Antibacterial, Antifungal, and Antioxidant Activities and Polyphenol Content of the Edible Seeds of *Archidendron bubalinum*

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Keywords: Antibacterial, Antifungal, Antioxidant, *Archidendron bubalinum*, Total Phenolic Content

Abstract: This study assessed the kernel extracts of *Archidendron bubalinum* (family *Leguminosae*) seeds for antibacterial, antifungal, and antioxidant activities as well as polyphenol content. The kernels were macerated using hexane, ethyl acetate, ethanol, and water sequentially and produced four extracts for analysis. All the extracts showed bactericidal effects against five of the six species of bacteria tested with minimum bactericidal concentrations of 0.63-2.50 mg/mL, and fungicidal effects against five species of fungi with minimum fungicidal concentrations of 0.31-2.50 mg/mL. All the extracts also exerted antioxidant activities. The hexane extract exhibited the lowest oxygen radical absorbance capacity (ORAC) and ferric-reducing antioxidant power (FRAP) values of 81.63 mmol Trolox equivalent/g of extract and 25.93 mmol Fe^{2+} equivalent/g of extract, respectively. In contrast, ethyl acetate extract exhibited the highest ORAC (300.13 mmol Trolox equivalent/g of extract) and FRAP (341.36 mmol Fe^{2+} equivalent/g of extract) values. Polyphenols were detected in all extracts with the total phenolic content (TPC) of 3.22-20.19 mg gallic acid equivalent/g of extract and the total flavonoid content (TFC) of 0.97-4.42 mg quercetin equivalent/g of extract. Association analysis between ORAC/FRAP and TPC/TFC revealed significant strong positive correlations (r = 0.937-0.995; all p<0.001). In conclusion, the edible seeds of *A. bubalinum* possessed antimicrobial and antioxidant properties and could be promoted as a healthy food.

SCIENCE AND TECHNOLOGY PUBLICATIONS

1 INTRODUCTION

Communicable diseases caused by microorganisms such as bacteria, fungi, viruses, and parasites are one of the major contributors to human morbidity and mortality worldwide. In 2019, more than 26.1 billion incident cases of communicable diseases were reported, of which ~93% of the cases involved respiratory and enteric systems, contributing to 4.2 million death globally (GBD 2019 Diseases and Injuries Collaborators, 2020). Bacteria have been recognized as a main etiological agent for human respiratory and enteric infections. They can be broadly classified into two groups; Gram-positive bacteria have a thick peptidoglycan layer anchored with teichoic acids in the cell wall without an outer membrane while Gram-negative bacteria possess a cytoplasmic membrane and a lipopolysaccharides-containing outer membrane with a thin peptidoglycan layer in between the membranes (Fisher and Mobashery, 2020). Beta-lactams, tetracyclines, oxazolidinones, macrolides, aminoglycosides, sulfonamides, and quinolones are among the antibacterial agents developed for therapeutic use. However, the misuse and overuse of these agents as well as the natural evolutionary ability of microorganisms have driven the increased prevalence of antibacterial resistance (Chokshi et al., 2019; Christaki et al., 2020). Resistance to antibacterial therapy has resulted in patients with more serious illnesses, prolonged hospitalization, and treatment failure, as well as increased costs and resource utilization of the healthcare system (Ahmad and Khan, 2019). It has been estimated that the total economic cost of antibacterial resistance caused by major human pathogens in the United States of America was $2.9 billion (Shrestha et al., 2018).

Fungi produce a wide spectrum of infections in humans, ranging from superficial (e.g., skin, hair, nail, and keratitis), mucosal (e.g., oral and

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Teh, K., Wong, Y. and Sit, N.
Antibacterial, Antifungal, and Antioxidant Activities and Polyphenol Content of the Edible Seeds of Archidendron bubalinum.
DOI: 10.5220/0011595600003430
In *Proceedings of the 8th International Conference on Agricultural and Biological Sciences (ABS 2022)*, pages 40-49
ISBN: 978-989-758-607-1; ISSN: 2795-5893
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vulvovaginal candidiasis), allergic (e.g., rhinosinusitis and severe asthma with fungal sensitization), chronic severe (e.g., chronic pulmonary aspergillosis), to invasive infections (e.g., invasive candidiasis, cryptococcosis, and aspergillosis). People with weakened or compromised immunity such as cancer patients or transplant recipients are more susceptible to fungal infections (Bongomin et al., 2017). Each year, over 300 million people are afflicted with an episode of fungal infection, resulting in more than 1.6 million deaths globally (Urban et al., 2021; GAFFI, 2022). Thus, effective antifungal treatment is important to reduce the mortality rate. Yet, only polyenes, flucytosine, azoles, and echinocandins are available as antifungal agents for clinical use. The emergence of fungal resistance, drugs' adverse effects, and undesirable drug-drug interactions hinder the outcomes of antifungal treatment (Revie et al., 2018).

In the human body, when the antioxidative protection system is unable to counteract reactive species production, oxidative stress ensues (Pisoschi et al., 2021). Hydroxyl radical, superoxide anion, singlet oxygen, and peroxynitrite are examples of these reactive species produced as the byproducts of aerobic cellular metabolism, or as the response of the body to exposure to cigarette smoke, ultraviolet radiation, pesticides, and ozone. Meanwhile, superoxide dismutase, glutathione peroxidase, metal-trapping proteins, and vitamins A, C, and E constitute important elements of the antioxidative protection system (Rosado-Pérez et al., 2018). The excess reactive species modify the structures and modulate the functions of proteins, lipids, and nucleic acids. Damages to these biomolecules lead to non-communicable diseases such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders (Liguori et al., 2018). Due to the concern of side effects from synthetic antioxidants (Ito et al., 1985), natural resources such as plants could offer an alternative source for the growing demand for exogenous antioxidants.

Edible fruits have long been recognized as an important source of nutrients such as proteins and minerals for human health as well as ingredients for medicines. Different classes of phytochemicals or secondary metabolites such as alkaloids, terpenoids, or polyphenols are present in fruits, which endorse them with various biological properties, including antimicrobial and antioxidant activities (Forni et al., 2019). Fruit with a high amount of procyanidins such as cranberries has been shown to reduce bacterial or fungal infections of the urinary tract (Vostalova et al., 2015; Sundararajan et al., 2018). Regular intakes of flavonoids through diets have been reported to significantly decrease the risks of cardiovascular diseases (Wang et al., 2014). In another study, a high intake of antioxidant-rich fruits and vegetables in healthy adults lowers the oxidized low-density lipoprotein level, which is considered a biomarker of cardiovascular diseases (Bacchetti et al., 2019).

Being one of the tropical countries with mega-biodiversity, Malaysia has at least 355 species of trees and 165 species of non-trees bearing edible fruits or seeds (Milow et al., 2014). More than two-thirds of them are wild or non-exclusively planted. These fruits and seeds present a vast opportunity for exploration as healthy foods.

Archidendron bubalinum (Jack) I.C.Nielsen (family *Leguminosae*; synonym *Pithecellobium bubalinum*) is a wild evergreen tree native to Peninsular Malaysia, Thailand, and Indonesia. It has many vernacular names, for example, 'kerdas' or "keredas" in Malaysia, 'neing-nok' in Thailand, and 'kabau' or 'julang-jaling' in Indonesia (Lim, 2012). The fruit has a woody pod and contains six to eight seeds. The seeds are creamy-white, ellipsoid to ovoid, and laterally flattened, which turn to a shining reddish-brown color when mature. The seeds have a strong pungent smell like jering and stink bean but the odor disappears on cooking (Lim, 2012). The kernels can be eaten raw after removing the hard husk. The husk of the seeds has been extensively researched for various biological activities, including antibacterial, antidiabetic, anti-uric acid, and antioxidant (Hanafi et al., 2018; Irawan et al., 2018; Styani et al., 2018; Riasari et al., 2019). However, the edible kernel has relatively been less studied. Recently, Riasari and colleagues (2021) reported that the kernel extract exerts antihyperglycemic activity in diabetic rats. The Temuan tribe of aborigines in Peninsular Malaysia use the seeds for treating diabetes (Ong and Azliza, 2015).

This study evaluated the antibacterial, antifungal, and antioxidant activities of the kernel extracts of *Archidendron bubalinum* seeds. The polyphenol content of the kernel extracts was also quantified. The results of this study provide scientific information on the exploration of this wild edible seed as a healthy food.

2 METHODOLOGIES

2.1 Sample Processing

Approximately 600 grams of fresh *Archidendron bubalinum* seeds were bought from a wet market in

Bachok, Kelantan, Malaysia on 29th May 2017. A few seeds were kept as a specimen voucher with the code number UTAR/FSC/17/001.

The husks were removed from the seeds after cleaning with water. The kernels were then cut into small pieces with a knife and extracted sequentially using hexane, ethyl acetate, ethanol, and distilled water. The maceration was performed at 110 rpm and ambient temperature for 24 h and the process was repeated two times. The hexane, ethyl acetate, and ethanol filtrates were concentrated to dryness at 40°C using a rotary evaporator whereas the water extract was freeze-dried. All dry extracts were kept at –20°C pending bioassay. The percentage of yield for each extract was calculated based on fresh weight.

2.2 Antibacterial Activity

The antibacterial activity of kernel extracts was evaluated using a colorimetric broth microdilution method (Chan et al., 2018) with slight modifications. The Gram-positive bacteria used were *Bacillus cereus* ATCC11778 and *Staphylococcus aureus* ATCC6538. While the Gram-negative bacteria tested were *Acinetobacter baumannii* ATCC19606, *Escherichia coli* ATCC35218, *Klebsiella pneumoniae* ATCC13883, and *Pseudomonas aeruginosa* ATCC27853. All the bacterial species were obtained from the American Type Culture Collection (ATCC) and cultured on Mueller-Hinton agar (MHA). An extract stock solution was prepared by dissolving 20 mg of extract in 2 mL of a methanol-water mixture (2:1, v/v). Subsequent dilutions were performed using Mueller-Hinton broth (MHB) in a 96-well plate to produce eight concentrations (0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, and 2.50 mg/mL) for the assay. The bacterial inoculum was prepared at 1×10^8 cfu/mL by adjusting the absorbance value at 625 nm to 0.08-0.10 (equivalent to 0.5 McFarland turbidity standard) and diluted to 1×10^6 cfu/mL with MHB. After that, 50 µL of prepared bacterial inoculum was introduced to the wells containing 50 µL of extract and incubated at 37°C for 24 h. Positive (chloramphenicol), negative (bacterial inoculum), blank (MHB), and technical (kernel extracts) controls were incorporated into each plate. The microbial growth indicator *p*-iodonitrotetrazolium chloride (0.4 mg/mL; 20 µL) was pipetted to each well after incubation. After 30 min of incubation at 37°C, the wells were observed for the formation of a pink or purple precipitate, and the extract's minimum inhibitory concentration (MIC) was ascertained. For the determination of the extract's minimum bactericidal concentration (MBC), 20 µL of the well's content that showed inhibitory activity was spread on MHA, followed by incubation at 37°C for 24 h. MBC is the lowest concentration of kernel extract that kills ≥99.9% of bacterial inoculum. The experiment was conducted in triplicate.

2.3 Antifungal Activity

The antifungal activity of kernel extracts was assessed using a colorimetric broth microdilution method (Chan et al., 2018) with slight modifications. Five species of fungi, comprising three yeasts (*Candida albicans* ATCC90028, *Candida krusei* ATCC6258, and *Candida parapsilosis* ATCC22019) and two filamentous fungi (*Aspergillus fumigatus* ATCC204305 and *Trichophyton rubrum* ATCC28188), were tested. All the fungal species were obtained from the ATCC. The three yeasts were cultured on Sabouraud dextrose agar (SDA). The *A. fumigatus* and *T. rubrum* were maintained on potato dextrose agar (PDA) and oatmeal agar (OA), respectively. A serial dilution of the extract stock solution (10 mg/mL) was performed using Roswell Park Memorial Institute (RPMI)-1640 medium to produce a concentration range of 0.02-2.50 mg/mL for evaluation. For the fungal inoculum preparation, the absorbances for *Candida* spp. and *A. fumigatus* were adjusted to 0.12-0.15 and 0.09-0.11, respectively, at 530 nm. The inoculums were then diluted to 1.5×10^3 cfu/mL with RPMI-1640 medium. While for *T. rubrum*, the cell number was enumerated using a hemocytometer and adjusted to 2×10^3 cfu/mL (CLSI, 2008a; 2008b). The diluted fungal inoculum (50 µL) was then pipetted into the extract solution and incubated at 35°C and 48 h for *Candida* spp., 35°C and 72 h for *A. fumigatus*, and 30°C and 96 h for *T. rubrum*. Two antibiotics, griseofulvin for *T. rubrum* and amphotericin B for others, were used as positive controls. Blank (medium), negative (fungal inoculum), and technical (kernel extracts) controls were incorporated into each plate. The *p*-iodonitrotetrazolium chloride (20 µL) was pipetted to each well one day before completion of the incubation period and the MIC of the extract was determined after incubation. After that, the spread plate method using SDA/PDA was used to determine the minimum fungicidal concentration (MFC) of the active extracts.

2.4 Antioxidant Activity

2.4.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging assay was performed using the method of Pavithra and Vadivukkarasi (2015) with slight modifications. The kernel extract (4 mg/mL) and vitamin C (0.4 mg/mL), which served as a positive control, were diluted two-fold serially with the methanol-water mixture in a 96-well plate to produce final concentration ranges of 1000-8 μg/mL and 100-0.8 μg/mL, respectively. One hundred μL of 0.2 mM DPPH radical solution was pipetted to 100 μL of kernel extract/vitamin C solution, and the plate was maintained in the dark at ambient temperature for 30 min. DPPH radical solution and extract solution without the addition of DPPH were used as blank and sample blank, respectively. The absorbance value was recorded at 517 nm. The DPPH radical scavenging activity was expressed in percentage and plotted against the concentration of kernel extract. The half-maximum inhibitory concentration (IC_{50}) of the extract was then determined graphically.

2.4.2 Oxygen Radical Absorbance Capacity (ORAC) Assay

The peroxyl radical scavenging activity of the kernel extracts was evaluated using the ORAC assay (Heng et al., 2020). Six concentrations (3.13, 6.25, 12.5, 25, 50, and 100 μg/mL) were prepared for each extract. Fifty μL of extract and one hundred fifty μL of 0.08 μM fluorescein were pipetted into a black 96-well plate. Sodium phosphate buffer (75 mM, pH 7.0) was used as a blank while Trolox solutions at 0.78, 1.56, 3.13, 6.25, and 12.5 μM were used to generate a calibration curve. Following the addition of 2,2-azobis (2-methylpropionamidine) dihydrochloride, the fluorescence intensity of the fluorescein at 485 nm (λ_{ex}) and 520 nm (λ_{em}) was monitored at 37°C every 1.5 min for 1 h. The area under the fluorescence intensity versus time curve (AUC) was then calculated. The ORAC value was interpolated from the plot of the net AUC value versus the concentration of Trolox. The equation and regression coefficient for the plot were $y = 1.7577x + 1.998$ and 0.9932, respectively. The ORAC value was expressed as mmol Trolox equivalent/g of extract. The assay was conducted in three independent experiments.

2.4.3 Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted using the method of Benzie and Strain (1996) with slight modifications. The standard curve was constructed using 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mM of ferrous sulfate. Vitamin C at 1 mg/mL served as a positive control whereas the methanol-water mixture was deployed as a negative control. A 270 μL of FRAP reagent was pipetted to 30 μL of extract/standard/controls and incubated at 37°C. After 4 min of incubation, the absorbance was read at 593 nm. The FRAP reagent contained 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3$ solution, and 10 mM 2,4,6-tri(2-pyridyl)-*s*-triazine solution at a ratio of 10:1:1 (v/v/v). The equation and regression coefficient for the standard curve were $y = 2.7655x + 0.0953$ and 0.9990, respectively. The FRAP value for each extract was interpolated from the standard curve and expressed as mmol Fe^{2+} equivalent/g of extract.

2.5 Polyphenol Content

2.5.1 Total Phenolic Content (TPC) Assay

The Folin-Ciocalteu method was used to determine the TPC of each kernel extract in triplicate (Herald et al., 2012). Eight gallic acid solutions (2.5, 5, 10, 20, 40, 80, 160, and 320 μg/mL) were used to generate a calibration curve. A 10 mg/mL of extract (25 μL), deionized water (75 μL), and 50% Folin-Ciocalteu reagent (25 μL) were mixed in a 96-well plate and shaken at 70 rpm for 6 min. For the sample blank, the 50% Folin-Ciocalteu reagent was replaced with deionized water. The methanol-water mixture was deployed as blank. Following the addition of 100 μL of 700 mM sodium carbonate, the plate was maintained in the dark at ambient temperature for 90 min, prior to the absorbance measurement at 765 nm. The actual absorbance value of sample was obtained after subtracting its absorbance value from the absorbance value of the sample blank. The equation and regression coefficient for the gallic acid calibration curve were $y = 0.0065x – 0.0031$ and 0.9977, respectively. The TPC of each kernel extract was expressed as mg gallic acid equivalent/g of extract.

2.5.2 Total Flavonoid Content (TFC) Assay

The aluminium chloride method was used to determine the TFC of each kernel extract in triplicate (Herald et al., 2012). A 725 mM of sodium nitrite (10 μL), 10 mg/mL of extract (25 μL), deionized water

(100 µL), and 750 mM of aluminium chloride (15 µL) were mixed in a 96-well plate and shaken at 70 rpm for 6 min. Quercetin (7.8, 15.6, 31.3, 62.5, 125, and 250 µg/mL) was used for constructing a calibration curve whereas the methanol-water mixture was used as a blank. The aluminium chloride in the mixture was substituted with deionized water for the sample blank. Each well was then added with 50 µL of 1 M sodium hydroxide, followed by 50 µL of deionized water. The plate was maintained in the dark at ambient temperature for 60 min before the absorbance was read at 420 nm. The equation and regression coefficient for the quercetin calibration curve were $y = 0.0014x + 0.0068$ and 0.9979, respectively. The TFC of each extract was expressed as mg quercetin equivalent/g of extract.

2.6 Data Analysis

The results obtained from the ORAC, FRAP, TPC, and TFC assays were examined for statistical significance using one-way analysis of variance (ANOVA). Subsequently, the significant difference between the kernel extracts within an assay was determined using Duncan's multiple range test. Correlation analysis between ORAC or FRAP and TPC or TFC was examined using the Pearson correlation test (Chan, 2003). The significance level was set at $p<0.05$. All statistical analyses were performed using the IBM SPSS Statistics for Windows Version 23.0 software.

3 RESULTS

3.1 Yield of Extraction

Four extracts were obtained from the extraction of the kernel of *A. bubalinum* seeds. The percentages of the yield of hexane, ethyl acetate, ethanol, and water extracts were 0.01%, 0.05%, 5.33%, and 3.58% (w/w), respectively. The total yield of extraction was 8.97% (w/w).

3.2 Antibacterial Activity

The bacteriostatic and bactericidal effects of an extract are designated by its MIC and MBC values, respectively. All the kernel extracts showed a bacteriostatic effect against the six species of bacteria. The MIC values ranged from 0.31 to 2.50 mg/mL (Table 1). However, the extracts required higher concentrations to exert their bactericidal effects. Moreover, none of the extracts exerted the bactericidal effect against *E. coli* (Table 1), suggesting an extract concentration higher than 2.50 mg/mL might be needed to kill this species. All the bacterial species were susceptible to chloramphenicol (positive control) with MIC values of 4 µg/mL against the Gram-positive bacteria and 2-64 µg/mL against the Gram-negative bacteria.

3.3 Antifungal Activity

All the kernel extracts possessed antifungal activity against the three species of yeasts and two species of filamentous fungi (Table 2). Their MIC values were the same as the MFC values, indicating the extracts possessed direct fungicidal effects on the fungi evaluated. The fungicidal effects were slightly stronger on the yeasts (MFC: 0.31-1.25 mg/mL) than that of the filamentous fungi (MFC: 0.63-2.50 mg/mL). All the fungi were susceptible to the positive controls, amphotericin B and griseofulvin, with MIC values of 1-2 and 0.50 µg/mL, respectively.

Table 1: Antibacterial activities of the kernel extracts of *Archidendron bubalinum* seeds.

Extract	Gram-positive bacteria		Gram-negative bacteria			
	Bacillus cereus ATCC11778	*Staphylococcus aureus* ATCC6538	*Acinetobacter baumannii* ATCC19606	*Escherichia coli* ATCC35218	*Klebsiella pneumoniae* ATCC13883	*Pseudomonas aeruginosa* ATCC27853
Minimum inhibitory concentration (mg/mL)						
Hexane	1.25	1.25	0.31	0.63	0.63	0.63
Ethyl acetate	1.25	1.25	0.31	0.63	1.25	0.63
Ethanol	2.50	1.25	0.31	1.25	1.25	0.63
Water	2.50	2.50	0.31	1.25	1.25	0.63
Minimum bactericidal concentration (mg/mL)						
Hexane	1.25	2.50	2.50	>2.50	0.63	2.50
Ethyl acetate	1.25	2.50	2.50	>2.50	1.25	2.50
Ethanol	2.50	2.50	2.50	>2.50	1.25	2.50
Water	2.50	2.50	2.50	>2.50	2.50	2.50

Notes: The concentrations are shown in mean values of triplicate.

3.4 Antioxidant Activity

Two radical scavenging activity assays (DPPH and ORAC) and an ion reducing activity assay (FRAP) were deployed to assess the antioxidant activities of the kernel extracts. Out of the four extracts, only ethyl acetate extract was able to reduce >50% of DPPH radicals when the concentration exceeded 0.5 mg/mL (Figure 1), and the IC_{50} value for this extract was 0.41 ± 0.04 mg/mL, much higher than the positive control, vitamin C (IC_{50} = 2.51 ± 0.12 µg/mL). Besides that, the highest ORAC value was exhibited by the ethyl acetate extract (300.13 ± 13.15 mmol Trolox equivalent/g of extract), followed by the ethanol extract. Meanwhile, the ORAC values for the hexane and water extracts were the lowest and similar ($p>0.05$). As shown in the FRAP assay (Table 3), the kernel extracts of *A. bubalinum* seeds also possessed the ability to reduce the ion Fe^{3+} to Fe^{2+}. The ethyl acetate extract showed the highest FRAP value (341.36 ± 20.23 mmol Fe^{2+} equivalent/g of extract), followed by the ethanol, water, and hexane extracts.

3.5 Polyphenol Content

All four kernel extracts of *A. bubalinum* seeds contained polyphenols. The TPC of the extracts ranged from the lowest 3.22 ± 0.35 mg gallic acid equivalent/g of extract for the hexane extract to the highest 20.19 ± 0.23 mg gallic acid equivalent/g of extract for the ethyl acetate extract (Table 3). While for TFC, the ethyl acetate extract also recorded the highest amount with a value of 4.42 ± 0.08 mg quercetin equivalent/g of extract. Although the ethanol extract had the lowest TFC, it was not significantly different ($p>0.05$) from that of the hexane and water extracts (Table 3).

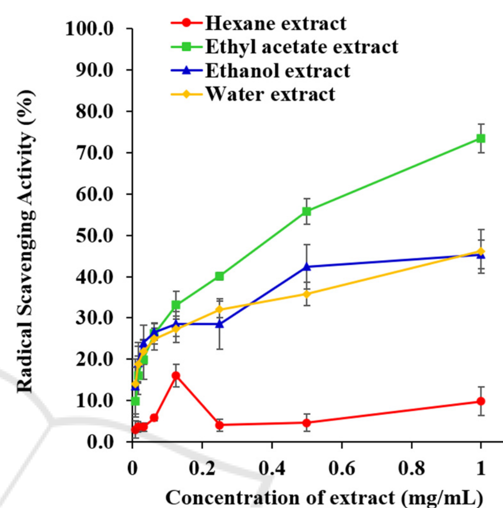

Figure 1: DPPH radical scavenging activity of the kernel extracts of *Archidendron bubalinum* seeds. Each value is shown in mean ± standard deviation of triplicate.

Table 2: Antifungal activities of the kernel extracts of *Archidendron bubalinum* seeds.

Extract	Yeasts			Filamentous Fungi	
	Candida albicans ATCC90028	*Candida krusei* ATCC6258	*Candida parapsilosis* ATCC22019	*Aspergillus fumigatus* ATCC204305	*Trichophyton rubrum* ATCC28188
Minimum inhibitory concentration (mg/mL)					
Hexane	0.63	0.63	0.31	0.63	1.25
Ethyl acetate	1.25	0.63	0.63	1.25	2.50
Ethanol	1.25	1.25	0.63	1.25	2.50
Water	1.25	1.25	0.63	2.50	2.50
Minimum fungicidal concentration (mg/mL)					
Hexane	0.63	0.63	0.31	0.63	1.25
Ethyl acetate	1.25	0.63	0.63	1.25	2.50
Ethanol	1.25	1.25	0.63	1.25	2.50
Water	1.25	1.25	0.63	2.50	2.50

Notes: The concentrations are shown in mean values of triplicate.

Table 3: Antioxidant activities and polyphenol content of the kernel extracts of Archidendron bubalinum seeds.

Extract	DPPH radical scavenging activity, half-maximum inhibitory concentration (mg/mL)	Oxygen radical absorbance capacity (mmol Trolox equivalent/g of extract)	Ferric-reducing antioxidant power (mmol Fe^{2+} equivalent/g of extract)	Total phenolic content (mg gallic acid equivalent/g of extract)	Total flavonoid content (mg quercetin equivalent/g of extract)
Hexane	Nil	81.63 ± 2.08^{a}	25.93 ± 4.70^{a}	3.22 ± 0.35^{a}	1.25 ± 0.23^{a}
Ethyl acetate	0.41 ± 0.04	300.13 ± 13.15^{c}	341.36 ± 20.23^{d}	20.19 ± 0.23^{d}	4.42 ± 0.08^{b}
Ethanol	Nil	118.90 ± 5.41^{b}	118.13 ± 10.18^{c}	8.73 ± 0.10^{c}	0.97 ± 0.11^{a}
Water	Nil	88.62 ± 0.26^{a}	75.22 ± 3.47^{b}	6.79 ± 0.11^{b}	1.16 ± 0.12^{a}

Notes: Each value is shown in mean ± standard deviation of triplicate. Values with different alphabetical superscripts denote significant differences ($p<0.05$) among the extracts by one-way ANOVA test.

3.6 Correlations between Antioxidant Activities and Polyphenol Content

The correlation analyses unveiled significant strong positive correlations between ORAC value and TPC ($r = 0.979$; $p<0.001$) or TFC ($r = 0.971$; $p<0.001$) (Figure 2). Similarly, strong positive correlations were noted between FRAP value and TPC ($r = 0.995$; $p<0.001$) or TFC ($r = 0.937$; $p<0.001$).

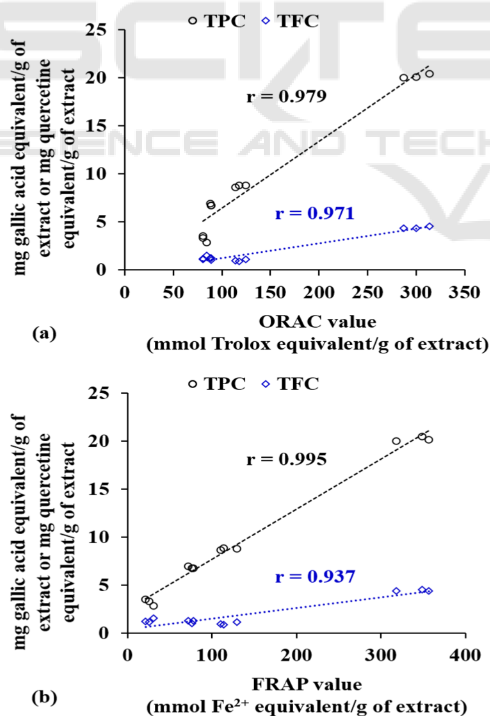

Figure 2: Association analysis between total phenolic content (TPC) or total flavonoid content (TFC) and (a) oxygen radical absorbance capacity (ORAC) and (b) ferric-reducing antioxidant power (FRAP) of the kernel extracts of *Archidendron bubalinum* seeds using the Pearson correlation test.

4 DISCUSSIONS

The results of this study indicated that the edible seeds of *A. bubalinum* possessed antibacterial, antifungal, and antioxidant activities. The ability of all the kernel extracts to exert antibacterial and antifungal activities implied that different classes of phytochemicals were present in the kernels and contributed to the activities. During the sequential solvent extraction, phytochemicals in the kernels are segregated according to the polarity of the solvent used. Non-polar solvents such as hexane and chloroform commonly remove alkaloids, fatty acids, sterols, terpenoids, etc. from plants. Phytochemicals such as anthraquinones, flavones, polyphenols, saponins, tannins, and terpenoids are obtained by ethyl acetate and ethanol, which are more polar solvents. The most polar solvent water could yield secondary metabolites like polypeptides and lectins (Heng et al., 2020).

The polyphenols quantified in all the kernel extracts may account, at least in part, for the antibacterial and antifungal activities. Polyphenols constitute one of the biggest groups of phytochemicals with approximately 8000 different structures. They are classified into flavonoids, stilbenes, phenolic acids, coumarins, anthraquinones, tannins, and xanthones (Forni et al., 2019). Polyphenols from plants have been reported to have inhibitory or killing effects against bacteria and fungi (Kumar et al., 2021; Manso et al., 2022). Polyphenols exert antimicrobial activities via various mechanisms, such as damaging fungal cell wall or bacterial lipopolysaccharide layer, formation of pores in cell

membrane leading to leakage of cytoplasmic content, inhibition of metabolic enzymes, ergosterol biosynthesis, or efflux pumps, repression of genes, disruption of ionic imbalance, induction of cell death, and inhibition of biofilm formation (Seleem et al., 2017; Simonetti et al., 2020; Kumar et al., 2021). The kernel extracts exhibited a direct killing effect on the fungi evaluated, suggesting the antifungal components of *A. bubalinum* seeds may mainly disrupt the integrity of the cell wall and cause cytolysis of the fungal cells.

Riasari et al. (2019) reported the DPPH radical scavenging activity of the ethanol extracts of the seeds of *A. bubalinum* from Lampung and South Sumatra (Indonesia) with half-maximum inhibitory concentrations of 163 and 446 µg/mL, respectively. In contrast, the ethanol kernel extract of *A. bubalinum* seeds used in this study exerted weak DPPH radical scavenging activity (<50% inhibition at 1 mg/mL). The differences could be attributed to agro-geographical reasons and/or extraction techniques used. Ghasemzadeh et al. (2018) studied the stink beans collected from different regions of Peninsular Malaysia and found that the sample collected from Perak had the highest DPPH radical scavenging and FRAP activities than the ones collected from Negeri Sembilan and Johor. Another study found that 18 metabolites from black bean and 11 metabolites from soybean were different significantly when their metabolite profiles were compared using two different extraction techniques (Maria John et al., 2018).

All four kernel extracts of *A. bubalinum* seeds exhibited antioxidant activities via the ORAC and FRAP assays. These antioxidant activities were likely contributed by polyphenols including flavonoids in the kernels due to the significant results from the correlation analyses. Significant strong positive correlations ($r > 0.80$) between ORAC values and polyphenol content (TPC and TFC) have also been documented for adlay seeds (Xu et al., 2017), seeds and fruit of *Phoenix dactylifera* (Djaoudene et al., 2021), and fruit of *Eleiodoxa conferta* (Go et al., 2021). Similarly, strong positive correlations between FRAP and TPC as well as between FRAP and TFC have been reported for stink beans (Ghasemzadeh et al., 2018), kiwifruit (Wang et al., 2018), and peels and seeds of pomegranates (Sabraoui et al., 2020). The existence of at least one phenyl ring in the molecular structure allows polyphenols to have free radical scavenging, singlet oxygen quenching, and metal ion reducing or chelating properties (Ullah et al., 2020). As the ethyl acetate extract was the most active among all kernel extracts, further analysis using gas/liquid chromatography-mass spectrometry could shed some light on the identity of antioxidants in the extract.

5 CONCLUSIONS

This study suggests that the edible seeds of *A. bubalinum* could be promoted as healthy food due to its health-promoting activities. All four kernel extracts (hexane, ethyl acetate, ethanol, and water) from the seeds had bactericidal activities against Gram-positive and Gram-negative bacteria and fungicidal activities against yeasts and filamentous fungi. The antimicrobial activities of the seeds are likely to be contributed by different classes of phytochemicals. All the kernel extracts also possessed antioxidant activities, as revealed by the ORAC and FRAP assays. Among the extracts, ethyl acetate extract showed the strongest antioxidant activity by having the highest ORAC and FRAP values. The antioxidant activities were attributable mainly to the polyphenols present in the kernels, due to the significant strong positive correlations between antioxidant activities and polyphenol content. Further work is essential to identify the bioactive components and elucidate the mechanisms of action. More research is needed if *A. bubalinum* is to be cultivated sustainably as a crop in Malaysia, as currently the fruit is mainly collected from forests for sales in wet markets.

ACKNOWLEDGEMENTS

The authors thank Dr. Sugumaran Manickam (Faculty of Science, University of Malaya, Malaysia) for the identification of plant species and Lee Vwen Tan for sourcing the plant material. The work is supported by the UTAR Research Publication Scheme (6251/S02).

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