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Antifungal and cytotoxic activities of extracts obtained from underutilised edible tropical fruits

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ABSTRACT

Objective: To evaluate antifungal and cytotoxic activities of four underutilised fruit species, *i.e. Artocarpus altilis* (breadfruit), *Cynometra cauliflora* (nam-nam), *Mangifera pajang* (*M. pajang*) (Bambangan) and *Physalis minima* (wild gooseberry). **Methods:** Extracts from the fresh flesh of *Artocarpus altilis* and *Cynometra cauliflora*, the flesh and kernel of *M. pajang*, and the whole fruit of *Physalis minima* were obtained by sequential extraction using hexane, chloroform, ethyl acetate, ethanol, methanol and distilled water. Each extract was assessed against six species of human fungal pathogens using a colourimetric broth microdilution method. The cytotoxicity was evaluated using African monkey kidney epithelial (Vero) cells. **Results:** All 30 extracts showed inhibitory activity against *Cryptococcus neoformans*. However, none of the extracts were active against *Aspergillus fumigatus*. The ethanol, methanol and water extracts from the kernel of *M. pajang* fruit showed the strongest activity against three species of *Candida* and *Trichophyton interdigitale*, with a minimum inhibitory concentration range of 0.001 – 0.630 mg/mL. The corresponding mean 50% cytotoxic concentrations for these three extracts were 358.7, 158.4 and 261.3 μg/mL, respectively against Vero cells. In contrast, the flesh of *M. pajang* fruit (hexane, chloroform and ethyl acetate extracts) showed statistically significant ($P<0.001$; ANOVA) strong toxicity against the cells, with 30.6, 13.5 and 22.2 μg/mL of mean values of 50% cytotoxic concentrations, respectively. **Conclusions:** The results suggest that the bioactivity of the kernel of *M. pajang* fruit is more selective towards fungi and thus is a potential source of new antifungal agents.

1. Introduction

Countries in the tropical region such as Malaysia have a rich botanical diversity. According to Milow *et al.*[1], 355 species of trees and 165 species of non-trees bearing edible fruits or seeds are found in Malaysia. Fruits are frequently used as a food source and as ingredients in traditional medicine. However, some of these fruits have been less utilised commercially. Underutilised tropical fruits are those fruits with less popularity, highly seasonal and have

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underexploited potential for contributing to food security, nutritional or medicinal health, income generation, and environmental services[2].

Artocarpus altilis (*A. altilis*) (Parkinson ex F.A.Zorn) Fosberg (family Moraceae) is a tropical fruit tree native to South/Southeast Asia and Australasia[3]. It is known as 'Sukun' locally or breadfruit in English. In Malaysia, ripe fruits are peeled, sliced and fried in syrup or palm sugar until they are crisp and brown before consumption. Several reviews on *A. altilis* have highlighted that extracts or phytochemicals isolated from the fruit possess antibacterial, anticancer, antihypergylcaemic, anti-inflammatory and antioxidant properties[3,4].

Cynometra cauliflora (*C. cauliflora*) L., commonly known as "Nam-nam", is a member of the bean family Leguminosae. This fruit tree is indigenous to eastern Peninsular Malaysia and is widely distributed in Southeast Asia, India and Sri Langka[5]. The fruit has a savoury taste and may be eaten raw or cooked with sugar to make a sweet compote, or fried in batter. The fruit has been used in folk medicine to treat loss of appetite[6]. Pharmacological studies on the fruit extracts reveal the presence of antioxidative[7], anti-lipase[8] and antiproliferative effects against human promyelocytic leukaemia cells[9].

Mangifera pajang (*M. pajang*) Kosterm. of the family Anacardiaceae is a mango species popularly known as "Bambangan" or "Pajang". It originated from Borneo Island, which comprises Sarawak, Sabah (Malaysia), Brunei and Kalimantan (Indonesia)[10]. The flesh is aromatic, juicy, sweet-sour in taste, fibrous in texture and can be eaten fresh or make into juice. The grated kernel, together with the cut flesh is used by the Kadazan-Dusun community in Sabah to make a pickle called 'nonsom bambangan'[10]. Besides nutritional values, the fruit extracts or compounds isolated from the fruit possess antioxidative[11-13], anticancer[12,14], antibacterial activities[12], and cytoprotective effects against liver cells[15].

Physalis minima (*P. minima*) L. is a herbaceous annual plant belonging to the family Solanaceae with many common names such as wild gooseberry, wild cape gooseberry, sunberry, ground cherry, *etc*[16]. It is believed to have originated from tropical America and is now distributed pantropically. The fruit is juicy and has been used in traditional medicine as a diuretic, a purgative, to relieve stomach pain, and to treat spleen disorders and constipation[17]. However, most of the studies on pharmacological activities and phytochemicals for *P. minima* have focused either on the leaves or the entire plant[16,18]. The fruit has thus far been only studied for antibacterial activity[19,20].

Despite the reported pharmacological properties of these four underutilised fruits, very little information is available regarding their antifungal potential against human pathogens. The escalation of invasive fungal infections in immunocompromised or immunosuppressed patients, the development of drug-resistant fungal strains, the significant side effects associated with synthetic antifungal drugs during chemotherapy and the relative high cost of treatment[21,22] have justified the search for newer, safer and cheaper antifungal agents among natural resources. Since plants produce a diverse variety of secondary metabolites such as alkaloids, anthraquinones, flavonoids, tannins, triterpenes, *etc*. as part of their defence mechanisms against microbial infections[23], they are promising sources of new antifungal compounds. This study was therefore conducted to evaluate the antifungal activity of the extracts obtained from the fruits of *A. altilis*, *C. cauliflora*, *M. pajang* and *P. minima* against a panel of human fungal pathogens. The fruit extracts were also assessed for cytotoxicity using African monkey kidney epithelial (Vero) cell line.

2. Materials and methods

2.1. Fruit species

The mature fruit (before fully ripened) of *A. altilis* was obtained from an orchard in Sitiawan, Perak, Malaysia while the ripe fruit (brownish yellow colour) of *C. cauliflora* was plucked from a garden in Tanah Merah, Kelantan, Malaysia. The fully ripened fruit of *M. pajang* with yellow flesh was bought from a wet market in Miri, Sarawak, Malaysia while the ripe fruit (yellow colour) of *P. minima* was purchased from a farm in Cameron Highlands, Pahang, Malaysia. The species identity of the fruits was confirmed by Professor Hean Chooi Ong, an ethnobotanist affiliated with Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia.

2.2. Preparation of fruit extracts

Only the edible parts of the fruits were used for extraction. The fresh flesh of *A. altilis* (1 564.32 g) and *C. cauliflora* (657.90 g), the whole fresh fruit of *P. minima* (360.73 g), and the fresh flesh (1 933.18 g) and kernel (275.93 g) of *M. pajang* were subjected to sequential solvent extraction using hexane, chloroform, ethyl acetate, ethanol, methanol and distilled water. All the organic solvents used were of analytical grade. The maceration process was carried out at room temperature and with agitation using an orbital shaker (Yihder Technology, New Taipei City, Taiwan) at 120 rpm for three cycles (one day/cycle). The collected extract solvents were removed using a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland) to obtain the dry extracts while the water extract was lyophilised using a freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). All the dry extracts were kept at -20 ℃ prior to bioassay.

2.3. Antifungal assay

Four species of yeasts [*Candida albicans* (*C. albicans*) ATCC® 90028™, *Candida parapsilosis* (*C. parapsilosis*) ATCC® 22019™, *Candida krusei* (*C. krusei*) ATCC® 6258™, *Cryptococcus neoformans* (*C. neoformans*) ATCC® 90112™] and two species of filamentous fungi [*Aspergillus fumigatus* (*A. fumigatus*) ATCC® 204305™, *Trichophyton interdigitale* (*T. interdigitale*) ATCC® 9533™] were tested in the study. All the species were obtained from American Type Culture Collection. The yeasts were grown on Sabouraud dextrose agar (SDA) (Merck, Darmstadt, Germany) while the filamentous fungi were maintained on potato dextrose agar (PDA) (Merck, Darmstadt, Germany).

A colourimetric broth microdilution method deploying *p*-iodonitrotetrazolium chloride as the growth indicator was used for the antifungal assay with modifications[24]. The stock solution for each fruit extract was prepared at 10 mg/mL in a methanol-water mixture (2:1, v/v), and filter-sterilised using 0.45 μm syringe filters. The fruit stock solution was then diluted two-fold serially with RPMI-1640 medium (Biowest, Nuaille, France) in 96-well, U-shaped microplates (Sigma-Aldrich, St. Louis, Missouri, USA) to obtain eight final concentration levels, *i.e.* 0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25 and 2.50 mg/mL. Dilutions beyond 0.02 mg/mL were performed whenever necessary. Medium, growth (fungus only), negative (extract only) and positive (antibiotic griseofulvin/amphotericin B) controls were included in each microplate during the assay. The final volume for each well was 100 μL. The inoculum preparations and the incubation conditions for yeasts and filamentous fungi were carried out according to the Clinical and Laboratory Standards Institute guidelines M27-A3 and M38-A2, respectively[25,26]. After the designated incubation periods, 20 μL of *p*-iodonitrotetrazolium chloride (Sigma-Aldrich, St. Louis, Missouri, USA) at 0.4 mg/mL was added to each well to determine the minimum inhibitory concentration (MIC) value. Subsequently, 20 μL of the content from those wells which did not show any growth was inoculated onto SDA or PDA plates using a spread plate method. The lowest concentration of an extract corresponds to no fungal colonies observed on the plates is taken as the minimum fungicidal concentration (MFC) of the extract. The assay was conducted in triplicate.

2.4. Cytotoxicity assay

The African monkey kidney epithelial (Vero) cell line (ATCC® CCL-81™) was used for cytotoxicity study. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, Missouri, USA) supplemented with 5% of foetal bovine serum (FBS) (Biowest USA, Riverside, Missouri, USA), 1% of penicillin solution (10 000 U/mL), 1% of streptomycin solution (10 mg/mL) (EMD Millipore, Darmstadt, Germany) and 3.7 g/L of sodium bicarbonate (Merck, Darmstadt, Germany). The cells were maintained at 37 ℃ in a humidified incubator (Memmert GmbH, Schwabach, Germany) with 5% carbon dioxide.

For the cytotoxicity assay, the fruit stock solution was prepared at 256 mg/mL in a dimethyl sulfoxide-ethanol mixture (60:40, v/v) and filter-sterilised using 0.45 μm syringe filters[27]. The stock solution was then diluted two-fold serially in DMEM (supplemented with 1% FBS) to obtain eight different final concentrations, *i.e.*, 5, 10, 20, 40, 80, 160, 320 and 640 μg/mL. The dimethyl sulfoxide-ethanol mixture in the microplate was maintained at a final concentration of less than 0.25% (v/v) to avoid any toxicity to the Vero cells[28]. The cells (4×10^4 cells/well) were seeded in 96-well, flat-bottom microplates (TPP Techno Plastic Products AG, Trasadingen, Switzerland) and incubated at 37 ℃ and 5% CO_2 for 24 h. One hundred microliters of the prepared extract solution was then added to the cells. The treated cells were incubated at 37 ℃ and with 5% CO_2 for another 72 h. Medium and cell controls were included in each microplate. After incubation, the viability of Vero cells was measured by the Neutral Red uptake assay[29]. The absorbance for each well was read at 540 nm using a microplate reader (BMG Labtech GmbH, Ortenberg, Germany). The assay was conducted in three independent experiments with duplicate in each experiment.

2.5. Data analysis

The MIC and MFC values are expressed as the mean of three consistent replicates. Fungal susceptibility index (FSI), expressed in % value is calculated as 100 × number of extracts effective against each fungal strain ÷ number of total extracts. Fifty percent cytotoxic concentration (CC_{50}) of an extract was determined from the plot of percentage of cell viability versus extract concentration. The data were analysed with one-way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows software, Version 20.0 (IBM, Armonk, New York, USA). The significance level was set at $P<0.001$. A post hoc test, either using the Tukey's (equal variance assumed) or Dunnett's (equal variance not assumed) test was conducted to determine which concentration of extract produced a significant result.

3. Results

3.1. Antifungal assay

By considering one extract against one fungus as one bioassay, as shown in Table 1, 45.6% of the bioassays (82/180) showed fungistatic activity whereas 26.1% (47/180) of the bioassays displayed fungicidal

Table 1

MIC and MFC of underutilised tropical fruit extracts against human fungal pathogens.

Extracts		MIC[a]						MFC[a]					
		C. albicans	*C. parapsilosis*	*C. krusei*	*C. neoformans*	*A. fumigatus*	*T. interdigitale*	*C. albicans*	*C. parapsilosis*	*C. krusei*	*C. neoformans*	*A. fumigatus*	*T. interdigitale*
A. altilis	Hexane	1.25	1.25	1.25	0.31	NA	2.50	2.50	1.25	1.25	1.25	–	NA
	Chloroform	0.63	2.50	1.25	0.31	NA	0.63	0.63	NA	1.25	1.25	–	1.25
	Ethyl acetate	NA	1.25	0.16	0.31	NA	NA	–	NA	0.31	NA	–	–
	Ethanol	NA	NA	NA	0.31	NA	NA	–	–	–	NA	–	–
	Methanol	NA	NA	NA	0.31	NA	NA	–	–	–	NA	–	–
	Water	NA	NA	NA	0.63	NA	NA	–	–	–	NA	–	–
C. cauliflora	Hexane	NA	2.50	NA	0.63	NA	NA	–	NA	–	NA	–	–
	Chloroform	2.50	1.25	NA	1.25	NA	NA	NA	NA	–	2.50	–	–
	Ethyl acetate	1.25	1.25	NA	0.31	NA	1.25	NA	1.25	–	0.63	–	2.50
	Ethanol	1.25	NA	NA	0.31	NA	NA	NA	–	–	1.25	–	–
	Methanol	2.50	NA	NA	0.31	NA	NA	NA	–	–	2.50	–	–
	Water	1.25	NA	NA	0.31	NA	NA	NA	–	–	NA	–	–
M. pajang (flesh)	Hexane	NA	1.25	2.50	0.31	NA	NA	–	1.25	2.50	1.25	–	–
	Chloroform	1.25	1.25	2.50	0.31	NA	NA	NA	1.25	2.50	2.50	–	–
	Ethyl acetate	NA	0.63	NA	0.31	NA	NA	–	0.63	–	NA	–	–
	Ethanol	NA	NA	NA	0.16	NA	NA	–	–	–	NA	–	–
	Methanol	NA	NA	NA	0.31	NA	NA	–	–	–	NA	–	–
	Water	NA	NA	NA	0.31	NA	NA	–	–	–	NA	–	–
M. pajang (kernel)	Hexane	0.31	0.31	0.63	0.08	NA	0.63	NA	0.31	0.63	0.16	–	NA
	Chloroform	0.31	0.04	0.02	0.04	NA	0.31	NA	0.04	0.02	0.08	–	0.63
	Ethyl acetate	0.08	0.02	0.01	0.02	NA	0.08	2.50	0.02	0.01	0.63	–	0.08
	Ethanol	0.08	0.003	0.001	0.16	NA	0.08	NA	0.01	0.001	1.25	–	0.16
	Methanol	0.31	0.003	0.001	0.31	NA	0.08	NA	0.01	0.001	1.25	–	0.16
	Water	0.63	0.005	0.003	0.31	NA	0.16	NA	0.02	0.003	2.50	–	0.16
P. minima	Hexane	2.50	NA	NA	0.63	NA	NA	NA	–	–	2.50	–	–
	Chloroform	2.50	NA	NA	2.50	NA	NA	NA	–	–	NA	–	–
	Ethyl acetate	NA	NA	NA	0.63	NA	NA	–	–	–	NA	–	–
	Ethanol	1.25	NA	NA	0.63	NA	NA	NA	–	–	NA	–	–
	Methanol	NA	NA	NA	0.63	NA	NA	–	–	–	NA	–	–
	Water	NA	NA	NA	1.25	NA	NA	–	–	–	NA	–	–
Antibiotic[b]		0.5–1.0	0.5–1.0	1–2	0.13– 0.25	0.5–2.0	1–4	–	–	–	–	–	–

[a]The MIC and MFC values for extracts are presented as mean of three consistent replicates in mg/mL. [b]The antibiotic griseofulvin was used for *T. interdigitale* while amphotericin B was used for other fungal strains, and the MIC values are expressed in μg/mL. "NA" denotes no activity while "–" denotes not performed.

activity. The dimorphic yeast *C. neoformans* was the most susceptible fungus as its growth was inhibited by all 30 extracts evaluated in this study with a MIC range of 0.02 to 2.50 mg/mL. Hence, FSI for *C. neoformans* was 100%. Half of these extracts were also able to kill the yeast but at a higher MFC range, which was 0.08 to 2.50 mg/mL. In contrast, the filamentous fungus *A. fumigatus* was the most resistant fungus (FSI = 0%) as none of the extracts showed activity against it. The calculated FSI values for *C. albicans*, *C. parapsilosis*, *C. krusei* and *T. interdigitale* were 56.7%, 50.0%, 36.7% and 30.0%, respectively.

Comparing the flesh and kernel of *M. pajang* fruit, all extracts from the kernel part were active against all the fungi, except *A. fumigatus*, with lower MIC and MFC ranges of 0.001-0.630 and 0.001-2.500 mg/mL, respectively. Extracts from the flesh part were only active against the four species of yeasts, with MIC and MFC ranges of 0.16-2.50 and 0.63-2.50 mg/mL, respectively. Besides *A. fumigatus*, all extracts of *P. minima* did not exhibit any antifungal activity against *C. parapsilosis*, *C. krusei* and *T. interdigitale*. Using the classification of antimicrobial potency proposed by Saraiva *et al.*[30], only the six extracts from the kernel of *M. pajang* were considered to be highly active against the fungi (depending on the species) tested, in which the achievable MIC value was less than 0.1 mg/mL. The ethanol and methanol extracts from the kernel exhibited the lowest MIC or MFC in this study, with a value of 0.001 mg/mL against *C. krusei* (Table 1).

3.2. Cytotoxicity assay

The cytotoxicity of the fruit extracts was assessed on the Vero cells. This well-established cell line has been widely used for evaluating the effects of chemicals, toxins and other substances, including plant extracts on mammalian cells at the molecular level. The fruits of *C. cauliflora* and *P. minima* were considered non-toxic as their extracts did not exhibit any statistically significant toxicity ($P>0.001$) on the Vero cells, except at the highest concentration 640 μg/mL for the chloroform extract of *C. cauliflora* and the ethyl acetate extract of *P. minima* (Figure 1). For the *A. altilis* fruit, only the hexane and chloroform extracts at the concentration higher than 160 and 320 μg/mL, respectively showed statistically significant ($P<0.001$) toxicity to the cells. The

corresponding CC_{50} values (mean±SD) for these two extracts were (118.6 ± 2.6) and (253.4 ± 4.5) μg/mL, respectively.

The flesh and kernel parts of *M. pajang* fruit showed distinct differences in the toxicity towards the Vero cells. For the flesh part, extracts obtained using less polar solvents (hexane, chloroform and ethyl acetate) were toxic to the cells when their concentrations exceeded 10 μg/mL. The CC_{50} values (mean±SD) for these three extracts were (30.6 ± 1.1), (13.5 ± 0.4) and (22.2 ± 2.5) μg/mL, respectively. On the other hands, the toxicity for the kernel part was mainly derived from the three more polar extracts, including hexane, ethanol, methanol and water. The strength of toxicity for these extracts were much lower, as evident by their higher CC_{50} values (mean±SD) which were (481.6 ± 14.2), (358.7 ± 18.4), (158.4 ± 7.6) and (261.3 ± 8.9) μg/mL, respectively.

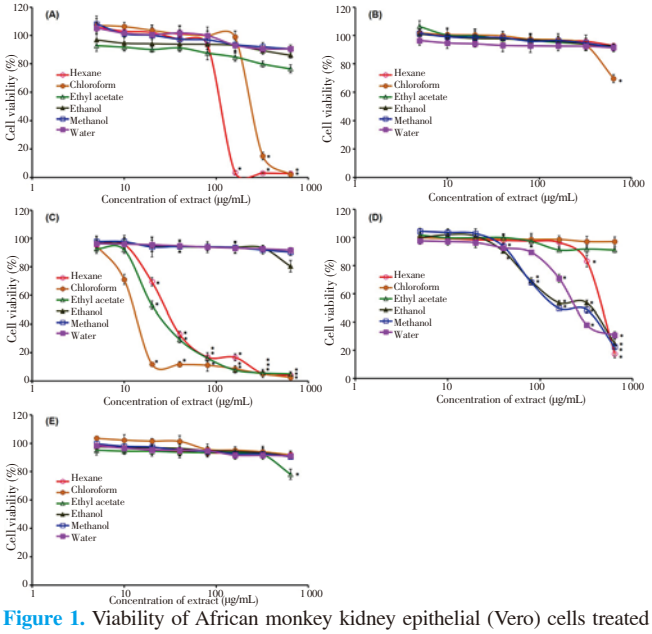

Figure 1. Viability of African monkey kidney epithelial (Vero) cells treated with different concentrations of underutilised tropical fruit extracts derived from (A) *A. altilis*, (B) *C. cauliflora*, (C) flesh of *M. pajang*, (D) kernel of *M. pajang* and (E) *P. minima*.
The percentage is expressed as mean ± standard deviation of three independent experiments. The asterisk mark denotes statistically significant difference (P<0.001) with one-way ANOVA test. The x-axis is in a log scale.

4. Discussion

To the best of our knowledge, this is the first report of *C. cauliflora* fruit extracts possessing antifungal activity against human pathogens. More studies need to be carried out to evaluate the phytochemicals and other antimicrobial activities such as antibacterial activity of this fruit. In a study using a normal mouse fibroblast (NIH/3T3) cell line as a model for cytotoxicity study, the cell viability assessed using MTT assay was reduced to 80.0% when the cells were treated with the methanol extract of the fruit at 30 μg/mL[9]. However, our study indicates that the methanol extract was not toxic (P>0.001) to the Vero cells (assessed using the Neutral Red uptake assay) even at the highest concentration of 640 μg/mL. The differences could be due to the types of cell line or the assay used to measure the cell viability[27]. Although the fruit samples for both studies were obtained from the east coast of Peninsular Malaysia, the fresh flesh was used in the current study while Tajudin *et al.*[9] studied the dried whole fruit. The difference in the material used could also be a variable that contributes to the differences in cytotoxicity.

This study reveals that the fruit of *P. minima* possesses limited antifungal property whereby the extracts were only active against two species of fungi, *i.e. C. albicans* and *C. neoformans*. In contrast, the fruit is known to have antibacterial property. Extracts obtained from the fresh or dried fruits using solvents of different polarity (hexane, chloroform, diethyl ether, ethyl acetate, acetone, ethanol, methanol, aqueous, or water) have shown different degrees of inhibitory activity against Gram-positive and Gram-negative bacteria[19,20]. The results of this study have provided us with a broader understanding on the antifungal potential of *A. altilis* fruit on different species of fungal pathogens. Our findings are in agreement with the study by Jalal *et al.*[31] who evaluated the antimicrobial activities of the hexane, dichloromethane and methanol extracts derived from the dry pulp (flesh) of *A. altilis* fruit. *C. albicans* was the only fungus studied and the MIC range reported was 0.5-4 mg/mL.

The flesh and kernel extracts of *M. pajang* fruit showed different antifungal activity and cytotoxicity against Vero cells, suggesting that the phytochemicals distribution between the two parts is highly different. The antifungal activity of the kernel extracts was more active and stronger against the fungi tested compared to that of the flesh extracts. Similar observations have been documented by other studies. Abu Bakar *et al.*[11] found that the 80% methanol extract of the kernel possesses significantly higher antioxidant activity than the flesh, as measured by DPPH radical scavenging and ferric reducing antioxidant power assays. The ethanol extract of the kernel, but not the flesh displays anticancer activity against colon, liver and ovarian cancer cell lines[14] and cytoprotective effect on human hepatocellular HepG2 cell line against oxidative damage[15]. However, Ahmad *et al.*[12] reported that none of the extracts (petroleum ether, chloroform, ethyl acetate and methanol) from the dried kernel powder showed antifungal activity against *C. albicans*, *Aspergillus ochraceaus* and *Saccharomyces cerevisiae*. Our study indicates that all six extracts from the fresh kernel were active against the three *Candida* spp., *C. neoformans* and *T. interdigitale* with MIC values ranging from 0.001 to 0.630 mg/mL. It is unclear whether these differences are due to the fungal species or the type of material (fresh vs dry) used in the study. Several aromatic esters (benzaldehyde, benzyl alcohol, methyl gallate), flavonoids (diosmin, hesperidin, naringin, rutin), phenolic acids (caffeic, chlorogenic,

p-coumaric, ferulic, gallic, synapic acids) and a sterol (β -sitosterol) have been isolated from the kernel[14,15]. Phenolic compounds such as flavonoids and phenolic acids from plants have been known to be active against human pathogens[32,33]. The phytochemicals present in the kernel that are responsible for the antifungal activity remain to be studied.

Despite the fact that the fresh flesh of *M. pajang* or the fruit juice has been consumed by the indigenous community regularly without any report of adverse effects, our study shows that the less polar extracts (hexane, chloroform and ethyl acetate) caused significant toxicity towards the Vero cells. Further studies using human cell lines are required to corroborate this observation. The toxicity caused by the flesh extracts of *M. pajang* fruit on the Vero cells was mainly due to phytochemicals with low polarity. On the other hands, the non-cytotoxic property for the more polar extracts supports the safe consumption of juice and the development of functional drink using *M. pajang* fruit[13]. As for the kernel part, phytochemicals with higher polarity in nature are responsible in reducing the viability of Vero cells. The kernel of *M. pajang* fruit is usually grated together with the cut flesh to make a pickle, which is then left to ferment for weeks before being eaten[10]. Toxicity has not been reported following the consumption of the pickle by the indigenous community. It is possible that the process of making the pickle might alter the toxicity of the phytochemicals present in the kernel and flesh.

Among the four types of underutilised tropical fruits evaluated in this study, the kernel extracts of *M. pajang* fruit are promising sources of new lead compounds with potent fungistatic or fungicidal activity against human pathogens and low or non-toxic to eukaryotic cells. Further studies are warranted to identify the active compounds and their mechanisms of action. It is also of interest to identify the chemical profile of each fruit extract. Continuous exploration and exploitation of underutilised tropical fruits is necessary to increase the economic value of the fruits, and to ensure optimal use of local resources.

Conflict of interest statement

We declare that there is no conflict of interest.

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