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Antimicrobial activity of *jambu mawar* [*Syzygium jambos* (L.) Alston] leaf extract against foodborne pathogens and spoilage microorganisms

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Abstract

The present work evaluated the antimicrobial potential of the ethanolic extract of *jambu mawar* [*Syzygium jambos* (L.) Alston] leaves against various foodborne pathogens and spoilage microorganisms *via* the disc diffusion assay (DDS) and the time-kill curve assay. These microorganisms included bacteria (*Klebsiella pneumoniae* ATCC13773, *Listeria monocytogenes* ATCC19112, *Proteus mirabilis* ATCC21100, *Pseudomonas aeruginosa* ATCC9027, *Staphylococcus aureus* ATCC29737, and *Vibrio parahaemolyticus* ATCC17802), yeasts (*Candida albicans* ATCC10231, *C. krusei* ATCC32196, *C. glabrata* ATCC2001, and *C. parapsilosis* ATCC22019), and moulds (*Aspergillus fumigatus* ATCC26430, *A. niger* ATCC9029, *Rhizopus oligosporus* ATCC22959, and *R. oryzae* ATCC22580). The inhibition zone of DDA ranged from 7.00 ± 0.23 to 10.25 ± 0.29 mm. The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal (MBC/MFC) of the ethanolic leaf extract were obtained at the concentrations of 0.01 to 2.50 and 0.01 to 5.00 mg/mL, respectively. The time-kill curve assay showed that except for *P. mirabilis*, other microorganisms were completely killed at MIC concentrations ranging from 0.5 to 4× MIC. In comparison, *P. mirabilis* showed a growth reduction of > 3 $\log_{10}$ CFU/mL for 4 h. Meanwhile, the conidial germination of *A. fumigatus* was fully inhibited at 0.5× MIC. Though not fully inhibited, the ethanolic leaf extract significantly reduced the conidial germination of *A. niger*, *R. oryzae*, and *R. oligosporus* to 7.0, 7.0, and 11.0%, respectively. Overall, the ethanolic leaf extract of *S. jambos* exhibited antimicrobial activity against foodborne pathogens and spoilage microorganisms.

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Introduction

Transporting food supplies worldwide poses a great challenge to food safety and security by creating appropriate conditions for the emergence, re-emergence, and proliferation of foodborne pathogens. Consequently, it is becoming more difficult to anticipate, detect, and effectively respond to foodborne threats (Kalyoussef and Feja, 2014). In Malaysia, unfortunately, the primary concern is on the food taste rather than the safety and hygiene of the foods. The drastic increase in food poisoning cases over the years reflects the indifference of Malaysians to food safety (New *et al.*, 2017).

Most foods and food products are perishable with a limited shelf life. Therefore, they need to be well protected from spoilage during their preparation, storage, and distribution. In particular, they are easily contaminated by various microorganisms such as bacteria and fungi, causing unacceptable responses that spoil their flavour, odour, colour, and textural properties. Above all, some of these microorganisms could potentially lead to severe foodborne diseases (Del Nobile *et al.*, 2012). Several preservation techniques such as thermal processing, acidification, salting, and drying have been developed in the food industry to inhibit the growth of spoilage and

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pathogenic microorganisms in foods (Davidson and Taylor, 2007).

Considerable efforts have also been directed at finding natural alternatives for inhibiting microbial growth in foods instead of chemical preservatives since majority of these are synthetic, with several of them have been found to be toxic, and cause potential health problems such as hypersensitivity, allergy, asthma, hyperactivity, neurological damage, and cancer (Anand and Sati, 2013). There are various naturally occurring antimicrobials that could be used in food preservatives. In general, they comprise a combination of compounds extracted from plants and animals or microorganisms with specific antimicrobial characteristics. They include essential oils, flavour compounds, bacteriocins, protamines, endolysins, lysozymes, lactoferrins, chitosans, and isothiocyanates. They are often used on fresh and processed fruits and vegetables (Galvez *et al*., 2010).

Plants are the most important natural sources of antimicrobial substances (Tajkarimi *et al*., 2010). They provide antimicrobials, antioxidants, flavours, and colour enhancers to enhance the shelf life and sensory acceptability of food products. Therefore, these compounds play indispensable roles in inhibiting the growth of foodborne pathogens, thus lowering the risks of illnesses (Rohani *et al*., 2011). Majority of plant extracts have the "generally recognised as safe" (GRAS) and "qualified presumption of safety" (QPS) statuses in the USA and EU, respectively (Burt, 2004). Plant extracts are widely permitted to be applied as preservatives in food products to ensure safety.

Syzygium jambos (L.) Alston (synonym: *Eugenia jambos* L.) is known for its antimicrobial, anti-inflammatory, and antipyretic properties (Sharma *et al*., 2013). It belongs to the family Myrtaceae, with several common local names such as *jamrosa, jainrosa*-tree, rose apple, and *jambu mawar* (Jahan, 2019). This species is a native widespread in Southeast Asia (Malaysia, Nepal, India, and Bangladesh), but now widely distributed in the tropics such as Africa (Benin, Democratic Republic of Congo, and Cameroon), Central America (Guatemala; Kuiate *et al*., 2007), Australia, and New Zealand. The present work thus evaluated the potential of the ethanolic extract of *S. jambos* leaves for antimicrobial activity against various bacteria, yeasts, and moulds.

Material and methods

Sample collection

Fresh green leaves of *S. jambos* were collected from Taman Pertanian, Universiti Putra Malaysia. They were washed and shade-dried for 3 d. The leaves were further dehydrated in an oven at 55°C for 3 h, and kept in sealed plastic bags at room temperature for further processing.

Extraction of S. jambos leaves

Leaves of *S. jambos* were extracted following the method of Rukayadi *et al.* (2008). Specifically, 100 g of dried leaves were ground using a dry blender (Panasonic MK-5087M, Osaka, Japan). The ground leaves were then soaked in four parts (v/v) of absolute ethanol (R and M Chemicals, Selangor, Malaysia) at a ratio of 1:4 for 24 h at 30°C in a shaking water bath (Saintifik Maju, Selangor, Malaysia). The soaked leaves were then vacuum-filtered through Whatman filter paper No. 2 (Whatman International Ltd., Middlesex, England) using an aspirator pump (EYELA A-1000S, Tokyo Rikakikai Co., Tokyo, Japan). The filtrate was concentrated using a rotary vacuum evaporator (Heidolph laborota 4000 efficient, Heidolph Instruments GmbH and Co. KG, Schwabach, Germany) at 50°C and 150 rpm for 20 min. The crude extract was stored at 4°C for further analysis.

Preparation of S. jambos leaf extract

Next, 100 mg of crude extract was dissolved in 1.00 mL of dimethylsulfoxide (DMSO) (R and M Chemicals, Selangor, Malaysia) to yield a 100 mg/mL (10%) ethanolic leaf extract. This mixture was further diluted in distilled water (1:10; v/v) to give a stock solution with a final concentration of 10 mg/mL (1.00%).

Bacterial and fungal cultures

The present work examined 14 strains of frequently reported foodborne pathogens and food spoilage microorganisms encompassing bacteria, yeasts, and moulds. They were *Klebsiella pneumoniae* ATCC13773, *Listeria monocytogenes* ATCC19112, *Proteus mirabilis* ATCC21100, *Pseudomonas aeruginosa* ATCC9027, *Staphylococcus aureus* ATCC29737, *Vibrio parahaemolyticus* ATCC17802, *Aspergillus fumigatus* ATCC26430, *Aspergillus niger* ATCC9029, *Rhizopus oligosporus* ATCC22959, *Rhizopus oryzae* ATCC22580, *Candida albicans* ATCC10231, *Candida krusei* ATCC32196, *Candida*

glabrata ATCC2001, and *Candida parapsilosis* ATCC22019, which were purchased from the American Type Culture Collection (ATCC; Maryland, United States). Bacterial cultures (*K. pneumoniae*, *L. monocytogenes*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, and *V. parahaemolyticus*) were maintained *via* sub-culturing on nutrient agar (NA; Difco, USA), yeasts (*C. albicans, C. krusei, C. glabrata,* and *C. parapsilosis*) on sabouraud dextrose agar (SDA; Difco, USA), and moulds (*A. fumigatus*, *A. niger*, *R. oligosporus,* and *R. oryzae* A) on potato dextrose agar (PDA; Difco, USA).

Disc diffusion assay (DDA)

The antimicrobial activity of ethanolic *S. jambos* leaf extract was evaluated using the DDA method prescribed by the Clinical and Laboratory Standards Institute (CLSI, 2017a; 2018a). Bacteria and yeasts were adjusted to the standard of 0.5 McFarland with concentrations ranging from 10^6 to 10^8 CFU/mL. Moulds were inoculated following the method of NCCLS M38-A (CLSI, 2002; 2017b). Briefly, moulds were grown on PDA at 35°C for 7 d, after which, approximately 1 to 2 mL of 0.85% sterile saline was dispensed to cover the grown fungal culture, and the colonies were gently probed with the tip of Pasteur pipette. The mixture containing conidial and hyphal fragments was collected and transferred to a sterile tube, and the heavy particles were allowed to settle for 5 to 10 min. The homogenous suspensions of the mixture at the top of the tube were collected and vortexed for 15 s. The optical density (OD) of conidial suspensions was assayed using a spectrophotometer at 530 nm. Since the transmittance of *Aspergillus* spp. ranged from 80.9 to 81.1%, while *Rhizopus* spp. ranged from 67.5 to 70%, thus, the conidial suspensions were diluted in 1:50 with sterile distilled water, yielding the inoculum dilutions to 2× density, *i.e.*, about (0.4 - 5.0) × 10^4 CFU/mL. The inoculums of each bacterial species, yeast species, and mould species were spread evenly using a sterile cotton swab on the dried surface of Mueller Hinton agar (MHA), SDA, and PDA, respectively.

Sterile Whatman filter paper discs with a diameter of 6 mm were fixed on the top of the culture, and 10 µL of 10 mg/mL (w/v) leaf extract was loaded on these paper discs. The positive control for yeasts and moulds was 0.1% Amphotericin B, while for bacteria it was 0.1% commercial chlorhexidine (CHX). By contrast, 10% DMSO served as the negative control for all species. These plates were incubated at different temperatures and durations for bacteria (37°C for 24 h), yeasts (35°C for 24 - 46 h), and moulds (30°C for 3 - 7 d). A clear zone around the filter discs would be indicative of inhibition of microbial growth, and the diameter of the zones was measured in mm.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC)

The MIC was evaluated using the two-fold standard broth microdilution method with an inoculum of approximately 10^6 - 10^8 CFU/mL (bacteria and yeasts) and 0.4 - 5 × 10^4 CFU/mL (moulds) on a disposable sterile 96-well, U-shaped microtiter plate. An aliquot of 100 µL inoculums was placed into each well of the microtiter plate from columns 12 to three that were two-folded with 100 µL of the extract. Wells of column 1 were each filled with 200 µL of plain broth as the negative control, while wells of column 2 were each filled with 100 µL of broth plus 100 µL of inoculum suspension as the positive control.

The microdilution was conducted at the extract concentrations ranging from 0.5 mg/mL in column 12 to 0.0009 mg/mL in column 3 for bacteria, and from 5.00 mg/mL in column 12 to 0.009 mg/mL in column 3 for yeasts and moulds. The microtiter plates were incubated at different durations and temperatures for bacteria (24 h at 37°C), yeasts (24 - 48 h at 35°C), and moulds (3 - 7 d at 30°C). The results of MIC were then sub-cultured on MHA, SDA, and PDA to determine the MBC/MFC. An aliquot of 10 µL from each well of the MIC suspension were dispensed onto the agar plates from columns 1 to 12. The plates were incubated at different durations and temperatures for bacteria (24 h at 37°C), yeasts. (24 - 48 h at 35°C), and moulds (3 - 7 d at 30°C) to evaluate the mean value of MIC and MBC/MFC.

Determination of time-kill curve

The time-kill curve assay was performed on bacteria and yeasts only following the CLSI (2018b) method based on the estimated MIC values in the microdilution with five extract concentrations (0, 0.5, 1.0, 2.0, and 4.0× MIC). The final concentration of each bacterial and yeast species was attained by diluting the extract with MHB/SDB containing approximately 10^6 - 10^8 CFU/mL of the inoculums. The mixture was assayed at different incubation intervals (0.0, 0.5, 1.0, 2.0, and 4.0 h). An aliquot of

100 µL of the mixture was serially diluted into 1% phosphate-buffered saline (PBS), and streaked evenly onto MHA/SDA. For bacteria, the streaked MHA/SDA plates were incubated at 37°C for 24 h, and yeasts at 35°C for 24 - 48 h. The total plate-count (TPC), expressed in $\log_{10}$ CFU/mL, was plotted against time.

Inhibition of conidial germination assay

The inhibition of conidial germination was assayed following the method of Rukayadi and Hwang (2007). The inoculum suspension of 5×10^4 CFU/mL was diluted with the PBD medium at a ratio of 1:10 ratio to give a final inoculum concentration of 5×10^3 CFU/mL. Meanwhile, the extract at different concentrations (0, 0.5, 1, 2, and 4× MIC) were also diluted with the PDB medium containing 5×10^3 CFU/mL in a ratio of 1:10 to yield an initial conidial inoculum of 4.5×10^3 CFU/mL. An aliquot of 1 mL of these cultures was each incubated at 35°C for 48 h for *A. niger, A. fumigatus, R. oligosporus,* and *R. oryzae*. The number of conidia was then determined by plating the cultures on PDA. The percentage of the conidial germination inhibition was calculated using Eq. 1 (Jin *et al*., 2004):

$$Germination\ inhibition\ \% = \frac{Average\ conidial\ germination\ \%\ of\ control - Average\ conidial\ germination\ \%\ of\ treatment}{Average\ conidial\ germination\ \%\ of\ control}\ x\ 100\% \quad \text{(Eq. 1)}$$

Statistical analysis

All experiments were carried out three times with three replications each ($n = 3 \times 3$). The one-way analysis of variance (ANOVA) was performed using the Minitab software (version 17.1). Statistical differences in antimicrobial activities and MIC were evaluated using Tukey's test. Results were interpreted as mean ± standard deviation (SD) of the replicates.

Results and discussion

Yield of extract

A 100 g of dried *S. jambos* leaves were extracted by a maceration method using ethanol as solvent in this experiment. The extraction yielded 9.16 g of semi-viscous crude in dark greenish colour with, thus giving 9.16% of total yield.

Disc diffusion assay (DDA)

Table 1 shows the DDA results of ethanolic *S. jambos* leaf extract against various foodborne pathogens and food spoilage microorganisms.

Table 1. Inhibition zone of ethanolic extract of *S. jambos* leaves against foodborne pathogens and food spoilage microorganisms.

Bacterial strains	Inhibition zone (mm)		
	***S. jambos* extract**	**CHX**	**DMSO**
K. pneumoniae ATCC13773	9.63 ± 0.17^{Bb}	14.00 ± 0.00^{Ae}	N.A.
L. monocytogenes ATCC 19112	10.25 ± 0.29^{Ba}	18.00 ± 0.92^{Ab}	N.A.
P. aeruginosa ATCC9027	9.38 ± 0.95^{Bb}	15.67 ± 0.53^{Ad}	N.A.
P. mirabilis ATCC 21100	9.88 ± 0.85^{Bb}	12.33 ± 0.53^{Af}	N.A.
S. aureus ATCC29737	9.50 ± 0.50^{Bb}	19.67 ± 0.53^{Aa}	N.A.
V. parahaemolyticus ATCC17802	9.25 ± 0.96^{Bb}	16.00 ± 0.92^{Ac}	N.A.
Fungal strains	***S. jambos* extract**	**Amp B**	**DMSO**
C. albicans ATCC10231	8.00 ± 0.00^{Bb}	16.67 ± 0.53^{Ab}	N.A.
C. glabrata ATCC2001	8.00 ± 0.00^{Bb}	14.50 ± 0.46^{Ac}	N.A.
C. krusei ATCC32196	7.00 ± 0.00^{Bc}	19.33 ± 0.53^{Aa}	N.A.
C. parapsilosis ATCC22019	7.00 ± 0.00^{Bc}	16.00 ± 0.00^{Ab}	N.A.
A. niger ATCC9029	8.00 ± 0.00^{Bb}	13.50 ± 0.46^{Ad}	N.A.
A. fumigatus ATCC26430	9.67 ± 0.33^{Ba}	13.33 ± 0.53^{Ad}	N.A.
R. oligosporus ATCC22959	7.00 ± 0.32^{Bc}	12.00 ± 0.00^{Ae}	N.A.
R. oryzae ATCC22580	8.67 ± 0.67^{Bb}	12.17 ± 0.27^{Ae}	N.A.

N.A.: no activity; diameter of inhibition zones in mm (including disc); positive control: CHX = 0.1% and Amp B = 0.1%; negative control: DMSO = 10%; results were expressed as means ± standard deviation (SD); $n = 3 \times 3$. Mean values ± standard deviation with different lowercase letters in the same column are significantly different ($p < 0.05$). Mean values ± standard deviation with different uppercase letters in the same row are significantly different ($p < 0.05$).

R. oligosporus showed the smallest inhibition zone (7.00 ± 0.32), while *L. monocytogenes* yielded the largest (0.25 ± 0.29 mm). In general, the larger the inhibition zone of a species, the higher the antimicrobial activity of the extract against that species. Overall, the DDA results showed that the ethanolic leaf extract of *S. jambos* possessed antimicrobial activities despite being at a lower intensity than the positive control, which was a commercial antimicrobial agent.

The outer membranes (cell envelope) of microorganisms serve as the first line of resistance against antimicrobial agents, especially Gram-negative bacteria with an additional layer of lipopolysaccharide (LPS) that prevents the hydrophilic antibacterial compounds from penetrating the cell. For an antimicrobial agent to work effectively, it must pass through the cell envelope, and present at an adequately high concentration at the target site, where it launches its antimicrobial action. Besides, microorganisms may possess another defence mechanism to help the disposal of antimicrobial compounds across the barrier via the efflux pump (Lambert, 2002).

In the present work, *S. aureus* showed an inhibition zone of 9.50 ± 0.50 mm in DDA. This finding was consistent with the result of Mohanty and Cock (2010) who found that the methanolic extract of *S. jambos* leaves was active against *S. aureus* (9.00 ± 0.00 mm), *Alcaligenes faecalis* (12.60 ± 0.50 mm), *Aeromonas hydrophilia* (9.70 ± 0.80 mm), and *Bacillus cereus* (10.20 ± 0.50 mm). However, contradictory to the finding of the present work, the leaf extract did not show any antimicrobial activity against *Citrobacter freundii, Escherichia coli, K. pneumoniae, P. mirabilis, P. fluorescens, Salmonella* Newport, *Serratia marcescens*, and *Shigella sonnei*. The causes for this discrepancy remained unknown. Ghareeb *et al.* (2017) fractionated the methanolic extract of *S. jambos* leaves, and isolated eight compounds namely quercetin-3-O-rutinoside, prenylbenzoic acid 4-β-D-glucoside, morolic acid 3-O-caffeate, 5,4'-dihydroxy, 7-methoxy, 6-methyl-flavone, 3,4,5-trihydroxybenzoic acid (gallic acid), quercetin, isoetin-7-O-β-D-glucopyranoside, and (4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4"-hydroxy-3", 5" dimethoxylbenzoate)]-glucopyranoside). They tested these compounds against four pathogenic microorganisms namely *S. aureus* (with inhibition zones 10.5, 16.0, 17.0, 9.0, 12.5, 15.0, 17.0, and 18.0 mm, respectively), methicillin-resistant *S. aureus* (MRSA) (with inhibition zones 11.5, 13.0, 18.0, 12.0, 10.5, 17.0, 15.0, and 19.0 mm, respectively), *P. aeruginosa* (with inhibition zones 17.5, 11.5, 18.0, 13.0, 11.5, 16.0, 19.0, and 15.0 mm, respectively), and *C. albicans* (with inhibition zones 14.5, 12.0, 19.0, 13.0, 12.5, 14.0, 9.0, and 18.0 mm, respectively).

Disc diffusion test may give inaccurate results, perhaps due to some restriction factors such as the ability of the extract particles to pass through the pore of the discs, and the inability of hydrophobic compounds to diffuse into the agar medium (Othman *et al.*, 2011). Besides, some active compounds of the extract might be trapped in the disc pores, thus failing to pass through the inoculated media, and could not express their activities (Gangoué-Piéboji *et al*., 2009).

Determination of MIC and MBC/MFC

MIC determines the lowest concentration of an antimicrobial agent in inhibiting the growth of a test microorganism in a broth microdilution plate; it is a reference method to evaluate the susceptibility of microorganisms towards antimicrobial agents. The test microorganism is examined for its capability to produce visible growth in microwells of broth containing serial microdilutions of the antimicrobial agents (Rodriguez-Tudela *et al*., 2003). In comparison, MBC/MFC is the least antimicrobial density required to kill the microorganisms, *i.e.*, microbicidal (Abedon, 2011). This evaluation can be performed by sub-culturing the broth dilutions that inhibit the growth of the microorganisms. Table 2 shows the MIC and MBC/MFC values of the ethanolic *S. jambos* leaf extract on the tested microorganisms. The MIC values ranged from 0.01 to 2.50 mg/mL, thus suggesting that the ethanolic *S. jambos* leaf extract possessed a broad-spectrum activity against all the selected microorganisms. Among these microorganisms, *K. pneumoniae* and *L. monocytogenes* were the most susceptible pathogens with a MIC value of 0.01 mg/mL each. By contrast, *R. oligosporus* was the most resistant, with a MIC value of 2.50 mg/mL. Meanwhile, the MBC/MFC values ranged from 0.01 to 5.00 mg/mL, with *K. pneumoniae* and *L. monocytogenes* giving the lower MBC values (x), and *R. oligosporus* the highest MFC value (y).

A study has been carried out previously showing that the ethanolic extract of *S. jambos* leaves

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of ethanolic extract of *S. jambos* leaves against foodborne pathogens and spoilage microorganisms.

Bacterial strains	MIC (mg/mL)	MBC (mg/mL)
K. pneumoniae ATCC13773	0.01[d]	0.01[c]
L. monocytogenes ATCC19112	0.01[d]	0.01[c]
P. aeruginosa ATCC9027	0.08[c]	0.16[b]
P. mirabilis ATCC21100	1.25[a]	1.25[a]
S. aureus ATCC29737	0.63[a]	1.25[a]
V. parahaemolyticus ATCC17802	0.63[a]	1.25[a]
Fungal strains	**MIC (mg/mL)**	**MFC (mg/mL)**
C. albicans ATCC10231	1.25[b]	1.25[c]
C. glabrata ATCC2001	1.25[b]	1.25[c]
C. krusei ATCC32196	1.25[b]	1.25[c]
C. parapsilosis ATCC22019	1.25[b]	1.25[c]
A. niger ATCC9029	1.25[b]	2.50[b]
A. fumigatus ATCC26430	1.25[b]	1.25[c]
R. oligosporus ATCC22959	2.50[a]	5.00[a]
R. oryzae ATCC22580	1.25[b]	1.25[c]

Mean values with different lowercase letters in the same column are significantly different ($p < 0.05$).

had inhibited the bacterial growth and demonstrated a noteworthy MIC value of 0.0312 mg/mL against *Propionibacterium acnes* (Sharma *et al*., 2013). Also, Wamba *et al*. (2018) showed that the methanolic *S. jambos* leaf extract possessed antibacterial activity against 26 strains of *S. aureus*, four strains of *K. pneumoniae*, and two strains of *P. aeruginosa*, with the MIC values ranging from 0.032 to 0.512 mg/mL. For Gram-negative bacteria, the lowest MIC value was 0.064 mg/mL against *K. pneumoniae* K24 strain. Meanwhile, the MBC values against all the 26 tested *S. aureus* strains and Gram-negative bacteria ranged from 0.128 to 1.024 mg/mL.

Mohanty and Cock (2010) reported a MIC value of 0.35 mg/mL against *S. aureus* in their methanolic *S. jambos* leaf extract. Unfortunately, to date, there are no antifungal data of *S. jambos* leaf extract from other studies for comparison. Results of MIC were only available for the methanolic (M) and aqueous (W) extract of *Buchanania obovata* fruit tested against *A. niger* (M = 1.28, W = NA mg/mL), *C. albicans* (M = 0.865, W = 0.146 mg/mL), and *Saccharomyces cerevisiae* (M = 0.655, W = 0.882 mg/mL). The results indicated that all the tested fungi were susceptible to the methanolic extract (Mazerand and Cock, 2019). In another study, the methanolic extract of *S. malaccese* leaves was reported to have antifungal activity against *C. albicans* and *C. tropicalis* with MIC value of 2 µg/L and MFC value of 8 µg/L (Savi *et al*., 2020).

One of the bioactive compounds found in of the leaf extract of *S. jambos* was phenolic compounds, which could be categorised into flavonoid polyphenolics (flavonoids), non-flavonoid polyphenolics (tannins), and phenolic acids (hydroxycinnamic acids and phenylpropanoids). In general, the leaf extract of *S. jambos* showed the highest concentration of phenolic compounds (Gavillán-Suárez *et al*., 2015). Therefore, the antimicrobial activity found in the present and previous studies might be related to phenolic compounds identified in the *S. jambos* leaf extracts (Tohma *et al*., 2016; Ghimire *et al*., 2017).

Time-kill curve assay

Figures 1 and 2 show the time-kill curves of the ethanolic *S. jambos* leaf extract against various microorganisms (bacteria and yeasts). The number of microorganisms for the samples incubated without exposing to the leaf extract of *S. jambos* from 0 to 4 h (0× MIC) increased parallel with time. The results of 0× MIC were similar to the samples exposed to 0.5× MIC of the leaf extract, but with a slight log reduction in *L. monocytogenes*, *K. pneumoniae*, *P. aeruginosa*,

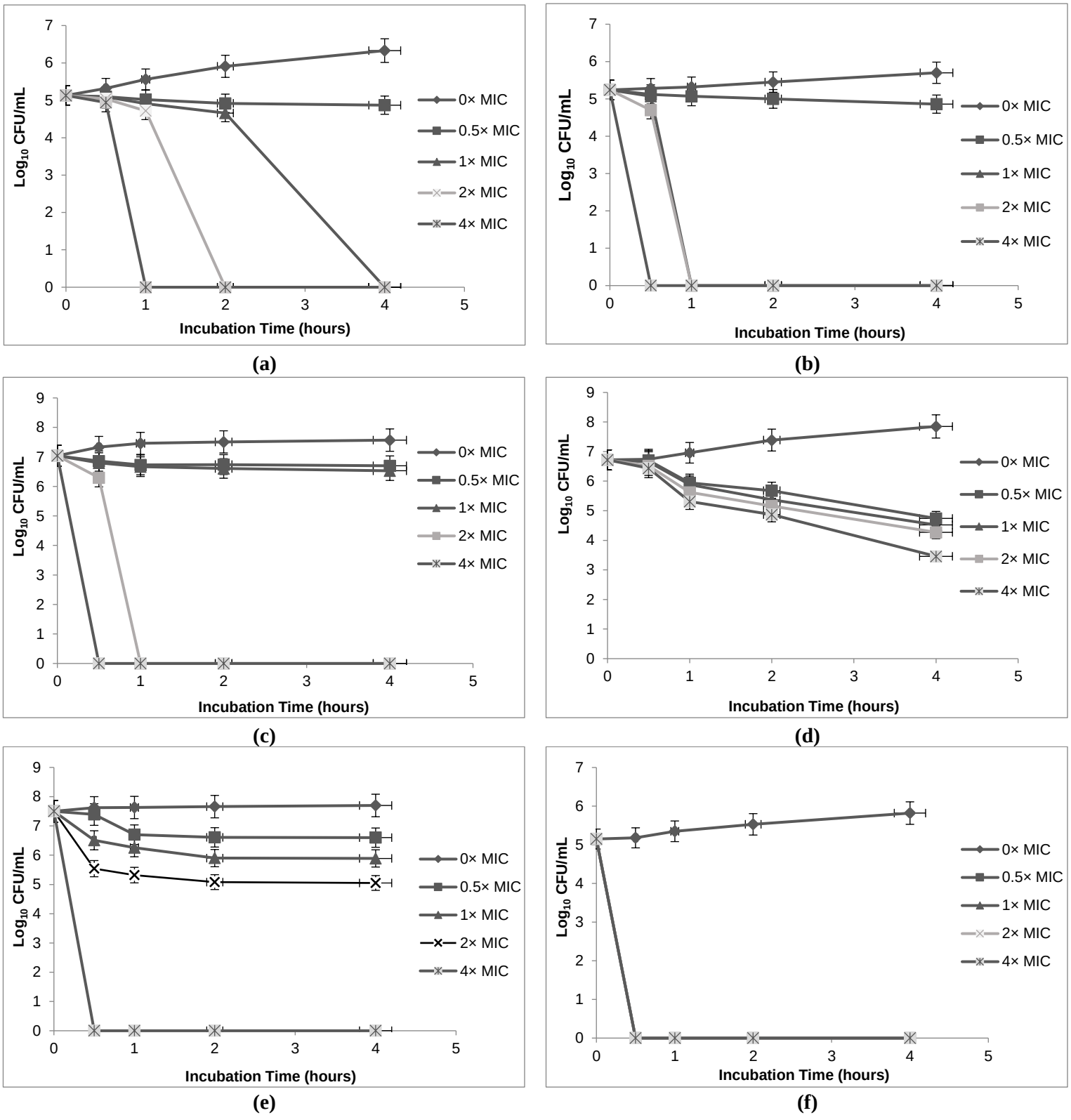

Figure 1. Time-kill curve plots of ethanolic extract of *S. jambos* leaves at concentrations of 0, 0.5, 1, 2, and 4× MIC at 0 to 4 h incubation time; **(a)** *K. pneumonia*, **(b)** *L. monocytogenes*, **(c)** *P. aeruginosa*, **(d)** *P. mirabilis*, **(e)** *S. aureus*, and **(f)** *V. parahaemolyticus*.

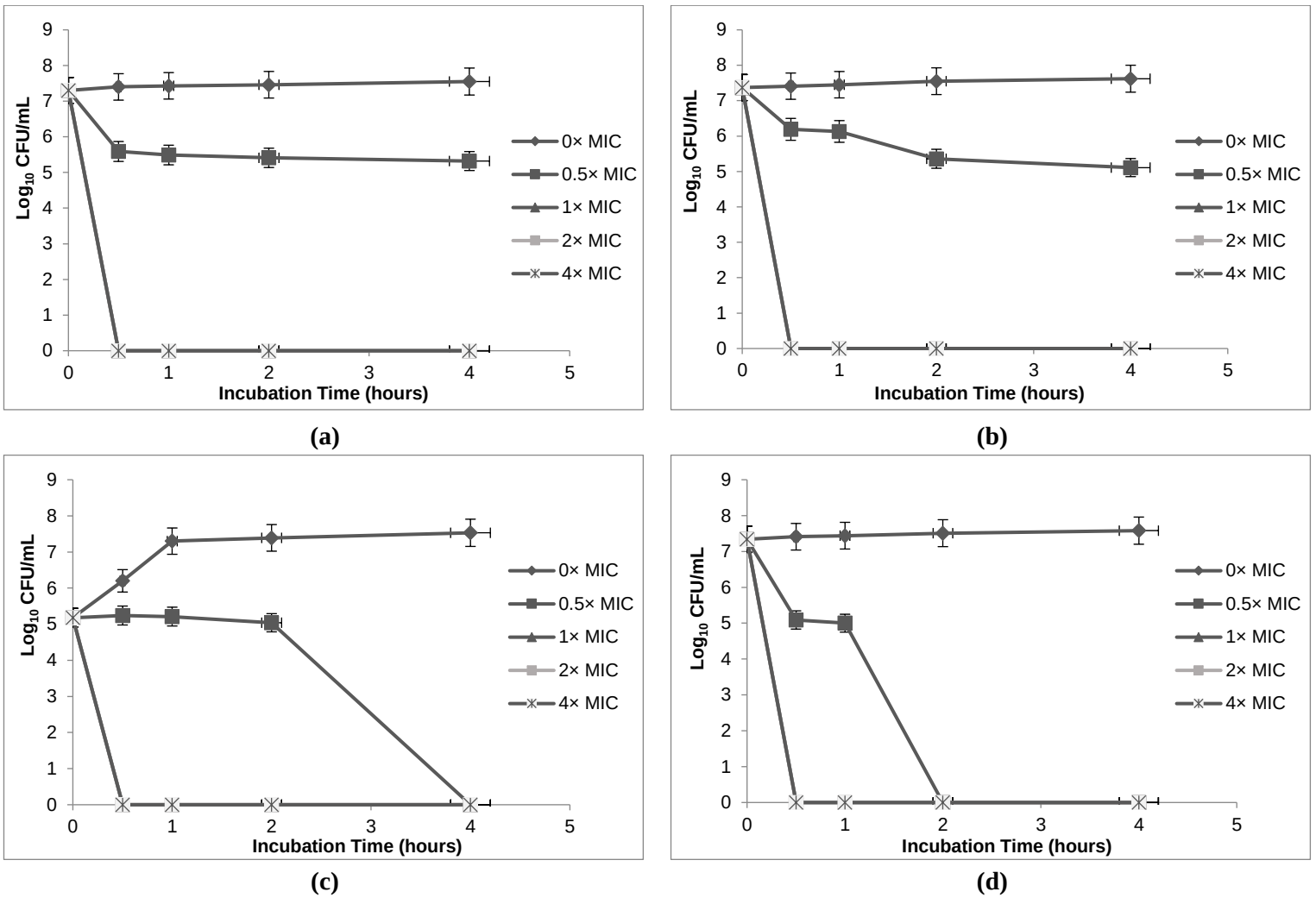

Figure 2. Time-kill curve plots of ethanolic extract of *S. jambos* leaves at concentrations of 0, 0.5, 1, 2, and 4× MIC at 0 to 4 h incubation time; **(a)** *C. albicans*, **(b)** *C. glabrata*, **(c)** *C. krusei*, and **(d)** *C. parapsilosis*.

C. glabrata, and *C. albicans*. However, it was nearly similar with 0.5, 1, and 2× MIC for *S. aureus* in the first 30 min of incubation time.

By contrast, *V. parahaemolyticus*, *C. parapsilosis*, and *C. krusei* were completely killed in 0.5× MIC at the incubation periods of 0.5, 2, and 4 h, respectively, in 0.63 and 6.25 mg/mL of the leaf extract of *S. jambos*. These three species were completely killed earlier than others. Penduka and Okoh (2012) reported that *L. monocytogenes* was completely killed with 0.314 mg/mL methanolic extract of *Garcinia kola* seed in 0.5 h of incubation. In comparison, the ethanolic leaf extracts of *S. jambos* killed *L. monocytogenes* with 0.01 mg/mL in 1 h in the present work, thus suggesting that the leaf extract of *S. jambos* possessed higher antibacterial activity against *L. monocytogenes* than the methanolic extract of *G. kola*.

In another study, Alwash *et al*. (2013) reported that *Melastoma malabathricum* (Malabar melastome or *senduduk*) extract killed *P. aeruginosa* completely at a concentration of 1.56 mg/mL within 8 h, and the leaf extract of *S. jambos* in the present work killed the same species at 0.16 mg/mL in 1 h. Besides, Sharaf (2020) reported that when treated with different concentrations (25, 12.5, and 6.25 mg/mL) of ethyl acetate extract of *Deverra tortuosa* (Desf) plant and incubation at 37°C for 24 h, the CFU of *C. albicans* was reduced in a higher proportion as the concentration of the plant extract increased. In comparison, the ethanolic leaf extract of *S. jambos* in the present work killed all the *Candida* species completely at concentrations ranging from 0.5 to 1× MIC within 0.5 to 4 h of exposure.

The results of the present work also concur with the findings of Phumat *et al.* (2020) in the antimicrobial activity of the extracts against *C. albicans*. Phumat *et al*. (2020) reported in their study that 4-allylpyrocatechol from the *Piper betle* (betel) leaf extract showed vigorous antifungal activity against *C. albicans*, and extracts of 1, 2, and 4× MIC (400, 800, and 1,600 μg/mL) killed *C. albicans* completely within 24, 12, and 2 h, respectively. Interestingly, the *S. jambos* ethanolic leaf extract showed broad antimicrobial activities against Gram-negative and Gram-positive bacteria, and yeasts.

Inhibition of conidial germination

Figure 3 shows the inhibition of conidial germination assay at different concentrations (0, 0.5, 1, 2, and 4× MIC) of the ethanolic *S. jambos* leaf extract. At 0.5× MIC, it was found to completely inhibit the conidial germination of *A. fumigatus*. Meanwhile, though not fully inhibited, *A. niger*, *R. oryzae*, and *R. oligosporus* showed a significant reduction from 100% to 7.0, 7.0, and 11.0%, respectively (Figure 3). In short, the ethanolic *S. jambos* leaf extract showed excellent inhibition against the conidial germination of the tested moulds.

Begum *et al*. (2010) reported that the conidial germination of *Rhizopus* spp. was reduced by 72% when treated with 5% the *Azadirachta indica* (neem) leaf extract. In comparison, the leaf extract of *S. jambos* in the present work showed a higher inhibitory effect against *R. oryzae* and *R. oligosporus*, and the conidial germination was reduced to 7 and 11%, respectively. Also, Rukayadi and Hwang (2007) reported that the xanthorrhizol isolated from *Curcuma xanthorrhiza* (Roxb.) (Javanese turmeric or *temulawak*) reduced the conidial germination when added into suspensions containing *A. flavus*, *A. fumigatus*, *A. niger*, *F. oxyxporum*, *R. oryzae*, and *Trichophyton mentagrophytes* with an average of 22, 18, 16, 24, 18, and 22%, respectively, at 4× MIC. By contrast, the present work showed a slightly higher reduction in the conidial germination of the tested moulds. In general, the ethanolic *S. jambos* leaf extract showed higher efficiency of antifungal activity against various moulds. The present work provided the first experimental finding on the inhibitory effect of the ethanolic *S. jambos* leaf extract on the conidial germination of moulds. This finding might be valuable for controlling the infections of pathogenic fungi.

Conclusion

The present work demonstrated that the ethanolic extract of *S. jambos* leaves exhibited antimicrobial activity against a broad spectrum of microorganisms that cause foodborne illnesses and food spoilage. The leaf extract exhibited microbicidal activity against *K. pneumoniae*, *L. monocytogenes*, *P. aeruginosa*, *S. aureus*, *V. parahaemolyticus*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *A. fumigatus*, while showing microbiostatic activity against *P. mirabilis*, *A. niger*, *R. oryzae*, and *R. oligosporus*. Therefore, the *S. jambos* leaf extract could be promoted to further tests and evaluations, and applied in food industries as a sanitiser or preservative in a wide range of foods.

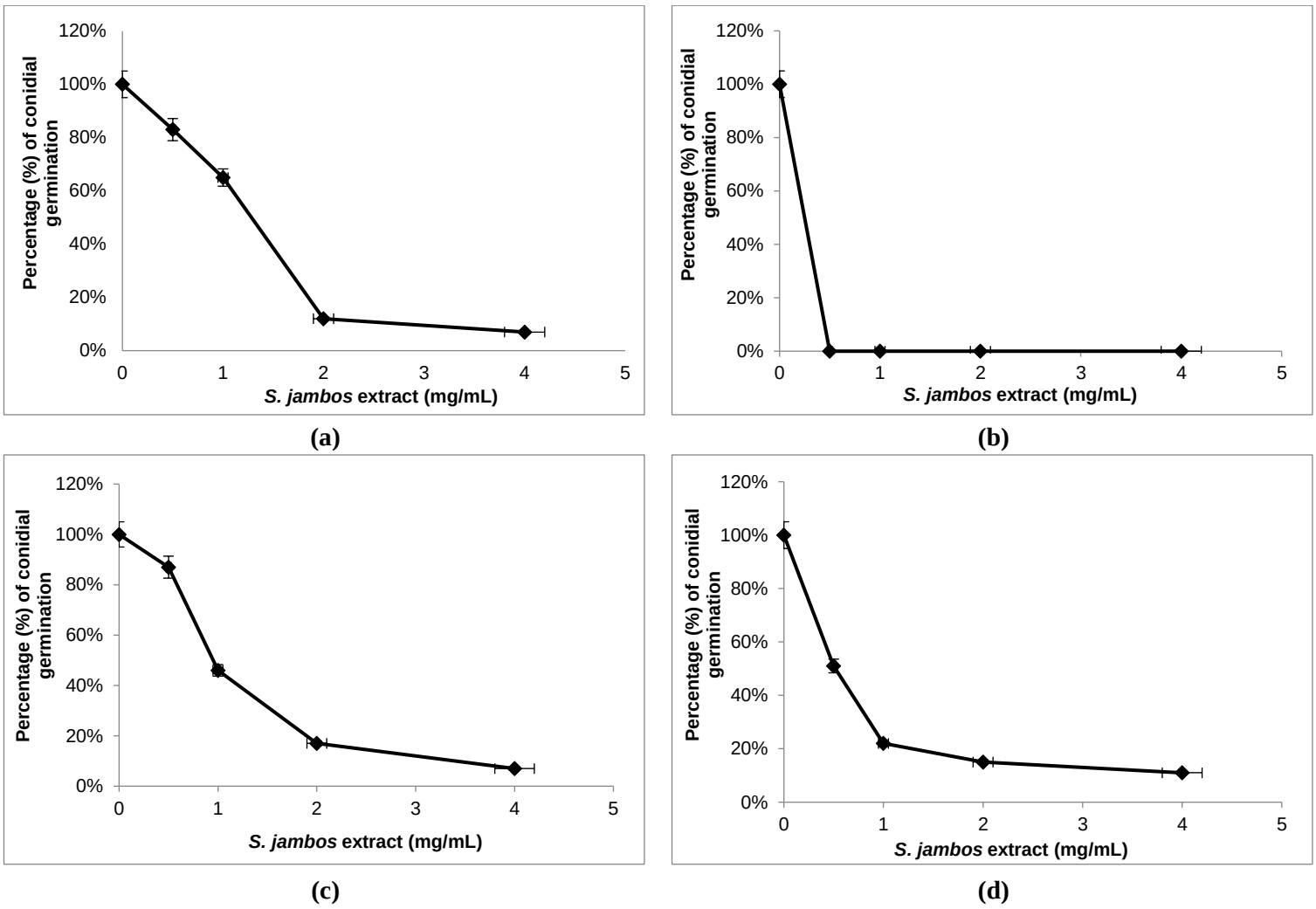

Figure 3. Effect of ethanolic extract of *S. jambos* leaves at concentrations of 0, 0.5, 1, 2, and 4× MIC; **(a)** *A. niger*, **(b)** *A. fumigatus*, **(c)** *R. oryzae*, and **(d)** *R. oligosporus* at concentrations of 0, 0.5, 1, 2, and 4× MIC.

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