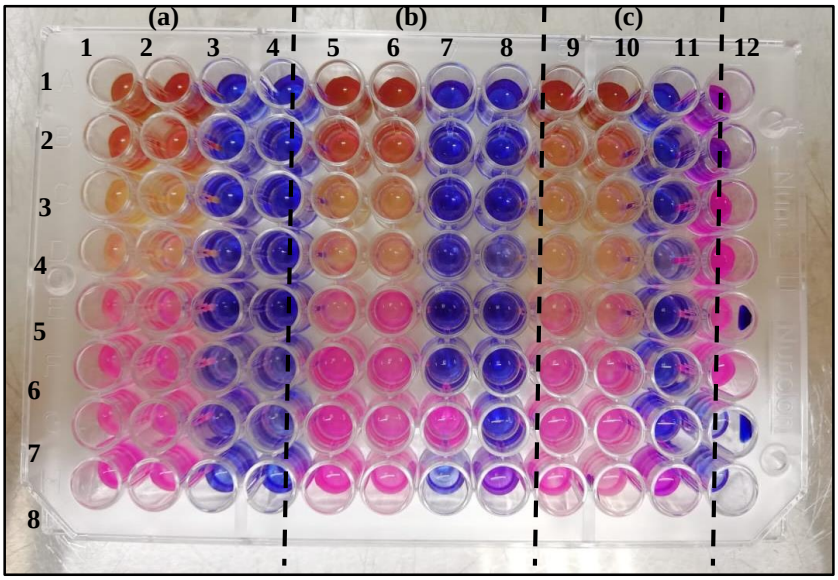

Figure 2: Antibacterial properties of the spray dried *A. malaccensis* aqueous leaf extract powder against (a) *E. coli* (b) *S. aureus* and (c) *B. cereus*. The spray dried *A. malaccensis* aqueous leaf extract powder was located in the 1st, 2nd, 5th, 6th, 9th and 10th columns whereas tetracycline was located in the 3rd, 4th, 7th, 8th and 11th columns. From the 1st to 8th rows, the concentrations of the spray dried *A. malaccensis* aqueous leaf extract powder are 128, 64, 32, 16, 8, 4, 2 and 1mg/ml whereas the concentrations of tetracycline are 128, 64, 32, 16, 8, 4, 2 and 1µg/ml. For the 12th column, the 1st to 6th rows served as the growth control while the 7th and 8th rows served as the sterility control. Blue color indicates inhibition of bacterial growth while pink color indicates growth of bacteria

The antifungal properties of the spray dried extract powder of *A. malaccensis* leaves were determined by using the broth microdilution assay. Based on the results presented in Table 2, Figure 3, Figure 4 and Figure 5, *A. niger, R. oryzae* and yeast demonstrated lower susceptibilities to the spray dried leaf extract powder of *A. malaccensis* as compared to miconazole. The MIC values of the spray dried *A. malaccensis* leaf extract powder (>128mg/ml) were higher than the MIC values of miconazole (0.0156 to 0.5mg/ml) against the selected fungi. More *A. malaccensis* spray dried leaf extract powder is needed to inhibit the fungal growth when compared with miconazole. The degree of antifungal activity could be attributed to the content of antifungal agents found in *Aquilaria* which its leaves consist of only 0.17% of saponins [15]. There is lack of research regarding the antifungal activities of *Aquilaria* leaves, although several studies have reported the antifungal activities from the other parts of *Aquilaria* plant. According to Zhang *et al*. (2014) [16], the agarwood essential oil recorded similar trend of MIC values ranged from 16 to >64mg/ml against fungi. It can be deduced that the spray dried *A. malaccensis* leaf extract powder has no significant effect as the antifungal agent against *A. niger, R. oryzae* and yeast.

Although the overall results have not shown antifungal effect, the spray dried ethanol leaf extract powder of *A. malaccensis* is more effective on the inhibition of bacterial growth in comparison to the spray dried aqueous leaf extract powder. The spray dried ethanol leaf extract powder of *A. malaccensis* (64mg/ml) recorded lower MIC values than the spray dried aqueous leaf extract powder (>128mg/ml) against the selected bacteria. When there is less spray dried *A. malaccensis* ethanol leaf extract powder required for inhibiting the growth of bacteria, it displays higher antimicrobial activity than the spray dried aqueous leaf extract powder. This phenomenon can be explained by the difference in the polarity of solvent used for extracting phytochemical from the leaves of *A. malaccensis*. Although water is more polar than ethanol, it is less efficient at dissolving and extracting the phenolic compounds compared to ethanol [17]. The phenolic compounds derived from *A. malaccensis* leaves exhibited antimicrobial activities [4]. Higher total phenolic content was found in the ethanol leaf extract of *A. malaccensis* with 39.99mg GAE/g followed by the aqueous leaf extract with 30.76mg GAE/g [7]. Hence, the spray dried ethanol leaf extract powder of *A. malaccensis* contains more phenolic compounds and thereby exhibits higher antimicrobial activities than the spray dried aqueous leaf extract powder.

Table 2: MIC ranges of the spray dried *A. malaccensis* leaf extract powder and miconazole against fungi

Fungi	MIC range mg/ml		
	Solvent leaf extract	Aqueous leaf extract	Miconazole
A. niger	>128	>128	0.5000
R. oryzae	>128	>128	0.0156 – 0.0313
Yeast	>128	>128	0.0313 – 0.0625

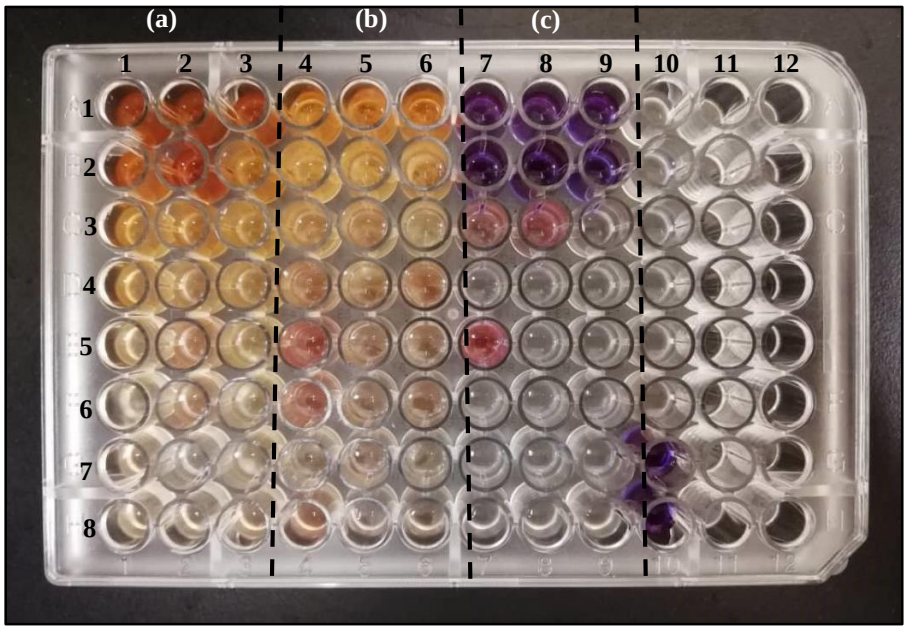

Figure 3: Antifungal properties of the spray dried *A. malaccensis* (a) solvent leaf extract powder, (b) aqueous leaf extract powder and (c) miconazole against *A. niger*. The spray dried *A. malaccensis* solvent leaf extract powder was located in the 1st to 3rd columns whereas the aqueous leaf extract powder was located in the 4th to 6th columns. Miconazole was located in the 7th to 9th columns. From the 1st to 8th rows, the concentrations of the spray dried *A. malaccensis* leaf extract powder are 128, 64, 32, 16, 8, 4, 2 and 1mg/ml whereas the concentrations of miconazole are 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 and 0.0078mg/ml. For the 10th column, the 1st to 6th rows served as the growth control while the 7th and 8th rows served as the sterility control. Purple color indicates inhibition of bacterial growth while pink color or colorless indicates growth of bacteria.

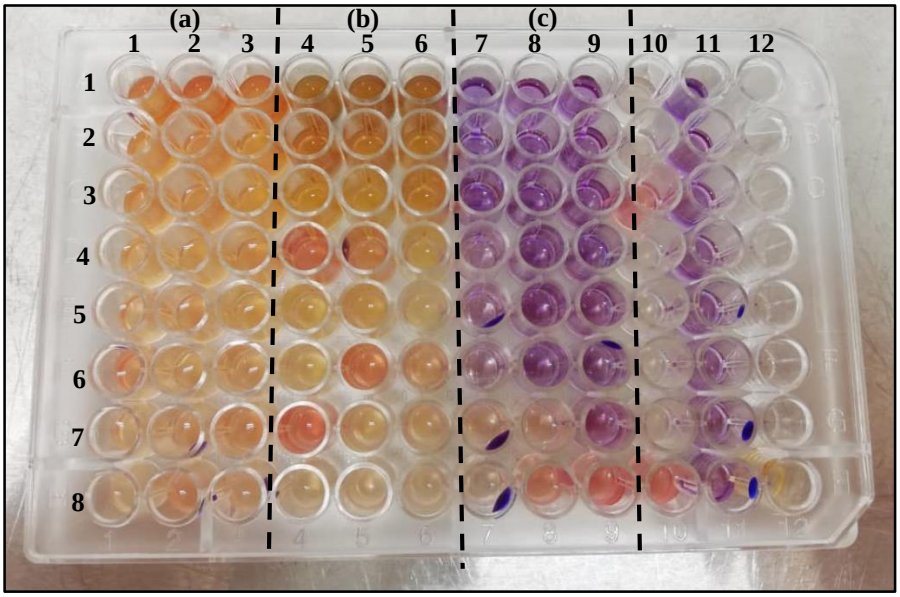

Figure 4: Antifungal properties of the spray dried *A. malaccensis* (a) aqueous leaf extract powder, (b) solvent leaf extract powder and (c) miconazole against *R. oryzae.* The spray dried *A. malaccensis* aqueous leaf extract powder was located in the 1st to 3rd columns whereas the solvent leaf extract powder was located in the 4th to 6th columns. Miconazole was located in the 7th to 9th columns. From the 1st to 8th rows, the concentrations of the spray dried *A. malaccensis* leaf extract powder are 128, 64, 32, 16, 8, 4, 2 and 1mg/ml whereas the concentrations of miconazole are 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 and 0.0078mg/ml. The 10th column was served as the growth control while the 11th column was served as the sterility control. Purple color indicates inhibition of bacterial growth while pink color or colorless indicates growth of bacteria.

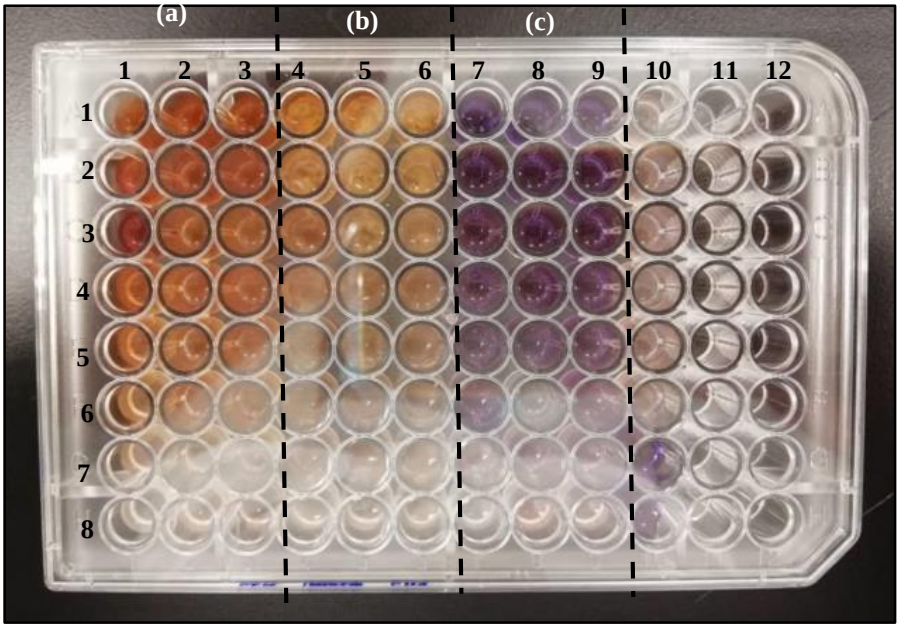

Figure 5: Antifungal properties of the spray dried *A. malaccensis* (a) solvent leaf extract powder, (b) aqueous leaf extract powder and (c) miconazole against yeast. The spray dried *A. malaccensis* solvent leaf extract powder was located in the 1st to 3rd columns whereas the aqueous leaf extract powder was located in the 4th to 6th columns. Miconazole was located in the 7th to 9th columns. From the 1st to 8th rows, the concentrations of the spray dried *A. malaccensis* leaf extract powder are 128, 64, 32, 16, 8, 4, 2 and 1mg/ml whereas the concentrations of miconazole are 1, 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.0156 and 0.0078mg/ml. For the 10th column, the 1st to 6th rows served as the growth control while the 7th and 8th rows served as the sterility control. Purple color indicates inhibition of bacterial growth while pink color or colorless indicates growth of bacteria

3.3 Shelf-life Evaluation by Standard Plate Count (SPC) Method

The shelf-life of a food product is the period during which the food product retains its acceptable qualities from the microbiological point of view [18]. The shelf-life of food product begins from the time the food product is prepared [19] and ends at the time the microbial count of food product is beyond the acceptable level [20]. As shown in Table 3, the spray dried *A. malaccensis* leaf extract powder contains less than 1.0×10^6CFU/g when examined for the presence of aerobic bacteria, fungi, *Staphylococci* and *E. coli*. According to NSW Food Authority (2009) [19], the total plate count of the spray dried *A. malaccensis* leaf extract powder falls within the acceptable range which is $<10^6$ to $<10^7$CFU/g. Spoilage will occur when the yeast and mold count reaches 10^6 to 10^7CFU/g [21]. Since the yeast and mold count of the spray dried *A. malaccensis* leaf extract powder was lower than 10^6CFU/g, food spoilage might not happen. Moreover, it was found that *Staphylococci* and *E. coli* were absent in the spray dried *A. malaccensis* leaf extract powder. The microbiological quality of the spray dried *A. malaccensis* leaf extract powder is classified as satisfactory because the levels of *Staphylococci* and *E. coli* were below 10^3CFU/g and 10^2CFU/g respectively. The microbial load of the spray dried *A. malaccensis* leaf extract powder is expected to cause no food safety concern [19].

This shelf-life study of the spray dried *A. malaccensis* leaf extract powder was carried out after 31 weeks of storage. In the present study, the total plate count, yeast and mold count, number of *Staphylococci* and *E. coli* were lower than the maximum acceptable values at the end of this shelf-life study. Therefore, the shelf-life of the spray dried solvent and aqueous leaf extract powder of *A. malaccensis* were determined at 33 weeks or more. The spray dried *A. malaccensis* leaf extract powder is considered a shelf stable food product due to its long shelf-life. Considering that the shelf-life study of the leaves of *Aquilaria* is lacking, the research on the other leaves are referred. This finding is in line

with that of the previous study which stated that the dried keffir lime leaf powder maintained their microbial safety during the ambient storage of 48 weeks [20].

The shelf-life of a food product can be affected by several factors such as storage condition, packaging and processing [19]. The spray dried *A. malaccensis* leaf extract powder was packaged in the polyethylene bag and light-impermeable container. Furthermore, it was stored under dark and room temperature. Spray drying was implemented in this study to destroy the microorganisms due to the application of heat treatment in which the spray dryer inlet temperature ranges from 120 to 170ºC. Moreover, the high temperature in the spray dryer drying chamber is expected to evaporate the water from the leaf extract of *A. malaccensis* [22]. The moisture content and water activity of the spray dried powder were reported as low as 2 to 5% and 0.2 to 0.6 respectively [23]. The deterioration of *A. malaccensis* leaf extract caused by the microorganism can be prevented at the water activity lower than 0.6 [24]. Therefore, the utilization of spray drying preserves the leaf extract of *A. malaccensis* from the microbial degradation and extends the shelf-life of the leaf extract.

Table 3: Total plate count, yeast and mold count, *Staphylococci* count and *E. coli* count in the spray dried *A. malaccensis* leaf extract powder

Sample	Week of shelf-life study	Total plate count on PCA plate	Yeast and mold count on PDA plate	*Staphylococci* count on MSA plate	*E. coli* count on EMB agar plate
		CFU/g			
Solvent leaf extract	32nd 33rd	$<1.0\times10^6$	$<1.0\times10^6$	$<1.0\times10^6$	$<1.0\times10^6$
Aqueous leaf extract	32nd 33rd	$<1.0\times10^6$	$<1.0\times10^6$	$<1.0\times10^6$	$<1.0\times10^6$

4. Conclusion

The data of this study indicated that the spray dried leaf extract powder of *A. malaccensis* has very low antibacterial effects compared with standard antibiotic, tetracycline against *E. coli*, *S. aureus* and *B. cereus*. This is proved by the appreciable higher MIC values of the spray dried *A. malaccensis* leaf extract powder (64 to >128mg/ml) than the MIC values of tetracycline (<1 to 4µg/ml). In addition, the spray dried *A. malaccensis* leaf extract powder had no significant antifungal activities against *A. niger*, *R. oryzae* and yeast. However, the results of antibacterial studies revealed that the spray dried ethanol leaf extract powder of *A. malaccensis* had slightly higher antimicrobial activities than the aqueous leaf extract powder. This is because ethanol is more effective in extracting the antimicrobial agents from the leaves of *A. malaccensis* than water. Long shelf-life (≥33 weeks) was recorded in the spray dried leaf extract powder of *A. malaccensis*. The reason for this could be the application of heat treatment that inactivates the microorganisms and reduces the water activitiy of *A. malaccensis* leaf extract during spray drying. In the future study, the antimicrobial activities of the spray dried *A. malaccensis* leaf extract powder can be determined using other microbial species in addition to *E. coli*, *S. aureus*, *B. cereus*, *A. niger*, *R. oryzae* and yeast. The shelf-life analysis of the spray dried *A. malaccensis* leaf extract powder can be carried out under accelerated condition.

Acknowledgement

The authors would like to thank the Synergy One Holding and Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for its support.

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