Original article

Antimicrobial activity of polyphenols extracted from Thai medical plants on extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from healthy dairy cows

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Abstract

Escherichia coli producing extended-spectrum beta-lactamase (ESBL) are antimicrobial--resistant Enterobacteriaceae important in the livestock production sector, especially dairy cows because these antimicrobial-resistant bacteria can be transferred to consumers via consumption. If antimicrobials are continually used in dairy farms, this may result in antimicrobial resistance. Therefore, investigation of antimicrobial resistance and finding new alternative methods for inhibiting ESBL-producing *E. coli* is essential. Hence, the aim of this study was to examine the ability of selected antimicrobials to inhibit *E. coli* ATCC 25922, control bacteria and ESBL-producing *E. coli* isolated from dairy farms. We also investigated the capacity of polyphenol extract from 10 varieties of medicinal plants to inhibit ESBL-producing *E. coli* using a broth microdilution method. It was found that control bacteria were susceptible to all antimicrobial agents, i.e., ampicillin, cefotaxime, ciprofloxacin, chloramphenicol, gentamycin, imipenem, nalidixic acid, tetracycline, and sulfamethoxazole/ trimethoprim. However, ESBL-producing *E. coli* exhibited both susceptibility and resistance to selected antimicrobials. The polyphenol extracted from *Psidium guajava* Linn at the lowest concentration was 4.5 mg/mL, which could inhibit control bacteria, but at the same concentration could not inhibit ESBL-producing *E. coli*. These phenomena indicated that ESBL-producing *E. coli* had both susceptibility and resistance to antimicrobials. Polyphenol, which could inhibit non-resistant *E. coli*, could not inhibit ESBL-producing *E. coli*.

Key words: antimicrobial resistance, *Escherichia coli* ATCC 25922, *Psidium guajava* Linn, Thailand

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Introduction

Antimicrobial resistance (AMR) has become a public health problem in many parts of the world (Pérez-Etayo et al. 2018). Antimicrobial use in food-producing animals for growth promotion and for disease treatment or prevention is probably a major contributor to the overall problem of resistance (Prestinaci et al. 2015). The main concern with resistant bacteria is the fear that resistance in the environment will be transferred to clinical pathogens, leading to untreatable infectious diseases (Tamhankar et al. 2019). *Escherichia coli* has become a general threat to public health because *E. coli* sometimes causes community-onset infectious diseases (Kawamura et al. 2017). In addition, most *E. coli* are commensal bacteria in the animal gut. Commensal *E. coli* can acquire AMR plasmids from AMR bacteria in the gut by horizontal transfer. Thus, they can switch from non-resistant bacteria to AMR bacteria in healthy animals (Proenca et al. 2017).

Extended-spectrum beta-lactamases (ESBL) are enzymes responsible for the hydrolysis of oxymino-beta-lactam drugs, which are important therapeutic agents for the treatment of serious human and animal infections. ESBL were first described in 1983 in Enterobacteriaceae (Palmeira and Ferreira 2020). At present, ESBL-producing *E. coli* have been reported in humans and animals worldwide (Hammerum et al. 2014, Pérez-Etayo et al. 2018, Palmeira and Ferreira 2020). ESBL-producing bacteria have been detected in meat samples and the transmission of ESBL-producing bacteria could drive from food animals and food handlers to consumers through the food chain (Lavilla et al. 2008). In addition, AMR Enterobacteriaceae can also be transmitted from live animals to humans (Dohmen et al. 2015). Cattle are one of the sources of protein and milk which are most consumed around the world. Cows are also one of the main sources of biological fertilizers, due to the high production of fecal mass of these animals. All these factors highlight the importance of cattle production in the context of the food chain and the contaminated environment as a reservoir and a transmitting/disseminating vehicle of ESBL-producing *E. coli* (Palmeira and Ferreira 2020). ESBL-producing *E. coli* contamination in dairy farms and milk products has been reported in many parts of the world, i.e., Japan, the United Kingdom, France, and South Korea (Tark et al. 2017), Germany, Iran, Indonesia, and the Czech Republic etc. (Bitrus et al. 2019). There are many reports showing that ESBL-producing *E. coli* were multi-drug resistant bacteria, for example to cefquinome, amoxicillin/clavulanic acid, aztreonam (Filioussis et al. 2020), ampicillin (AMP), (Ibrahim et al. 2016, Filioussis et al. 2020), colistin (Filioussis et al. 2020),

penicillin G, and streptomycin (Sudarwanto et al. 2017).

Polyphenols are natural compounds occurring in plants (Cardona et al. 2013). The mechanisms of antibacterial action of phenolic compounds involve many sites of action at the cellular level, such as the modification in the permeability of cell membranes, changes in various intracellular functions induced by hydrogen bonding of the phenolic compounds to enzymes, or by the modification of cell wall rigidity with integrity losses due to different interactions with the cell membrane (Bouarab-Chibane et al. 2019). It has been suggested that polyphenols exert their antibacterial effects in three ways; namely, direct killing of bacteria, synergistic activation of antimicrobials, and attenuation of bacterial pathogenicity (Xie et al. 2017). Alberto (2006) found that polyphenols extracted from the skin of two apple varieties, *Royal Gala* and *Granny Smith*, had an antimicrobial effect against *E. coli* ATCC 25922. Rodríguez-Pérez (2016), who studied the efficacy of polyphenol extracted from cranberry (*Vaccinium macrocarpon*) found that this compound was active against *E. coli*. From the previous studies mentioned above, we hypothesized that antimicrobials, which are used in Thailand, had the ability to inhibit non-resistant *E. coli* and ESBL-producing *E. coli.* Additionally, we would like to know the lowest concentration of polyphenols extracted from medicinal plants that could be effective against *E. coli* and ESBL-producing *E. coli*.

Therefore, the aims of this study were to examine the effect of antimicrobials in inhibiting *E. coli* and ESBL-producing *E. coli* and to investigate the efficacy of polyphenol extracts from 10 varieties of medicinal plants i.e., *Tamarindus indica* Linn, *Careya arborea* Roxb., *Manihot esculenta* Crantz, *Psidium guajava* Linn, *Ficus racemose* Linn, *Cocos nucifera* Linn, *Musa ABB*, Kluai 'Namwa', *Punica granatum* Linn, *Morus nigra* Linn, *Ficus cariaca* and *Careya arborea* Roxb. in inhibiting *E. coli* ATCC 25922 and ESBL-producing *E. coli*. Knowledge from the present study is important for the evaluation of antimicrobial resistance and finding a new antimicrobial compound or method for solving this problem.

Materials and Methods

Plants: sources and methods of polyphenol extraction

Plants used in this study were grown in Mahasarakham province, Thailand (16°11′3.00″N, 103°18′1.20″ E). All plants were collected during July – September 2020. The samples were placed in airtight plastic bags and

Table 1. Type of medicinal plants, sources, profile of polyphenolics, and methods of polyphenol extraction.

Type of plants (Sources of plants)	Profile of polyphenolics	Method of extraction
1. *Tamarindus indica* Linn (local market)	Apigenin, catechin, procyanidin B2, epicatechin, procyanidin dimer, procyanidin trimer, along with taxifolin, eriodictyol, naringenin (Bhadoriya et al. 2011)	The seeds were heated in a hot air oven at 140°C for 45 min, cooled and cracked to separate their outside brown layer. Only brown-red seed coats were collected, and these were then ground into fine powder. The polyphenols in the tamarind seed coat powder were extracted by using 95% ethanol as a solvent (1:5, w:v).
2. *Cocos nucifera* Linn (local market)	Catechins, epicatechins, tannins, flavonoids (Lima et al. 2015)	The coconut pith powder was extracted by using distilled water as a solvent (1:5, w:v).
3. *Musa ABB, Kluai 'Namwa'* (local market)	Gallic acid, glutaric acid, 2-hydroxyvaleric acid, protocatechuic acid, 1,4-Ipomeadiol (Jannoey et al. 2021)	The banana blossoms were washed with water and chopped. The fresh samples were heated in a hot air oven at 60°C for 48 hours, then ground into fine powder. The polyphenols in the banana blossoms powder were extracted by using distilled water as a solvent (1:5, w:v).
4. *Punica granatum* Linn (local market)	Anthocyanins, flavanones, flavones, flavonols, isoflavonoids, hydroxycinnamics, alkylphenols, tyrosol, curcuminoids, phenolic terpenes (Fellah et al. 2018)	Peel of *Punica granatum* Linn were collected. The fresh samples were heated in a hot air oven at 60°C for 48 hours, then ground into fine powder. The polyphenols in the peel of *Punica granatum* L. powder were extracted by using 95% ethanol as a solvent (1:5, w:v).
5. *Ficus racemose* Linn (botanical garden of the faculty of Veterinary Sciences, Mahasarakham University)	Anthocyanin, flavonoids, tannin (Sivakumar et al. 2019)	Leaves and fruits of *Ficus racemose* Linn were heated in a hot air oven at 60°C for 48 hours, then ground into fine powder. The polyphenols in the leaves and fruits of *Ficus racemose* Linn powder were extracted by using 95% ethanol as a solvent (1:5, w:v).
6. *Ficus cariaca* (fig garden in Maha Sarakham province)	Caffeoylmalic acid, rutin (Petruccelli et al. 2018)	The leaves were heated in a hot air oven at 60°C for 48 hours, then ground into fine powder. The polyphenols in the leaf powder of each medicinal plant were extracted by using 95% ethanol as a solvent (1:5, w:v).
7. *Manihot esculenta Crantz* (botanical garden of the faculty of Veterinary Sciences, Mahasarakham University)	Salicylic acid, syringic acid, benzoic acids, gallic acid, Protocatechiuc acid, Vanillic acid, Gentisic acid, P-catechuic, p-Hydroxybenzoic (Laya et al. 2020)	
8. *Careya arborea* Roxb. (local market)	Quercetin, ellagic acid, and gallic cid (Gupta et al. 2014)	
9 *Morus nigra* Linn. (mulberry garden in Maha Sarakham province)	Gallic acid, protocatechuic acid, protocatechuic, aldehyde, p-Hydroxybenzoic acid, vanillic acid, chlorogenic acid, syringic acid, syringealdehyde, p-coumaric acid, ferulic acid, m-coumaric acid (Memon et al. 2010)	
10. *Psidium guajava* Linn (botanical garden of the faculty of Veterinary Sciences, Mahasarakham University)	Quercetins, total myricetin, total catechins, gallic acid, ellagic acid and tannins (Chang et al. 2012)	

transferred to the laboratory of the Faculty of Veterinary Sciences, Mahasarakham University. The criteria for selecting the medicinal plants for use in the present study were (1) they are common plants that are easy to find, have a large quantity and can be easily grown on general farms, (2) these are plants that contain polyphe-nols, and (3) it has been reported in an academic journal that the extracts from these plants have antimicrobial properties. Details of plants, sources, the important polyphenol which is found in each plant, and methods of polyphenol extraction are shown in Table 1. The extracts were dried by heat in a hot air oven at 60°C

Table 2. Antimicrobial resistance profiles of 25 extended-spectrum beta-lactamases-producing *Escherichia coli* isolates from 4 dairy farms in Borabue district, Maha Sarakham province, Thailand.

Dairy farms	No. of positive samples	No. of ESBL producers	Antimicrobial resistance profiles[a]	No. of isolate(s)
A	4	10	CTX–AMP	3
			CTX–AMP–GEN	1
			CTX–AMP–SXT	1
			CTX–AMP–SXT–GEN	1
			CTX–AMP–SXT–TET	4
B	1	1	CTX–AMP–CHL–TET	1
C	3	6	CTX–AMP–CHL–TET	6
D	4	8	CTX–AMP	3
			CTX–AMP–TET	1
			CTX–AMP–SXT–GEN	4
Total	12	25		25

[a] CTX – cefotaxime, AMP – ampicillin, CHL – chloramphenicol, GEN – gentamicin, SXT – sulfamethoxazole/trimethoprim, TET – tetracycline

until dried. The dried extracts were transferred into plastic tubes and kept at 4°C before used.

Total phenolic contents were determined using the Folin-Ciocalteu method. Briefly, 10 ml of 0.2 M Folin-Ciocalteu reagent (Merck KGaA, Darmstadt, Germany) was adjusted to 100 ml using distilled water. 7.5 g of sodium carbonate (Ajax Finechem Pty Ltd, Taren Point, Australia) was then dissolved in distilled water and the volume adjusted to 100 ml. Five μg of each extract (from each of 10 medicinal plants) was then dissolved in distilled water and the volume adjusted to 100 ml. Extract (0.5 ml), Folin-Ciocalteu reagent (2.5 ml), and sodium carbonate solution (2.0 ml) were placed into a test tube, shaken well, left at room temperature for 1 hour, and then the absorbance then measured at 765 nm. Gallic acid was used as the control to express the total phenolic contents (mg GAE/g of extract). Polyphenol solution of each medicinal plant was prepared in distilled water at a concentration of 5.0 and 10.0 mg/ml for Trial 2 and 3 respectively. The solutions were then filtrated using bacterial syringe filters and stored at – 21°C before use.

Microorganisms

Ethics approval

This study was approved by the Ethics Committee on Animal Experimentation of Mahasarakham University (license number: IACUC-MSU-018/2020).

ESBL-producing *E. coli* isolates

Twenty-five ESBL-producing *E. coli* isolates were isolated from 12 healthy dairy cows reared on 4 dairy farms located in Borabue district, Maha Sarakham province, Thailand (Table 2). Briefly, the fecal samples were placed on MacConkey agar (Oxoid Ltd, Hampshire, UK) and MacConkey agar containing 2 μg/ml of cefotaxime (CTX) (Sigma-Aldrich Pte. Ltd, Singapore) and then incubated overnight at 37°C. The 3–5 pink colonies were picked from MacConkey agar containing 2 μg/ml of CTX to identify the bacterial species based on biochemical parameters using API20E (bioMerieux, Mercy-I'Etoile, France), and confirmed the phenotype of ESBL using a disk synergist test using amoxycillin/clavulanic acid (20/10 μg/disk), CTX (30 μg/disk), ceftazidime (30 μg/disk) and cefpodoxime sodium (10 μg/disk) (Luzzaro et al. 2020).

Non-resistant *E. coli* American Type Culture Collection (ATCC) 25922

The non-resistant reference strain bacteria used in this study was *E. coli* ATCC 25922 obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. This isolate was maintained in Tryptic Soy Broth (Becton Dickinson and company, Sparks, MD, USA) containing 10% glycerol and kept at – 80°C until used.

Experimental design and laboratory testing

The present study was divided into 3 trials as follows: Trial 1 tested antimicrobial susceptibility to in-

vestigate the AMR profiles of reference bacteria (non-resistant *E. coli* ATCC 25922) and ESBL-producing *E. coli* that were isolated from healthy dairy cows, to examine whether the two types of bacteria were resistant to antimicrobials. Trial 2 tested the ability of polyphenols extracted from 10 medicinal plants to inhibit reference bacteria (non-resistant *E. coli* ATCC 25922). Trial 3 tested the effect of polyphenols extracted from medicinal plants which had resistance effects against reference bacteria (non-resistant *E. coli* ATCC 25922) with ESBL-producing *E. coli*, using a commercial antimicrobial agent as a control group. The details of the trials were as follows.

Trial 1: AMR profiles of test microorganisms

AMR profiles of test microorganisms in the present study followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI) VET01-A4 (CLSI 2013). Determination of the susceptibility of bacteria to antimicrobial agents was based on CLSI supplement M100-S25 (CLSI 2015). The details of these tests were as follows: antimicrobial susceptibility testing of the reference bacteria (non-resistant *E. coli* ATCC 25922) and 25 ESBL-producing *E. coli* isolates using 2 methods that were (i) a disk diffusion method with 8 commercial antimicrobial agents including AMP (30 μg/disk), chloramphenicol (30 μg/disk, CHL), ciprofloxacin (5 μg/disk, CIP), gentamicin (30 μg/disk, GEN), imipenem (10 μg/disk, IMI), nalidixic acid (30 μg/disk, NAL), tetracycline (30 μg/disk, TET) and sulfamethoxazole/trimethoprim (23.75/1.25 μg/disk, SXT), and (ii) a broth microdilution method with one antimicrobial agent (CTX) to investigate the minimum inhibitory concentration (MIC). The steps of the MIC method were as follows: 200 μl of CTX diluted with cation-adjusted Mueller Hinton II broth (MHB II) (Becton Dickinson and company, Sparks, MD, USA) (0.5-1024 μg/ml) were added in sterile 96-well microplates and each well which contained CTX solution was then inoculated with 10 μl of bacterial solution at a concentration 10^7 CFU/ml. The final concentration of bacteria in each well was 10^5 CFU/ml. The negative and positive control group were a sterile MHB II and all dilutions of CTX, and the MHB II with bacteria, respectively. The microplate with cocktails of antimicrobial agents and bacterial suspension, and the negative and positive control groups were incubated at 35°C for 16 to 20 hours. The MIC was read as the lowest concentration that completely inhibited the bacterial growth in the well as assessed by the unaided eye. Subsequently, CTX were used as a control group for polyphenol efficacy testing by broth microdilution method in Trial 2 and 3.

Trial 2: Antimicrobial activity of polyphenol extracts against non-resistant bacteria (*E. coli* ATCC 25922)

Polyphenols (5 mg/ml concentration) extracted from 10 medicinal plants (i.e., *Careya arborea* Roxb., *Cocos nucifera* Linn, *Ficus cariaca*, *Ficus racemose* Linn, *Manihot esculenta* Crantz, *Morus nigra* Linn, *Musa ABB*, Kluai 'Namwa', *Psidium guajava* Linn, *Punica granatum* Linn, and *Tamarindus indica* Linn) were diluted by MHB II to achieve concentrations of 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 mg/ml for investigation of the MIC which inhibited non-resistance *E. coli* ATCC 25922 using a broth microdilution method (CLSI 2013).

Trial 3: Antimicrobial activity of polyphenol extracts against AMR bacteria (ESBL-producing *E. coli*)

According to the results of Trial 2, the extracts which could inhibit the growth of *E. coli* ATCC 25922 at MIC ≤ 4.5 mg/ml were selected to determine the antimicrobial activity against ESBL-producing bacteria. Ten mg/ml of polyphenols were diluted by MHB II to achieve concentrations of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, and 9 mg/ml, for investigation of MIC using a broth microdilution method (CLSI 2013). *Escherichia coli* ATCC 25922 was used as microorganism control.

Results

ESBL-producing *E. coli* isolates which were isolated from dairy farms and the reference bacteria, *E. coli* ATCC 25922 were tested for antimicrobial susceptibility by using 9 commercial antimicrobial agents: CTX, AMP, CHL, CIP, GEN, IMI, NAL, SXT, and TET. Subsequently, CTX from Trial 1 were used as controls for polyphenol efficacy testing in Trial 2 and 3. Polyphenols that had been extracted from 10 medicinal plants were then tested to find the lowest polyphenol concentration able to inhibit the reference bacteria, *E. coli* ATCC 25922. Lastly, polyphenol from the medicinal plant which had the lowest concentration that could inhibit the reference bacteria was tested for inhibition of ESBL-producing *E. coli* in Trial 3. The results were as follows:

Trial 1: AMR profiles of test microorganisms

E. coli ATCC25922 is susceptible to 9 commercial antimicrobial agents including AMP, CHL, CIP, GEN, IMI, NAL, SXT, TET and CTX (MIC ≤ 0.5 μg/ml).

Table 3. Minimum inhibitory concentration (MIC) of 10 polyphenol extracts (1 – 4.5 mg/ml) against non-resistant isolate (*Escherichia coli* ATCC 25922) using broth microdilution method.

Polyphenol extracts (1 – 4.5 mg/ml)	Results of minimum inhibitory concentration (MIC, mg/ml)[a]
	E. coli ATCC 25922
Tamarindus indica Linn	> 4.5
Cocos nucifera Linn	> 4.5
Musa ABB, Kluai 'Namwa'	> 4.5
Punica granatum Linn	> 4.5
Ficus racemose Linn	> 4.5
Ficus cariaca	> 4.5
Manihot esculenta Crantz	> 4.5
Careya arborea Roxb.	> 4.5
Morus nigra Linn	> 4.5
Psidium guajava Linn	4.5

[a] MIC – minimum inhibitory concentration

Table 4. Minimum inhibitory concentration (MIC) of *Psidium guajava* Linn extract against non-resistant isolate (*Escherichia coli* ATCC 25922) and 25 extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolates using a broth microdilution method.

Test microorganisms	Concentration of *Psidium guajava* Linn (mg/ml)	Results[a] (MIC, mg/ml)
A non-resistant *E. coli (E. coli* ATCC 25922)	3–9	4.5
Twenty-five ESBL-producing *E. coli* isolates	3–9	>9

[a] MIC – minimum inhibitory concentration

Twenty-five ESBL-producing *E. coli* isolates were from 12 individual fecal samples of dairy cows from 4 farms including 10 isolates from farm A (4 of 12 cows), an isolate from farm B (1 of 12 cows), 6 isolates from farm C (3 of 12 cows), and 8 isolates from farm D (4 of 12 cows) as shown in Table 2. The results of antimicrobial susceptibility of 25 ESBL producers showed that:

(i) ten isolates from farm A were resistant to 5 of 9 antimicrobial agents: CTX, AMP, GEN, SXT and TET with 5 AMR profiles that were CTX–AMP (3 isolates), CTX–AMP–GEN (1 isolate), CTX–AMP–SXT (1 isolate), CTX–AMP–SXT–GEN (1 isolate), and CTX–AMP–SXT–TET (4 isolates),

(ii) the isolate from farm B and 6 isolates from farm C were resistant to 4 of 9 antimicrobial agents with the same AMR profile that was CTX–AMP–CHL–TET, and

(iii) eight isolates from farm D were resistant to 5 of 9 antimicrobial agents: CTX, AMP, GEN, SXT and TET with 3 AMR profiles that were CTX–AMP (3 isolates), CTX–AMP–TET (1 isolate), and CTX–AMP–SXT–GEN (4 isolates).

Twenty-five ESBL producers were resistant to 5 of 9 antimicrobials: AMP (100%, 25/25), CTX (100%, 25/25), TET (48%, 12/25), SXT (44%, 11/25), CHL (24%, 6/25) and GEN (24%, 6/25), respectively. All 25 isolates had a total of 7 AMR profiles, which were CTX – AMP (24%, 6/25), CTX – AMP – GEN (4%, 1/25), CTX – AMP – SXT (4%, 1/25), CTX – AMP – TET (4%, 1/25), CTX – AMP – GEN – SXT (20%, 5/25), CTX – AMP – TET – SXT (20%, 5/25), and CTX – AMP – TET – CHL (24%, 6/25).

Trial 2: Antimicrobial activity of polyphenol extracts against non-resistant bacteria (*E. coli* ATCC 25922)

The polyphenol extracted from *Psidium guajava* Linn could inhibit *E. coli* ATCC 25922 with MIC at 4.5 mg/ml. In contrast, the MIC of polyphenols extracted from other medicinal plants that could inhibit *E. coli* ATCC 25922 were more than 4.5 mg/ml (Table 3).

Trial 3: Antimicrobial activity of polyphenol extracts against AMR bacteria (ESBL-producing *E. coli*)

From the result of Trial 2, polyphenol extracted from *Psidium guajava* Linn was selected for testing its efficacy to inhibit 25 ESBL-producing *E. coli* isolates.

It was found that the MIC of *Psidium guajava* Linn which could inhibit ESBL-producing *E. coli* isolates was more than 9 mg/ml (Table 4).

Discussion

Antimicrobial susceptibility studies of different strains of *E. coli* have sought to determine the type of antimicrobials that can still inhibit *E. coli*. Several studies have found that *E. coli* isolates from healthy and unhealthy dairy cows were susceptible and/or resistant to many antimicrobials, such as that of Sawant (2006) who studied commensal AMR *E. coli* which was isolated from healthy dairy cows in a farm in central and south-central Pennsylvania. They found that *E. coli* isolates were resistant to multiple antimicrobials: TET, florfenicol, AMP, CHL, spectinomycin, and ceftiofur. Also, Hang (2019) found that commensal *E. coli* isolates from healthy dairy cows in southern Vietnam were resistant to AMP, florfenicol, spectinomycin and TET. Similar to the study of Hinthong (2017), they reported that pathogenic *E. coli* isolates from the milk of subclinical mastitis cows in Thailand were resistant to multiple antimicrobials: AMP, carbenicillin, ceftazidime, ceftriaxone, CTX, cefuroxime, cefepime, cefoperazone, GEN, norfloxacin and SXT. Thus, in this study, commensal *E. coli* was a good indicator to identify AMR bacteria in healthy dairy cows and the *E. coli* ATCC 25922 was a good reference bacterium that represented the non-resistant bacteria.

ESBLs are hydrolytic enzymes produced by gram-negative bacteria, and they confer resistance to many important antimicrobials including penicillin as well as the first to fourth generation of cephalosporins and monobactams (Paterson et al. 2005). In recent years, there have been studies of the increasing emergence of ESBL-producing members of Enterobacteriaceae around the globe, which is in part a consequence of the extensive use of oxyimino-cephalosporins in the treatment of bacterial infections (Faruk et al. 2016). Ibrahim (2016), who studied the antimicrobial resistance of ESBL-producing *E. coli* isolated from a dairy farm, found that these bacteria were most resistant to AMP (56.3%), followed by oxytetracycline, streptomycin and sulfonamide (41.1, 39.6 and 37.3%, respectively). Also, Tark (2017) found that ESBL-producing *E. coli* isolated from a dairy farm in South-Korea was resistant to TET (23.3%), followed by streptomycin (17.1%), AMP (16.6%), neomycin (11.8%), and SXT (11.2%), respectively. In the present study, where the ESBL-producing *E. coli* was used to examine AMR profiles comprising 25 CTX-resistant isolates, it was found that all of them were susceptible to CIP and NA,

but not AMP that was found in AMR profiles of all isolates. Except for AMP, the highest percentage of antimicrobial resistance was TET (48%), followed by SXT (44%), CHL (24%), and GEN (24%), respectively. This phenomenon indicated that ESBL-producing *E. coli* obtained from dairy farms in the same district were resistant to many antimicrobials in several groups. Moreover, they had several antimicrobial profiles. Note that these 25 ESBL producers were obtained from different farms, but some of them had the same AMR profiles (Table 2). Although the reason for this phenomenon was not investigated in this study, it is possible that dairy farms located in this area were under the supervision of the same veterinarians and/or dairy cooperation unit. Thus, the antimicrobial uses on those farms may be similar. In addition, the lack of farm biosecurity could provide the transfer of AMR bacteria from farms to adjacent farms.

In the last few decades, the increase in the use of antimicrobials has led to the growing incidence of bacterial resistance, thereby prompting the search for new active compounds against AMR pathogens (Raphaelli et al. 2019). Phenolic compounds are known to possess different pharmacological activities among which antioxidant and antimicrobial effects have recently received more attention (Bahri-Sahloul et al. 2014). This study was designed to examine the efficacy of polyphenols extracted from 10 medicinal plants, i.e., *Careya arborea* Roxb., *Cocos nucifera* Linn, *Ficus cariaca*, *Ficus racemose* Linn, *Manihot esculenta* Crantz, *Morus nigra* Linn, *Musa ABB*, Kluai 'Namwa', *Psidium guajava* Linn, *Punica granatum* Linn, and *Tamarindus indica* Linn and to test the properties of these polyphenols against *E. coli* ATCC 25922, reference bacteria for screening the polyphenol from the medicinal plant which had the highest efficacy to inhibit reference bacteria. It was found that the polyphenol extracted from *Psidium guajava* Linn could inhibit *E. coli* ATCC 25922 with MIC 4.5 mg/ml, while the MIC of polyphenol extracts from other medicinal plants that could inhibit *E. coli* ATCC 25922 were less than 4.5 mg/ml. This result was similar to the study of Cheruiyot (2009) and Arollado (2017), who found that extracts from *Psidium guajava* Linn could inhibit *E. coli*. The study of Mailoa (2014) explained the effects as being due to those tannins (tannins are plant polyphenols) extracted from *Psidium guajava* Linn leaves being able to react with proteins to form hydrogen bonds, causing protein denaturation. Tannin also reacts with phospholipids in cell membranes, resulting damage into the cell membrane of *E. coli*.

From the result of Trial 2, polyphenol extracted from *Psidium guajava* Linn was selected for testing its efficacy to inhibit 25 isolates of ESBL-producing

E. coli (Table 3). In all cases, ESBL-producers had MIC of this polyphenol extract less than 9 mg/ml. Even if polyphenol extracted from *Psidium guajava* Linn could inhibit *E. coli* ATCC 25922, it was not effective against ESBL-producing *E. coli* at the same or up to twice the concentration. Therefore, this phenomenon indicated that ESBL-producing *E. coli* was resistant to a polyphenol concentration at less than 9 mg/ml (Table 4). However, it is possible that if the concentration of polyphenol extracted from *Psidium guajava* Linn was raised to a certain level there may be an inhibitory effect on ESBL-producing *E. coli*. In addition, other studies have shown that the combination of polyphenol with antibiotics enhances the action of the antimicrobials to inhibit ESBL-producing *E. coli* in combinations such as (−)-epigallocatechin-3-gallate (EGCG) and β-lactam antimicrobial CTX to inhibit ESBL-producing *E. coli*, and it was found that the combination of CTX and EGCG induced permanent cellular damage and eventually killed the cells (Cui et al. 2012). Cui (2012) explained this phenomenon that EGCG could induce the generation of reactive oxygen species (ROS) by an auto-oxidation mechanism. The inhibitory effect of β-lactam antimicrobials is also known to be related to endogenous hydroxyl radical (OHN) damage. Therefore, the mechanism for the synergistic effect between CTX and EGCG is a converging attack of exogenous and endogenous oxidative stress generated by EGCG and cefotaxime, respectively. In addition, oxidative stress not only initiates membrane degradation, but also causes cell wall collapse and significantly disrupts cellular proteins. In the present study, it was found that the use of polyphenol extracted from *Psidium guajava* Linn alone may not be able to inhibit ESBL-producing *E. coli*. This may be partly due to the polyphenol concentrations which were used for testing. Therefore, a further study may be to examine the use of polyphenols extracted from *Psidium guajava* Linn to demonstrate appropriate antimicrobial inhibition of ESBL-producing *E. coli*.

In conclusion, *E. coli* ATCC 25922 control bacteria were susceptible to AMP, CIP, CHL, CTX, GEN, IMI, NAL, SXT, and TET. While ESBL-producing *E. coli* isolates from dairy farms were susceptible and resistant to selected antimicrobials. The polyphenol extracted from *Psidium guajava* Linn could inhibit *E. coli* ATCC 25922. The MIC of polyphenol extracted from *Psidium guajava* Linn that inhibited *E. coli* ATCC 25922 was 4.5 mg/ml. However, it was found that the lowest concentration of polyphenol extracted from *Psidium guajava* Linn that could inhibit *E. coli* ATCC 25922, could not inhibit ESBL-producing *E. coli*.

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