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ฤทธิ์ต้านอนุมูลอิสระและต้านจุลชีพของสารสกัดด้วยเอทานอลของผลส้มแขก

Antioxidant and Antimicrobial Properties of Ethanolic Extract of Asam Gelugor Fruit (*Garcinia atroviridis*)

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บทคัดย่อ

การดื้อต่อยาปฏิชีวนะของจุลชีพเป็นปัญหาสำคัญทั่วโลกทั้งในทางสาธารณสุขและทางสัตวแพทย์ ทำให้สารธรรมชาติหลายชนิดถูกนำมาศึกษาฤทธิ์ต้านจุลชีพอย่างกว้างขวาง ซึ่งส้มแขกมีฤทธิ์ทางเภสัชวิทยาที่หลากหลาย โดยเฉพาะฤทธิ์ต้านอนุมูลอิสระและต้านจุลชีพ อย่างไรก็ตามข้อมูลเกี่ยวกับฤทธิ์ดังกล่าวของผลส้มแขกมีอยู่อย่างจำกัด ดังนั้นการศึกษานี้จึงมีจุดประสงค์ในการศึกษาฤทธิ์ต้านอนุมูลอิสระและต้านจุลชีพของผลส้มแขก ผลการศึกษาพบว่า สารสกัดด้วยเอทานอลของผลส้มแขกความเข้มข้น 2.5 มิลลิกรัม/มิลลิลิตร มีฤทธิ์ต้านอนุมูลอิสระ DPPH ได้มากกว่า tocopheryl acetate ที่มีความเข้มข้นเดียวกัน ถึง 1.13 เท่า (antioxidation index เท่ากับ 81.30±4.40% และ 72.06±0.83%, $p<0.05$) นอกจากนี้สารสกัดผลส้มแขกความเข้มข้น 50-200 มิลลิกรัม/มิลลิลิตร ยังมีฤทธิ์ต้านเชื้อแบคทีเรีย *Streptococcus agalactiae* ATCC 27956, *Staphylococcus aureus* DMST 4745, *S. epidermidis* DMST 12853, *S. intermedius* DMST 5024, *Bacillus subtilis* DMST 3763 และ *Escherichia coli* TISTR 073 ซึ่งศึกษาด้วยวิธี agar well diffusion assays แต่ไม่พบฤทธิ์ดังกล่าวต่อเชื้อยีสต์ *Candida albicans* ATCC 10231 และ *Malassezia pachydermatis* (คัดแยกจากสุนัข) เมื่อทดสอบด้วยวิธี broth microdilution พบค่า minimum inhibitory concentration (MIC) ต่อเชื้อแบคทีเรียอยู่ในช่วง 3.13-12.5 มิลลิกรัม/มิลลิลิตร และต่อเชื้อยีสต์ เท่ากับ 50 มิลลิกรัม/มิลลิลิตร ผลการศึกษานี้แสดงให้เห็นว่าสารสกัดด้วยเอทานอลของผลส้มแขกมีศักยภาพในการใช้เป็นสารต้านอนุมูลอิสระและต้านจุลชีพ โดยควรจะมีการศึกษาเพิ่มเติมเกี่ยวกับฤทธิ์ต้านจุลชีพชนิดอื่น ๆ และการพัฒนาสูตรตำรับยาที่เหมาะสม

คำสำคัญ : ฤทธิ์ต้านอนุมูลอิสระ ; ฤทธิ์ต้านจุลชีพ ; ผลส้มแขก

Abstract

Antimicrobial resistance is a worldwide problem in both public health and veterinary medicine. Therefore, many natural substances have been studied to find out more about their antimicrobial activities. The asam gelugor plant has various pharmacological properties, especially antioxidant and antimicrobial activities, but there is limited information about the bioactivities of asam gelugor fruit. This study investigated the antioxidant activity and antimicrobial effects of asam gelugor fruit extract. The DPPH radical scavenging capacity of 2.5 mg/ml ethanolic asam gelugor fruit extract was 1.13 times higher than 2.5 mg/ml tocopheryl acetate (antioxidation index 81.30±4.40% vs 72.06±0.83%, $p<0.05$). In addition, the fruit extract concentration of 50-200 mg/ml showed antimicrobial activity in agar well diffusion assays against *Streptococcus agalactiae* ATCC 27956, *Staphylococcus aureus* DMST 4745, *S. intermedius* DMST 5024, *S. epidermidis* DMST 12853, *Bacillus subtilis* DMST 3763 and *Escherichia coli* TISTR 073, but no activity against *Candida albicans* ATCC 10231 and *Malassezia pachydermatis* (isolated from dogs). The minimum inhibitory concentration (MIC) values determined via broth microdilution ranged from 3.13 to 12.5 mg/ml for the tested bacterial strains and were 50 mg/ml for both of the tested yeast isolates. The results demonstrated that ethanolic asam gelugor fruit extract could potentially be used as an antioxidant and antimicrobial agent. However, further study should be conducted to determine its activity against other microbial strains and to develop suitable formulations.

Keywords : antioxidant activity ; antimicrobial activity, asam gelugor fruit

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Introduction

Antimicrobial resistance is a worldwide problem in both the public health and veterinary fields, causing tremendous economic loss (Ventola, 2015). These problems occur from the extensive use of antibiotics in treating infectious diseases in humans and animals, especially from the long-term use and misuse of antibiotics (Wright, 2015; Venter *et al*., 2017). Microbial resistance to antibiotics causes the drugs to be ineffective resulting in treatment failure and new drugs must be developed to replace existing ones. However, most antibiotics are developed from existing antibiotics and tend to have a similar chemical basis as the original medication, which allows microorganisms to develop resistance rapidly (Aslam *et al*, 2018). A variety of infectious diseases in animals is the cause of an extensively using antimicrobial drugs in the veterinary field, especially the disease caused by many members of staphylococci, streptococci, Enterobacteriaceae and some pathogenic yeasts, leading to involve antimicrobial resistance problems (Palma *et al.*, 2020). Therefore, many natural substances have been studied to find out more about their antimicrobial activities, both as alternatives to current antibiotics and for use in combination with currently used antibiotics (Srivastava *et al*., 2014; Cheesman *et al*., 2017; Gupta & Birdi, 2017).

Asam gelugor (*Garcinia atroviridis* Griffith ex T. Anderson.) is a perennial tree in the Clusiaceae family. It is a tropical plant native to Malaysia, Thailand, Myanmar, and India (Assam). The fruit of asam gelugor has a sour taste and is used as an ingredient of many local foods (Lim, 2012). It also has various pharmacological properties, including anti-hyperlipidemia, antioxidant, anticancer, anti-inflammatory, anti-atherosclerosis, antimalarial and antimicrobial activities (Galih *et al.*, 2018). Previous studies have reported that extracts of asam gelugor roots, leaves, and fruits have antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Candida albicans* (Mackeen *et al.*, 2000; Widyowati & Rahman, 2010). However, there are limited studies of the antioxidant and antimicrobial activities of asam gelugor fruit extract, which an important role in infectious disease treatment by the extract. Therefore, the objectives of this study were to study the effect of ethanolic asam gelugor fruits extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and antimicrobial activities against some animal pathogenic bacteria and yeast including *S. aureus* DMST 4745, *Staphylococcus intermedius* DMST 5024, *Staphylococcus epidermidis* DMST 12853, *Streptococcus agalactiae* ATCC 27956, *E. coli* TISTR 073, *C. albicans* ATCC 10231 and *Malassezia pachydermatis* (isolated from dog; Animal hospital, Faculty of Veterinary Medicine, Khon Kaen University, Thailand). The bacterium *B. subtilis* DMST 3763 was also used for a model of gram-positive bacilli bacteria.

Methods

1. Materials

The fresh half-ripe fruits of asam gelugor (*Garcinia atroviridis* Griffith ex T. Anderson.) were purchased from local markets in Khon Kaen, Thailand. Gentamicin, cephalexin and ketoconazole were purchased from Sigma-Aldrich, Germany. Absolute ethanol was purchased from Merck, Germany. Tocopheryl acetate (vitamin E) was purchased from Namsiang Co., Ltd., Thailand. Butylated hydroxytoluene (BHT) was purchased from Riedel de Haen, Germany. 2,2-diphenyl-l-1-picrylhydrazil (DPPH) was purchased from Fluka, Germany. Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB), Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB) were purchased from Becton Dickinson, France.

2. Plant extraction

The fresh half-ripe fruits of asam gelugor were cut into small pieces, dried at 50°C for 48 h in a hot air oven (UF110, Memmert, Germany) and ground to powder by an electric stainless steel grinding machine (DMF-10A, Daming, China). Then, 500 g of powder was macerated with 95% ethanol in a ratio of 1:5 for 7 days. The solvent was evaporated from the filtrate (Whatman filter no. 4) using a rotary evaporator (Heidolph, Germany), the filtrate was freeze dried (Coolsafe 110-4 and CryoSafe 18-50, Scanvac, Denmark). A total of 12% yield was observed after extraction process and the extract was stored at -20 °C until use.

3. Microbial strains and culture conditions

Bacteria*: S. aureus* DMST 4745, *S. intermedius* DMST 5024, *S. epidermidis* DMST 12853, *S. agalactiae* ATCC 27956, *B. subtilis* DMST 3763, *E. coli* TISTR 073, and yeast*: C. albicans* ATCC 10231 and *M. pachydermatis (*isolated from dogs) were obtained from the Pharmacology and Toxicology Laboratory of the Faculty of Veterinary Medicine, Khon Kaen University, Thailand. The yeasts and bacteria were cultured in Sabouraud dextrose broth (SDB, Becton Dickinson, France) and Mueller Hinton broth (MHB, Becton Dickinson, France), respectively, and incubated at 37°C for 24 h. The optical density (OD) at 600 nm of microbial suspension was measured by Vis-spectrophotometer (Genesys 10 VIS, Thermo Scientific, USA) and adjusted to 10^6 CFU/ml, which was confirmed by viable counts (CLSI, 2008, 2013). The microbial calibration curve in an author laboratory showed the bacteria and yeasts at this concentration have $OD_{600\ nm}$ value of 0.03 and 0.17, respectively.

4. Determination of antioxidant activity by DPPH assay

The DPPH assay was performed according to the method previously described by Lertsatitthanakorn *et al.* (2006). Briefly, the asam gelugor extract was diluted with absolute ethanol to give a final concentration of 10 mg/ml and 50 µl was added to 50 µl of absolute ethanol in 96-well round-bottomed microtiter plates (Corning Incorporated, USA). Serial two-fold dilutions were done. Then 50 µl of a 0.0004 M DPPH solution was added into all

test wells and the contents were mixed for 5 min. The mixtures were incubated in the dark for 25 minutes at room temperature and their absorbance at 517 nm was recorded by microplate reader (EZ Read 400, Biochrom, UK). BHT and vitamin E were used as positive antioxidant controls. The percentage of DPPH radical inhibition (% antioxidation index; %AI) was calculated according to the following equation:

$$\%AI = \frac{Abs(ct)-(Abs(s)-Abs(b))}{Abs(ct)} x\ 100 \qquad (1)$$

Where Abs(ct) represents the absorbance of the sample containing all reagents except the antioxidant. Abs(s) is the absorbance of the samples containing an antioxidant. Abs(b) is the absorbance of the sample containing all reagents except antioxidant and DPPH.

5. Determination of antimicrobial activity by agar well diffusion method

The inhibition zones of asam gelugor extract against tested microorganisms were determined by the agar well diffusion method according to CLSI guidelines (2013) for bacteria and CLSI guidelines (2009) for yeasts, with modifications. Briefly, the microbial suspension concentration of 10^6 CFU/ml was inoculated onto MHA plates for bacteria or SDA plates for yeast (instead of using supplemented Mueller-Hinton agar to improve the growth of *M. pachydermatis*) by the streak plate technique. The wells were cut in the inoculated plates by a cork borer (6 mm diameter). Then, 20 µl aliquots of 50, 100 and 200 mg/ml asam gelugor extract diluted in absolute ethanol were added into the wells. The plates were incubated at 37°C for 24 h. The inhibition zones were measured using a mathematics ruler. All tests were performed in triplicate.

6. Determination of antibacterial activity by broth microdilution method

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of asam gelugor extracts against tested microorganisms were determined by the broth microdilution method according to CLSI guidelines (2008, 2013) with modifications. Briefly, serial dilutions of the asam gelugor extract (50-0.0977 mg/ml), gentamicin (125-0.24 µg/ml), cephalexin (125-0.24 µg/ml) or ketoconazole (125-0.24 µg/ml) were prepared with MHB for bacteria or SDB for yeast (instead of using RPMI 1640 medium to improve the growth of *M. pachydermatis*) in 96-well flat-bottomed microtiter plates (Costar®, Corning Incorporated) (50 µl per well). Then, a fifty microliters aliquot of a 10^6 CFU/ml bacterial suspension and 10^4 CFU/ml (OD_{600nm} 0.01) yeasts suspension was added into each well. Wells containing the microbial suspension without tested agents served as positive growth control wells and wells containing tested agents without the microbial suspension served as negative growth control wells. The plates were incubated at 37°C for 24 h. All tests were

performed in triplicate. We used gentamicin, cephalexin and ketoconazole as the standard antimicrobial agents since these drugs are used often clinically. The MIC was defined as the lowest concentration of antimicrobial agent that prevented visible growth after 24 h of incubation. Ten microliter samples from the wells with no visible growth were inoculated onto MHA (bacteria) or SDA (yeast) and incubated at 37°C for 24 h. The MBC or MFC was determined from the lowest concentration of antimicrobial agent that showed no growth on MHA or SDA.

7. Statistically analysis

The data was subject to one-way analysis of variance (One-way ANOVA) using SPSS software (α = 0.05) (KKU license, windows version 19.0, SPSS Inc., USA).

Results

1. Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of the ethanolic asam gelugor fruit extract is shown in Table 1. The 2.5 mg/ml extract showed an antioxidant effect 1.13 times higher than 2.5 mg/ml tocopheryl acetate The 0.03 mg/ml BHT, which is a positive control, had the highest antioxidant activity, 1.13 and 1.27 times more than asam gelugor extract and tocopheryl acetate, respectively.

Table 1 Antioxidation indices of asam gelugor extract, tocopheryl acetate and butylated hydroxytoluene (BHT).

Antioxidants	Antioxidation index (%)
2.5 mg/ml Asam gelugor extract	81.30±4.40^{b}
2.5 mg/ml Tocopheryl acetate	72.06±0.83^{c}
0.03 mg/ml BHT	91.78±3.00^{a}

Values represent the means of triplicate experiments ± SD. Superscript letters within a column indicate statistically significant differences between the means of triplicate experiments ($p<0.05$).

2. Determination of antimicrobial activity by agar well diffusion

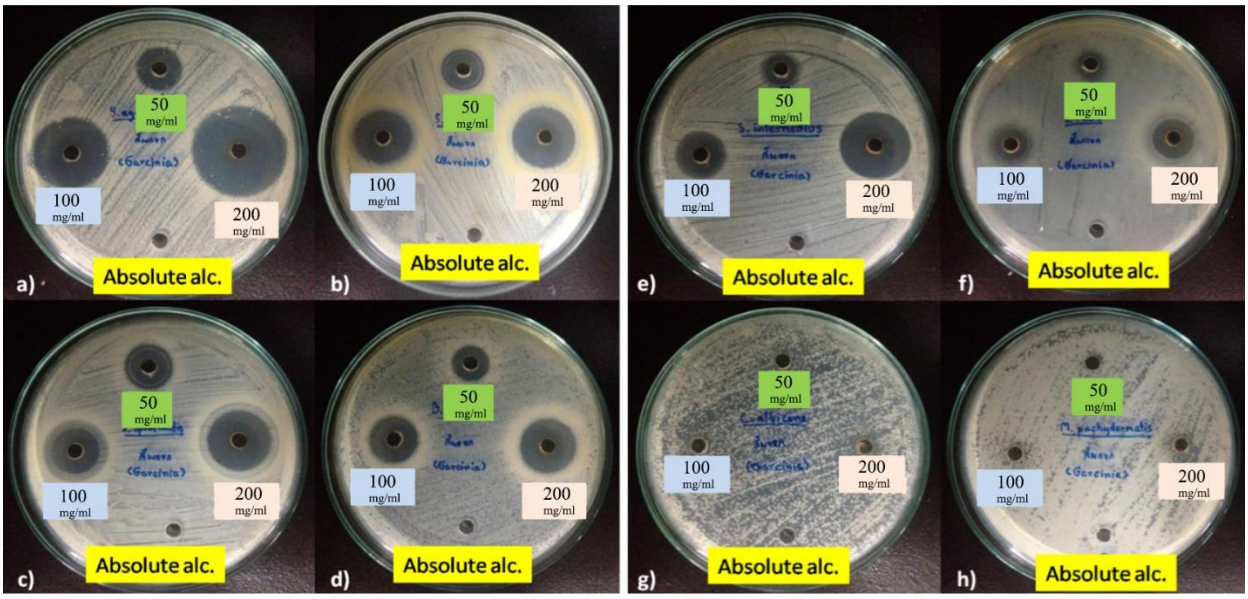
The inhibition zone diameters for the agar well diffusion assay are given in Table 2 and representative images are shown in Figure 1. The absolute ethanol (diluent control) showed no antimicrobial effect (inhibition zones $\leq$6.00 mm, Figure 1). The asam gelugor extract showed a concentration dependent antimicrobial effect against the tested bacteria, with 200 mg/ml having the largest inhibition zone diameters for all tested bacterial isolates, but did not affect the growth of *C. albicans* and *M. pachydermatis* (inhibition zones $\leq$6.00 mm). *S. agalactiae* had the largest inhibition zones (13.00±1.18 mm for 50 mg/ml, 21.87±0.51 mm for 100 mg/ml, and 27.30±1.00 mm for

200 mg/ml) followed by the staphylococci (*S. aureus*, *S. epidermidis* and *S. intermedius*), *B. subtilis*, and *E. coli* (Table 2).

Table 2 Antimicrobial activity of asam gelugor extract against tested microorganisms using agar well diffusion method.

Microbial strains	Inhibition zones diameter (mm)		
	50 mg/ml	100 mg/ml	200 mg/ml
S. agalactiae ATCC 27956	13.00±1.18[a]	21.87±0.51[a]	27.30±1.00[a]
S. aureus DMST 4745	12.00±0.30[a]	16.77±0.81[b]	20.53±1.08[b]
S. epidermidis DMST 12853	11.57±0.23[b]	14.90±0.17[b]	19.77±0.40[b]
S. intermedius DMST 5024	9.87±0.97[b]	12.57±0.81[b]	21.30±0.00[b]
B. subtilis DMST 3763	10.90±0.72[b]	14.23±1.08[b]	17.67±0.85[c]
E. coli TISTR 073	6.67±0.35[c]	8.97±0.57[c]	12.23±1.08[d]
C. albicans ATCC 10231	≤6.00±0.00[c]	≤6.00±0.00[d]	≤6.00±0.00[e]
M. pachydermatis isolated	≤6.00±0.00[c]	≤6.00±0.00[d]	≤6.00±0.00[e]

Note: Values represent the means of triplicate experiments ± SD. Superscript letters within a column indicate statistically significant differences between the means of triplicate experiments ($p<0.05$).



a) *S. agalactiae* ATCC 27956, b) *S. aureus* DMST 4745, c) *S. epidermidis* DMST 12853, d) *B. subtilis* DMST 3763, e) *S. intermedius* DMST 5024, f) *E. coli* TISTR 073 , g) *C. albicans* ATCC 10231, h) *M. pachydermatis* isolated, Absolute alc. = absolute ethyl alcohol

Figure 1 The inhibition zones of 50, 100 and 200 mg/ml asam gelugor extract against tested microorganisms.

3. Determination of antimicrobial activity by broth microdilution

Table 3 shows the MIC and MBC results of the antibacterial testing of asam gelugor extract and the antibiotics gentamicin and cephalexin. All strains of tested bacteria were susceptible to gentamicin (MIC ≤ 4 µg/ml) according to CLSI interpretive standards (2016) except *S. agalactiae*, which has no interpretive breakpoints for gentamicin. As there are no CLSI interpretive standards for cephalexin, the cephalexin susceptibility breakpoint for veterinary isolates (CLSI revision) of Papich & Lindeman (2018) were used. The bacteria *S. agalactiae, S. aureus* and *B. subtilis* (MIC ≤ 2 µg/ml) were classified as sensitive to cephalexin, while *S. epidermidis* and *S. intermedius* (MIC = 4 µg/ml) and *E. coli* (MIC ≥ 8 µg/ml) were classified as intermediate and resistant, respectively. The asam gelugor extract MICs ranged from 3.13 to 12.5 mg/ml and MBCs were 3.13 to more than 50 mg/ml. Among the bacteria tested, *S. agalactiae*, *S. aureus* and *S. intermedius* had the lowest MIC and MBC values (3.13 mg/ml), *S. epidermidis* had the second lowest MIC (6.25 mg/ml) and the MBC (12.5 mg/ml) was twice the MIC. *B. subtilis* and *E. coli* had the same MIC (12.5 mg/ml), but the *B. subtilis* MBC was > 50 mg/ml and the *E. coli* MBC was 25 mg/ml.

Table 3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of asam gelugor extract, gentamicin, and cephalexin against tested bacteria.

Bacterial strain	Antimicrobial agent					
	Asam gelugor extract (mg/ml)		Gentamicin (µg/ml)		Cephalexin (µg/ml)	
	MICs	MBCs	MICs	MBCs	MICs	MBCs
S. agalactiae ATCC 27956	3.13	3.13	0.49	0.49	0.98	0.98
S. aureus DMST 4745	3.13	3.13	0.49	0.49	0.49	0.49
S. epidermidis DMST 12853	6.25	12.50	0.49	0.49	3.91	3.91
B. subtilis DMST 3763	12.50	>50.00	0.49	0.49	0.49	1.95
S. intermedius DMST 5024	3.13	3.13	0.49	0.49	3.91	3.91
E. coli TISTR 073	12.50	25.00	3.91	3.91	15.63	125.00

The antifungal MIC and MFC activities of asam gelugor extract against *C. albicans* and *M. pachydermatis* are shown in Table 4. The ketoconazole gave a little difference MIC value between 2 yeast strains (7.81 and 3.91 µg/ml, respectively), while had higher MFC values against *C. albicans* (31.25 µg/ml) than *M. pachydermatis* (15.63 µg/ml). The asam gelugor extract had the same antifungal activity against *C. albicans* and *M. pachydermatis*; MIC and MFC values of 50 mg/ml.

Table 4 Minimum inhibitory concentrations (MICs) minimum fungicidal concentrations (MFCs) of asam gelugor extract and ketoconazole against tested yeasts.

Yeast strain	Antimicrobial agent			
	Asam gelugor extract (mg/ml)		Ketoconazole (µg/ml)	
	MICs	MFCs	MICs	MFCs
C. albicans ATCC 10231	50.00	50.00	7.81	31.25
M. pachydermatis isolated	50.00	50.00	3.91	15.63

Discussion

It is interesting that the ethanolic extract of the asam gelugor fruit had a radical scavenging capacity higher than tocopheryl acetate at the same concentration. The DPPH scavenging activity of the extract in our study was higher than an aqueous extract of asam gelugor fruit reported by Nursakinah *et al.*, 2012 (52% DPPH scavenging activity). In addition to the DPPH radical scavenging activity, asam gelugor has been reported to have good antioxidant activity in ferric reducing antioxidant power (FRAP), ferric thiocyanate (FTC) and thiobarbituric acid (TBA) assays with total reducing capacity dependent on the part of the plant used, the solvent or extraction method and the testing protocol (Hamidon *et al.*, 2017). Mackeen *et al.* (2000) reported that methanolic extract of root, bark, fruits and leaves of asam gelugor had antioxidant indices in the range of 64 to 90% by FTC method and 87-93% by the TBA method, both of which were higher than **α**-tocopherol, a commercially available antioxidant. The antioxidant activities of asam gelugor extract are presumably due to the phenolic compounds (Abdullah *et al.*, 2013). Asam gelugor fruit was shown to have total phenolic content of 9.25 to 16.23 mg gallic acid/g sample in aqueous extract and flavonoid content of 0.29 mg/g sample in aqueous-methanolic extract (Miean & Mohamed, 2001; Nursakinah *et al*., 2012). The main phenolic compounds found in asam gelugor fruits were luteolin, myricetin and quercetin (Miean & Mohamed, 2001). Phenolic compounds are aromatic ring molecules with at least 1 hydroxyl group. These substances act against DPPH phenoxyl radical (DPPH$^{\bullet}$) by a H-atom transfer mechanism (Mathew *et al.*, 2015). Phenolic compounds can transfer an H-atom from their hydroxyl group (aromatic-OH) to a free radical molecule, which has a single unpaired electron, changing the reactive DPPH phenoxyl radical to its inactive reduced form (DPPH-H). The resultant phenoxy radicals (Aromatic-O$^{\bullet}$) are rendered highly stable by hydrogen bonds, conjugation, and resonance to make them nonreactive phenoxyl radicals, stopping the chain reaction of oxidation (Gulcin, 2011; Leopoldini *et al*., 2011).

The results of the antimicrobial assays of the asam gelugor fruit extract revealed that the extract had little or no effect against *C. albicans*, *M. pachydermatis* and *E. coli*. These results were different to a previous study of

Basri *et al*. (2005) which reported that 1% w/v ethanolic extract of asam gelugor fruit, lower than used in the current study, had some antimicrobial activity against *E. coli* O157:H7 (inhibition zone 14.50 mm), and against two yeasts *Candida glabrata* and *Candida parapsilosis* (inhibition zone 7.80 mm). While Thongboon (2013) reported that ethanolic asam gelugor fruit extract had an MIC value of 1 mg/ml against *S. aureus* and *E. coli*, 3 to 12 times lower than the MICs in the current study. These differences may be due to a dissimilar susceptibility of each microbial strain. Moreover, plant sources and extraction methods also affect the results. However, some reports using different extraction methods may produce similar test results. Mackeen *et al.* (2000) employed a different extraction and testing method from the current study (methanolic extraction and disc diffusion), they showed similar antimicrobial activities for *S. aureus*, *B. subtilis* B28 (mutant) and B29 (wild type) and *E. coli* (minimum inhibitory dose; MID = 500 µg/disc) and no effect against *C. albicans* (MID > 1,000 µg/disc). This case may arise from the similar susceptibility of tested microbes to different extracts.

There is limited study of phytochemicals in asam gelugor fruits ethanolic extract. However, based on polarity and solubility properties of the solvents and biochemical compounds, constituents contained in the extract may similarly to aqueous and methanolic extract but different in proportion (Azwanida, 2015). Most phytochemicals contained in asam gelugor fruits are well extracted by ethanol and water/ethanol solvent especially flavonoid, steroid, terpenoid and tannin (Andayani *et al.*, 2020). There are many compounds reported from asam gelugor fruits extract including flavonoids (luteolin and quercetin), sesquiterpenoids ((-)-β-caryophyllene, β-caryophyllene alcohol and α-humulene) and organic acids (citric acid, tartaric acid, malic acid, ascorbic acid, pentadecanoic acid, nonadecanoic acid, dodecanoic acid and hydroxycitric acid) (Tan *et al.*, 2012; Adamczak *et al.*, 2020). The antimicrobial effect of asam gelugor fruit extract is presumably due to the phenolics present in the extract, especially flavonoids, which block nucleic acid production by inhibiting DNA gyrase and affect some crucial intracellular enzymes and functions of microorganisms (Cushnie & Lamb, 2005; Wang & Xie, 2010). In addition, sesquiterpenoids are the predominant volatile constituents of asam gelugor fruit, have also been shown to have antimicrobial activity by destroying microbial cell membranes and other membranous organelles (Jang *et al.*, 2020). Finally, the organic acids that are found in high levels in asam gelugor fruit may inhibit microbial growth by interfering with ion transport across the cell membrane (Hamidon *et al.*, 2017; Adamczak *et al.*, 2020). The previous toxicological study of asam gelugor fruit extract showed that it had a noncytotoxic effect towards CEM-SS (human T-lymphoblastic leukemia) cells, Raji (human B-lymphoblastoid) cells (convulsion dose 50% were >100 µM and >600 µM, respectively), and brine shrimp (lethal concentration 50% >300 µM) (Mackeen et al., 2012). Moreover, the extract also safe and nontoxic to Wistar rats (*Ratus norvegicus*) in the sub-chronic toxicity study (Milanda *et al.* 2019). Therefore, ethanolic asam gelugor fruit extract may be used as antioxidant and antimicrobial agents for

control infectious disease in animals. However, further study is required for other microbial strains, study suitable concentrations, type of medications and treatment patterns, and to determine the *in vivo* efficacy in experimental animals.

Conclusions

The ethanolic asam gelugor fruit extract had higher DPPH scavenging capacity than tocopheryl acetate at the same concentration. The extract showed antimicrobial activity against *S. agalactiae*, *S. aureus*, *S. epidermidis*, *B. subtilis*, *S. intermedius* and *E. coli*, but had low activity against *C. albicans* and *M. pachydermatis*. The MBC values were 1 to 4 times more than MIC for tested bacteria and yeasts except for *B. subtilis* DMST 3763 (MBC > 4 times of MIC). These results demonstrate that the ethanolic asam gelugor fruit extract has bactericidal and fungicidal effects and can potentially be used as an antioxidant and antimicrobial agent.

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References

Abdullah, A.R., Bakhari, N.A., & Osman, H. (2013). Study on the relationship of the phenolic, flavonoid and tannin content to the antioxidant activity of *Garcinia atroviridis*. *Universal Journal of Applied Science*, *1*(3), 95-100.

Adamczak, A., Ozarowski, M., & Karpinski, T.M. (2020). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine*, *9*(1), 109.

Andayani, R., Armin, F., & Mardhiyah, A. (2020). Determination of the phenolics and antioxidant activity in the rind extracts of *Garcinia mangostana* L., *Garcinia cowa* Roxb., and *Garcinia atroviridis* Griff. ex. T. Anders. *Asian Journal of Pharmaceutical and Clinical Research*, *13*(8), 149-152.

Aslam, B., Wang, W., Arshad, M., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, *11*, 1645-1658.

Azwanida, N.N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, *4*(3), 1000196.

Basri, D.F., Sharif, R., Morat, P., & Latip, J. (2005). Evaluation of antimicrobial activities of the crude extracts from *Garcinia atroviridis* and *Solanum torvum*. *Malaysia Journal of science*, *24*(1), 233-238.

Cheesman, M.J., Ilanko, A., Blonk, B., & Cock, I.E. (2017). Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution? *Pharmacognosy Reviews*, *11*(22), 57-72.

CLSI. (2008). *Reference method for broth dilution antifungal susceptibility testing of yeasts; approve standard-third edition. CLS document M27-A3*. Pennsylvania: Clinical and laboratory standard institute.

CLSI. (2009). *Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline-second edition. CLS document M44-A2*. Pennsylvania: Clinical and laboratory standard institute.

CLSI. (2013). *Performance standard for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard-fourth edition. CLS document VET01-A4*. Pennsylvania: Clinical and laboratory standard institute.

CLSI. (2016). *Performance standards for antimicrobial susceptibility testing. 26th edition. CLSI supplement M100S*. Pennsylvania: Clinical and laboratory standard institute.

Cushnie, T.P.T., & Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343-356.

Galih, S., Tiana, M., & Muchtaridi, M. (2018). Pharmacological activity of gelugur acid plant (*Garcinia atroviridis* Griff.): a study. *International Journal of Current Medical Sciences*, *8*, 406-410.

Gulcin, I. (2011). Antioxidant activity of eugenol: a structure-activity relationship study. *Journal of Medicinal Food*, *14*(9), 975-985.

Gupta, P.D., & Birdi, T.J. (2017). Development of botanicals to combat antibiotic resistance. *Journal of Ayurveda and Integrative Medicine*, *8*(4), 266-275.

Hamidon, H., Susanti, D., Taher, M., & Zakaria, Z.A. (2017). *Garcinia atroviridis* - A review on phytochemicals and pharmacological properties. *Marmara Pharmaceutical Journal*, *21*(1), 38-47.

Jang, H.I., Rhee, K.J., & Eom, Y.B. (2020). Antibacterial and antibiofilm effects of α-humulene against *Bacteroides fragilis*. *Canadian Journal of Microbiology*, *66*(6), 389-399.

Leopoldini, M., Russo, N., & Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry*, *125*(2), 288-306.

Lertsatitthanakorn, P., Taweechaisupapong, S., Aromdee, C., & Khunkitti, W. (2006). In vitro bioactivities of essential oils used for acne control. *International Journal of Aromatherapy*, *16*(1), 43-49.

Lim, T.K. (2012). *Edible Medicinal and Non-Medicinal Plants: Fruits*. Heidelberg: Springer.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L.Y., & Mohamed, S.M. (2000). Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. ex T. Anders. *Journal of Ethnopharmacology*, *72*(3), 395-402.

Mackeen, M.M., Mooi, L.Y., Amran, M., Mat, N., Lajis, N.H., & Ali, A.M. (2012). Noncytotoxic and antitumour-promoting activities of garcinia acid esters from *Garcinia atroviridis* Griff. ex T. Anders (Guttiferae). *Evidence-Based Complementary and Alternative Medicine*, *2012*, 829814.

Mathew, S., Abraham, T.E., & Zakaria, Z.A. (2015). Reactivity of phenolic compounds towards free radicals under *in vitro* conditions. *Journal of Food Science and Technology*, *52*(9), 5790-5798.

Miean, K.H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, *49*(6), 3106-3112.

Milanda, T., Safrudin, A.D., Samudra, G., Sumiwi, S.A., & Muchtaridi, M. (2019). Sub-chronic toxicity of *Garcinia atroviridis* Griff Fruit's ethanol extract on Wistar rats (*Ratus norvegicus*). *Journal of Advanced Pharmaceutical Technology & Research*, *10*(4), 178-183.

Nursakinah, I., Zulkhairi, H.A., Norhafizah, M., Hasnah, B., Zamree, Md.S., Shafeera, I.F., Dasiman, R., & Hamzah, F.H. (2012). Nutritional content and *in vitro* antioxidant potential of *Garcinia atroviridis* (asam gelugor) leaves and fruits. *Malaysian Journal of Nutrition*, *18*(3), 363-371.

Palma, E., Tilocca, B., & Roncada, P. 2020. Antimicrobial resistance in veterinary medicine: an overview. *International Journal of Molecular Sciences*, *21*(6), 1914.

Papich, M.G., & Lindeman, C. 2018. Cephalexin susceptibility breakpoint for veterinary isolates: Clinical Laboratory Standards Institute revision. *Journal of Veterinary Diagnostic Investigation*, *30*(1), 113-120.

Srivastava, J., Chandra, H., Nautiyal, A.R., & Kalra, S.J.S. (2014). Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDA_ms) as an alternative drug line to control infections. *3 Biotech*, *4*(5), 451-460.

Tan, W.N., Wong, K.C., Khairuddean, M., Eldeen, I.M., Asmawi, M., & Sulaiman, B. (2012). Volatile constituents of the fruit of *Garcinia atroviridis* and their antibacterial and anti-inflammatory activities. *Flavour and Fragrance Journal*, *28*(1), 2-9.

Thongboon, P. (2013). *Antibacterial efficacy of Garcinia extract and its application in hand cleaning product* [Thesis]. Songkla: Prince of Songkla University.

Venter, H., Henningsen, M.L., & Begg, S.L. (2017). Antimicrobial resistance in healthcare, agriculture and the environment: the biochemistry behind the headlines. *Essays in Biochemistry*, *61*(1), 1-10.

Ventola, C.L. (2015). The antibiotic resistance crisis part 1: causes and threats. *Pharmacy and Therapeutics*, *40*(4), 277-283.

Wang, Q., & Xie, M. (2010). Antibacterial activity and mechanism of luteolin on *Staphylococcus aureus*. *Acta microbiologica Sinica*, *50*(9), 1180-1184.

Widyowati, R., & Rahman, A. (2010). Kandungan Kimia dan Aktivitas Antimikroba Ekstrak *Garcinia celebica* l. terhadap *Staphylococcus aureus*, *Shigella dysenteriae* dan *Candida albicans*. *Majalah Farmasi Airlangga*, *8*(2), 23-27.

Wright, G.D. (2015). Solving the antibiotic crisis. *ACS Infectious Diseases*, *1*, 80-84.