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Research article

Antibacterial effect of ethanolic *Morus alba* Linn. leaf extract against mastitis-causing *Escherichia coli* and *Staphylococcus aureus in vitro*

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Abstract

This study was conducted to determine the *in vitro* antibacterial effect of an ethanolic *Morus alba* L. (mulberry) leaf extract against *Escherichia coli* and *Staphylococcus aureus* isolated from mastitis dairy cows and against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 reference strains. The antibacterial efficacy was evaluated by a microdilution method to determine the MIC, MBC, and a time-kill assay by using azithromycin as a positive control. The data were analyzed nonparametric tests with a significance level of $p<0.05$. The quercetin content of the mulberry leaf extract was 83.187 ± 0.272 µg/g dried plant material, as determined by HPLC. The extract had MICs of 10, 20, 10, and 10 mg/mL and MBCs of 20, 20, 20, and 10 mg/mL for *E. coli* ATCC 25922, *E. coli* ECCM62, *S. aureus* ATCC 25923, and *S. aureus* SACM62, respectively. Quercetin had relevant MIC of 10 mg/ml and MBC of 20 mg/mL to the extract for both ATCC strains. Time-kill tests showed a complete elimination of *S. aureus* ATCC 25923 and *S. aureus* SACM62 after a 30-min exposure to 10 and 20 mg/mL *M. alba* extract. *E. coli* ATCC 25922 and *E. coli* ECCM62 showed reductions of 6.8 log CFU/mL after a 360 min exposure to 40 mg/mL *M. alba* extract. In this study, the inhibitory effect was stronger against *S. aureus* than against *E. coli* ($p<0.05$). Overall, the *M. alba* L. extract showed appreciable *in vitro* antibacterial efficacy against mastitis-causing *E. coli* and *S. aureus* strains and against reference ATCC strains.

Keywords: *Escherichia coli*, Mastitis, *Morus alba* Linn., Quercetin, *Staphylococcus aureus*

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INTRODUCTION

Mastitis is a common health problem that affects milk quality and production in dairy cows and causes global economic losses (Royster and Wagner, 2015; Bi et al., 2016). *Escherichia coli* and *Staphylococcus aureus* are major pathogens causing bovine mastitis. The worldwide prevalence of mastitis varies depending on the pathogen. In Brazil, it is 70.3% for *S. aureus* (Mesquita et al., 2019); in Thailand, it is 7.3% for *S. aureus* (Kampa et al., 2009); in China, it is 28.6% for *E. coli* (Bi et al., 2016); and in clinical healthy cows, it is 6.6% for *E. coli* (Cervinkova et al., 2013). However, conventional antibiotic therapy results in intractable antibiotic residues and antibacterial resistance, raising public health concerns (Lee et al., 2018; Meade et al., 2019). Herbal medicine, as an alternative treatment approach to overcome this health issue, has been well recognized in various research models and in studies on the application of medicinal plants and herb extracts in animal health management (Manyi-Loh et al., 2018; Rahman et al., 2018).

One plant with phytopharmacological potential is mulberry, a member of the Moraceae (genus *Morus* L., species *M. alba*, *M. rubra*, *M. nigra*, *M. indica*, *M. australis*, and *M. cathayana*) (Hussain et al., 2017; Rohela et al., 2020). Mulberry has been well regarded as multipurpose plant due to recognition of its role in environmental safety as a medicinal plant (Rohela et al., 2020). The leaves of *M. alba* L. contain many pharmaceutically active substances, including a high abundance of flavonoid compounds, such as quercetin, rutin, kaempferol, and kuwanol, as well as phenolic acids (e.g., ferulic acid, gallic acid) (Hussain et al., 2017; Lin et al., 2017). Mulberry also has economic importance for the sericulture industry, as it is traditionally used to feed silkworms in Asia (Lamberti et al., 2019). Previous studies have demonstrated several medicinal properties of mulberry plants, including antihyperglycemic (Chen et al., 2018), antioxidant (Polumackanycz et al., 2019) and antibacterial (Abdel-Hamid et al., 2017) activities. Extracts of *M. alba* L. leaves have also shown beneficial effects against bacteria isolated from humans, such as *S. aureus* and *E. coli* (minimum inhibitory concentration [MIC] 0.32 mg/mL and minimum bactericidal concentration [MBC] 0.64 mg/mL) (Abdel-Hamid et al., 2017). A study of leaf extracts from two *Morus* species (*M. alba* and *M. rubra* L.) showed effects against gram-negative and gram-positive bacterial ATCC strains, including *S. aureus*, *S. epidermidis*, *S. pyogenes*, *E. faecalis*, *Propionibacterium acnes*, E. coli, *P. aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *K. pneumoniae*, with MIC and MBC values of 15.75–252.00 mg/mL (Miljkovic et al., 2018). However, information from cattle-related models is extremely limited.

Therefore, the aims of this study were to determine the major bioactive compounds in a *M. alba* leaf extract using high performance liquid chromatography (HPLC) and to establish the antibacterial efficacy (MIC, MBC, and time-kill) of mulberry leaf extracts against mastitis-causing *S. aureus* and *E. coli* strains.

MATERIALS AND METHODS

Ethics consideration

The current study complied with the ethical guidelines for animal care and use and approved by the Instructional Animal Care and Use Committee of Khon Kaen University, Khon Kaen, Thailand; Record no. IACUC-KKU-31/63.

Preparation of ethanolic M. alba L. leaf extract

The *M. alba* L. leaves were obtained from Khon Kaen Province and were identified with a voucher specimen (X. Phengvongsone 01), which was deposited in the Khon Kaen University Herbarium. The mulberry leaf extract was prepared by extraction in 95% ethanol (Peanparkdee, 2016; Pulbutr et al., 2018). Briefly, the leaves were washed, chopped, dried, ground into a coarse powder, and then macerated in 95% ethanol for 7 days. The macerate was filtered through muslin cloth and Whatman No. 1 filter paper under a 20 in Hg vacuum pressure. The ethanol in the liquid filtrate was removed using a rotary evaporator (Heidolph model MXO7R-20-HD2E, USA) at 160 rpm and 50 °C for 30–50 minutes. The crude extract was subsequently freeze-dried (Scanvac CoolSafe™ model 110-4, Denmark) and stored at -20 °C until use. The percentage yield of the freeze-dried extract was determined by the following formula: (weight of freeze-dried extract/weight of dry chopped leaves) ×100.

Chemical analysis of M. alba L. crude extract

The mulberry leaf crude extract was analyzed by HPLC (Tallini et al., 2015). Briefly, 0.1 g of the freeze-dried extract was thoroughly dissolved in 10 mL boiling deionized water, mixed for 15 min, and then filtered (Whatman No. 1 paper). The filtrate was partitioned with 20 mL ethyl acetate three times, centrifuged (NUVE NF 800R, Turkey) at 3,650×g for 5 min, evaporated at 50 °C, and subsequently dissolved in 1 mL methanol and 14 mL 37% (v/v) HCl. The sample was then hydrolyzed in an ultrasound bath for 2 h, partitioned with 20 mL dichloromethane three times, and centrifuged at 3,650×g for 5 min. The organic phase was evaporated, dissolved in 5 mL methanol, filtered through a 0.45 µm nylon syringe filter, and injected into the HPLC without dilution (model LC20A, Shimadzu Corporation, Japan). The HPLC system was equipped with a C18 reversed-phase column (GL Sciences Inc., Inertsil® ODS-3, 5 µm, 4.6 × 250 mm, Japan) and a guard-column (C18) held at a temperature of 35 °C, an ultraviolet (UV) detector (UV/VIS Waters 2487, USA) set at 370 nm, and a photodiode array detector (UV/VIS Waters 996, USA) run under the general-purpose mode on a Thermo Scientific Hypersil column to evaluate the specificity.

The mobile phase consisted of solution A (deionized water containing 0.01% (v/v) trifluoroacetic acid), and solution B (acetonitrile containing 0.08% (v/v) trifluoroacetic acid). It was passed through a 0.2 µm filter membrane and run at a flow rate of 0.6 mL/min, using the following gradient profile: 0 min at 50% B, 0–2 min at 60% B, 2–4 min at 80% B, and 4–7 min at 95% B. Authentic quercetin (1 mg) was dissolved in 1 mL methanol as a stock solution and stored in dark glass bottles at 4 °C. An analytical curve was prepared using a 5-point calibration at 20, 40, 60, 80, and 100 µg/mL of standard quercetin (HPLC

grade, Sigma-Aldrich, USA) in methanol and mobile phase. The standards were filtered through 0.45 µm nylon syringe filters and injected into the HPLC. The phenolic compound concentrations were calculated from the retention time and peak area.

Bacterial strains

Four pathogenic bacterial strains, including two field isolates (*E. coli* ECCM62 and *S. aureus* SACM62) and two reference strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923), were selected for this study. The field isolates were derived from mastitis milk from dairy cows that were primarily recruited using the California mastitis test (Hendrix and Sirois, 2007). The mastitis bacteria were microbiologically isolated and identified according to the method described previously (Quin et al., 2001). Briefly, 0.01 mL of the milk sample was cultured on 5% defibrinated sheep's blood agar and MacConkey's lactose agar plates at 37 °C for 18–24 h.

The isolates were further identified by molecular identification as *S. aureus* and E. coli. Single colonies of each bacterial isolate were selected for molecular identification (Quin et al., 2001; Wang et al., 2002; Strommenger et al., 2003). Genomic DNA was extracted using a GF-1 bacterial DNA extraction kit (Vivantis Technologies Sdn Bhd, Malaysia), according to the manufacturer's protocol. The *E. coli* and *S. aureus* 16S rRNA genes were amplified using the primers (Strommenger et al., 2003; Wang et al., 2002) synthesized by Humanizing Genomics macrogen, Korea. All PCR reactions were performed using 2×Taq Master Mix (Vivantis Technologies Sdn Bhd, Malaysia) in a thermal cycler (Bibby Scientific™ Techne™ TC-512 Gradient Thermal Cycler, UK). PCR amplicons were submitted for sequencing to Barcode Taq Sequencing by CELEMIC (The NGS-Based Sequencing Technology, Celemic, Korea), and then analyzed using the Basic Local Alignment Search Tool (BLAST) to search for biological sequence homology between *E. coli* or *S. aureus* in the GenBank database (The National Center for Biotechnology Information). The 16S rRNA gene sequences were aligned using CLUSTALW and a phylogenetic analysis was also performed according to modified Kumar et al. (2016) and Fahim et al. (2019) using neighbor-joining approach and 500-bootstrap consensus replication method based on 16S rRNA genes for *S. aureus* and *E. coli* strains in MEGA version 11 software.

The selected field isolates of *E. coli* ECCM62 and *S. aureus* SACM62 possessed 16S rRNA genes with high homology (>99% similarity) compared to gene sequences in GenBank database ─ *S. aureus* (MK945592.1 and NBNF01000010.1) and *E. coli* ─(MK503655.1 and KY095112.1) and published gene sequences for *E. coli* (Fahim et al., 2019) and *S. aureus* (Takahashi et al., 1997) strains, respectively, isolated from mastitis in dairy cows.

Determination of MIC and MBC of *M. alba* L. Leaf Extract

The antibacterial activity of the *M. alba* L. leaf extract was determined using a broth microdilution method and was reported as MIC and MBC (Budiman et al., 2017). Briefly, stock solutions of herbal extract and quercetin were prepared by dissolving the agents in 25% (v/v) dimethyl sulfoxide

(DMSO) to give a final concentration of 160 mg/mL. A 100 µL volume of Mueller Hinton broth (MHB) was loaded into each well of a 96-well round-bottomed microliter plate (Costar, Corning Inc., USA). The extract was then prepared as twofold serial dilutions of 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 mg/mL in triplicate. DMSO (0.006–52.5 µL/mL) and azithromycin (0.32–0.000625 mg/mL) were also used as the negative and positive controls, respectively. A 10 µL volume of the desired bacterial suspension, which had been adjusted to a 0.5 McFarland turbidity standard reading (approximately 1.5×10^8 CFU/mL), was added to each well, and the plate was covered with a lid and incubated at 37 °C for 24 h. The lowest extract concentration that showed no visible bacterial growth was considered the MIC. The MBC was determined by streaking the suspension in the 96-well plate that represented the MIC on MHB and incubating at 37 °C for 24 h. The MBC was recorded as the lowest concentration with no detectable bacterial growth.

Determination of antibacterial activity by the time-kill kinetic method

The antibacterial activity of the *M. alba* L. extract was determined against the four selected bacteria using a time-kill assay (Appiah et al., 2017). In brief, the extract was used at 1 × MIC and 2 × MIC concentrations for varying lengths of time. Three tubes were prepared for each tested strain (one growth control containing normal saline and two tubes containing the leaf extract). A 5×10^8 CFU/mL log-phase inoculum was added to each tube, along with MHB. The bacterial viability was determined at 8 time points (after 1, 5, 15, 30, 60, 120, 180, and 360 min incubations). Bacterial growth in each tube was determined by performing three consecutive 1:10 (v/v) dilutions of 0.1 mL aliquots from each tube with MHB, plating them, and incubating the plates at 37 °C for 24 h. Plates with 30–300 colonies were counted, and the kill rates were plotted as log10 viable counts (CFU/mL) versus time.

Statistical analysis

All statistical analyses were performed using the Statistics Package for the Social Sciences (SPSS version 26.0, IBM SPSS Statistics; USA). Nonparametric tests (independent-samples Mann-Whitney U test) were used to compare the MIC or MBC between *E. coli* and *S. aureus*. A value of $p<0.05$ was considered statistically significant.

RESULTS

Yield of *M. alba* L. leaf extract

After a 7-day maceration in 95% ethanol (v/v), the extract yield for all *M. alba* L. leaves in this study was 11.29%.

Quercetin determination in *M. alba* L. Extract

Quercetin was an active flavonoid substance found by HPLC analysis of the crude leaf extract and was extracted with a yield of 83.19 ± 0.27 µg/g of dried plant (Figure 1).

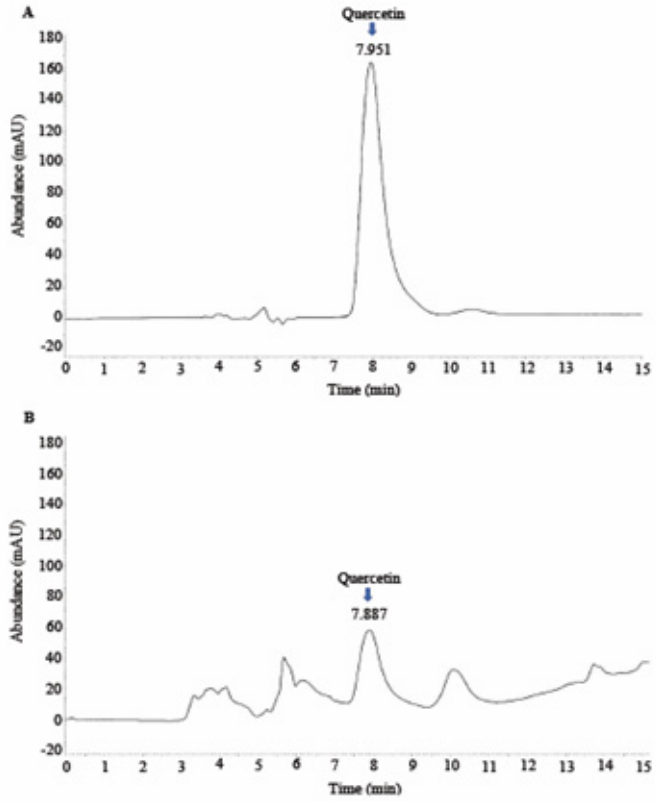

Figure 1 Chromatograms of quercetin by HPLC (Shimadzu model LC-20A) at 370 nm; (A) Quercetin standard, and (B) Quercetin in *M. alba* L. leaves crude extract.

Antibacterial activity by determination of the MIC and MBC

The *M. alba* L. leaf extract had antibacterial activity against field and ATCC bacterial strains, as confirmed by the MIC and MBC values shown in Table 1. The MIC and MBC were both 20 mg/mL for the *E. coli* ECCM62 mastitis isolate and 10 and 20 mg/mL, respectively, for the *E. coli* ATCC 25922 strain, whereas the MIC and MBC were both 10 mg/mL for the *S. aureus* SACM62 mastitis isolate and 10 and 20 mg/mL, respectively, for the *S. aureus* ATCC 25923 strain. The extract and quercetin had similar MIC and MBC values for both ATCC reference strains. The MIC and MBC values differed for the different pathogens, as shown by asymptotically significant p-values <0.05. The inhibitory effect was stronger against *S. aureus* than against *E. coli* (p<0.05).

Table 1 Antibacterial activity of *M. alba* L. leaf extract, azithromycin, and quercetin against *E. coli* and *S. aureus* pathogenic strains.

Bacteria	*M. alba* L. leaf extract (mg/mL)		Azithromycin (mg/mL)		Quercetin (mg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli ECCM62	20	20	0.04	0.04	n/a	n/a
E. coli ATCC 25922	10	20	n/a	n/a	10	20
S. aureus SACM62	10	10	0.005	0.005	n/a	n/a
S. aureus ATCC 25923	10	20	n/a	n/a	10	20

MIC = minimal inhibitory concentration, MBC = minimum bactericidal concentration n/a = not applicable

Antibacterial activity by the time-kill kinetic method

Time-kill analysis was performed to evaluate the kinetic killing profile of the *M. alba* extract at 1 × MIC and 2 × MIC concentrations following treatment of *S. aureus* and *E. coli* for 0, 1, 5, 15, 30, 60, 180, and 360 min. The killing ability of the leaf extract showed time dependence for all four bacterial strains. The bactericidal effects of the leaf extract on *E. coli* ATCC 25922 and the *E. coli* ECCM62 mastitis isolate are shown in Figure 2. The log reductions after a 360 min exposure to 20 and 40 mg/mL *M. alba* were 5.1 and 6.8 log CFU/mL, respectively, for *E. coli* ATCC 25922 and 3.9 and 6.8 log CFU/mL, respectively, for the *E. coli* ECCM62 mastitis isolate. The effects on *S. aureus* ATCC 25923 and the *S. aureus* SACM62 mastitis isolate are shown in Figure 3. The reduction rates after a 30 min exposure to 10 and 20 mg/mL leaf extract were 100% for both *S. aureus* ATCC 25923 and the *S. aureus* SACM62 mastitis isolate, indicating that the antibacterial ability of the *M. alba* leaf extract was more effective against *S. aureus* than against *E. coli* in terms of both concentration and time required to kill the bacteria.

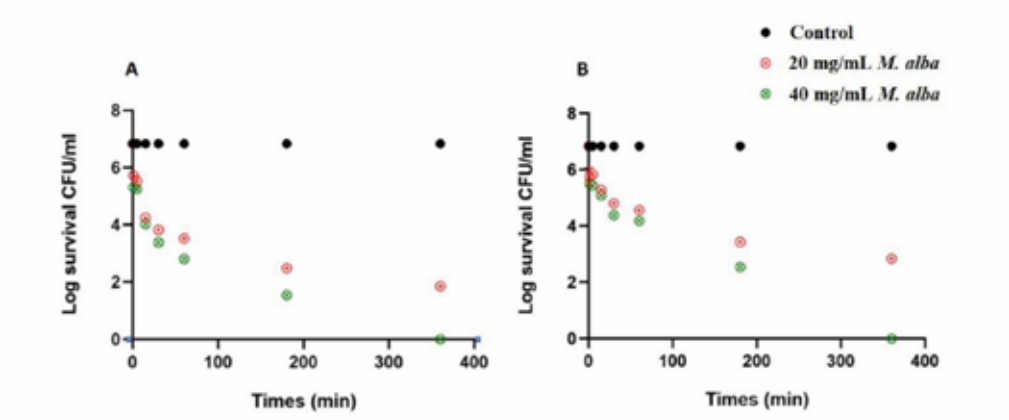

Figure 2 The bactericidal effect of *M. alba* on time-killing in *E. coli*. (A) *E. coli* ATCC 25922 and (B) *E. coli* ECCM62 field strains were exposed to normal saline (control), 20 and 40 mg/mL *M. alba* extract for 0, 1, 5, 15, 30, 60, 180 and 360 minutes.

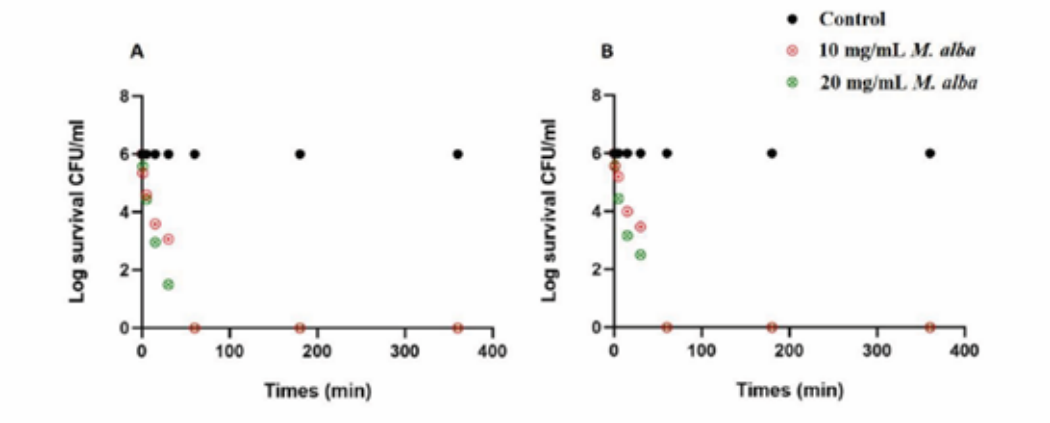

Figure 3 The bactericidal effect of *M. alba* on time-killing in *S. aureus*. (A) *S. aureus* ATCC 25923 and (B) *S. aureus* SACM62 field strains were exposed to normal saline (control), 10 and 20 mg/mL M. alba extract for 0, 1, 5, 15, 30, 60, 180 and 360 minutes.

DISCUSSION

Yield of *M. alba* L. leaf extract

In this study, *M. alba* L. leaf extraction yield higher than in a previously reported value (8.02%) that used a similar extraction and 2-day maceration of *M. alba* L. var. Buriram 60 leaves (Pulbutr et al., 2018). By contrast, a longer maceration period resulted in much lower yields (7.35–8.52%) for ethanolic extracts of *M. nigra* L. leaves after a 10-day maceration (de Freitas et al., 2016). These yield extract differences could reflect the effects of multiple factors, including the mulberry species, location of the sample, maceration period, and choice of extraction solvent (commonly 95% ethanol) (Kobus-Cisowska et al., 2020). However, a 7-day maceration could improve the yield obtained by ethanolic extraction of *M. alba* L. leaves.

Quercetin determination in *M. alba* L. extract

Quercetin is well known as a predominant flavonol glycoside in mulberry leaves (Peanparkdee et al., 2016; Zhai et al., 2018). A previous study showed that a *Morus* spp. leaf extract contained 102.16 ± 1.28 µg/mL quercetin, compared to luteolin at 100.52 ± 1.63 µg/mL and isoquercitin at 99.97 ± 1.06 µg/mL (de Freitas et al., 2016). Another study demonstrated that the ethanol concentration modulated the quercetin content extracted from *M. alba* leaves, as 95% ethanol yielded the highest amount of quercetin (1.52 µg/g of dry leaves), with lower amounts extracted with 50, 60, and 70% ethanol (Kobus-Cisowska et al., 2020). The highest average quercetin content, at 2323.90 ± 145.35 µg/g, was reported for hydrolyzed extracts from *M. nigra* L. (black mulberry), with kaempferol being the next highest extract component, at 1446.36 ± 59 µg/g (Zhai et al., 2018). Extraction of these active substances from mulberry leaves depends on many factors, such as the solvent concentration and type, the time and method of maceration, the temperature of fermentation, the species of *Morus*, and the location where the samples are collected (Peanparkdee et al., 2016; Pothinuch and Tongchitpakdee, 2019).

Antibacterial activity by determination of the MIC and MBC

This study demonstrated that ethanolic *M. alba* L. leaf extract had potentially antibacterial spectrum against the selected dairy mastitis pathogens and reference bacterial strains according to the MIC and MBC values. The inhibition activity of the extract was like authentic quercetin and clearly affected both bacterial strains, but stronger against *S. aureus* representing gram-positive bacteria than *E. coli*—gram-negative bacteria representative. A previous study showed similarly good inhibition of a 95% ethanolic *M. alba* leaf extract, with a reported MIC of 0.32 mg/mL for both *S. aureus* and *E. coli* strains (Abdel-Hamid et al., 2017). A mulberry extract at 0.32 to 1.28 mg/mL (MIC and MBC) was shown to inhibit *E. coli*,

S. aureus, and *Salmonella typhi* (Aelenei et al., 2019). A *M. alba* extract also inhibited the growth of *S. aureus* ATCC 33591 and ATCC 43300 at 250 µg/mL (MIC or MBC) (Chotigarpa et al., 2019). In accordance with previous studies on the antibacterial properties of some organic acids (Pangprasit et al. (2021) and herbal extract (Kuraeiad, et al., 2022) against reference and major mastitis pathogen strains of *E. coli* and *S. aureus*. Acetic acid had antibacterial property of against major dairy mastitis pathogens such as *S. aureus* and *E. coli* with MIC and MBC values of 1.25 mg/mL and 2.5–10 mg/mL (Pangprasit et al. (2021). Coconut oil extract could also enhance antibacterial activities against *S. aureus* ATCC 29523 and *E. coli* ATCC 29522 (Kuraeiad, et al., 2022).

Antibacterial activity by the time-kill kinetic method

Time killing can ascribed to interactions between the tested bacteria and antimicrobials present in the mulberry leaf extract during the time of exposure with the bacteria. In this study, the mulberry leaf extract completely eliminated the tested *S. aureus* strains, indicating that the antibacterial ability of the *M. alba* leaf extract was more effective against

S. aureus than against *E. coli* in terms of both concentration and time required to kill the bacteria. These findings agree with a prior study on the kinetics of the killing profile of lactic acid against both standard and mastitis pathogen strains of *E. coli* and *S. aureus* (Wu et al., 2019).

Another study reported some discouraging time-kill assay results for an ethanolic *M. alba* L. extract against *S. aureus* strain 25923 (a methicillin-susceptible strain) and *S. aureus* 33591 (a methicillin-resistant strain). Subjecting the bacteria to a concentration of 0.5 MIC at 6 time points (2, 4, 8, 12, 20, and 24 h) did not reduce the CFU counts of *S. aureus* even after 24 h (Pang et al., 2019). The mulberry fruit extracts had certain bactericidal activity against *S. aureus* ATCC 25923, taking 24 h to kill the bacteria (Suriyaprom et al., 2021). However, other research has reported the separation of specific substances from mulberries that show positive results against tested pathogens by the time-kill assay. For example, Sanggenon D was isolated from mulberry root bark extract and tested at 1 × MIC and 2 × MIC against *S. aureus*. At 1 × MIC and 2 × MIC, the extract decreased the colonies of *S. aureus* at 2.1 $\log_{10}$ CFU/mL for 2 h and after treatment for 8 h (Anim et al., 2015). These studies showed that quercetin inhibited *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 at a MIC of 10 mg/mL and an MBC of 20 mg/mL. Quercetin is a major substance and an active flavonoid compound (MIC 260 µg/mL) in *M. alba* L. leaves, and it shows activity against *S. aureus* clinical isolates in humans and against *S. aureus* ATCC 43300 (Yu et al., 2018).

This study firstly demonstrated the antibacterial of the ethanolic mulberry leaf extract containing quercetin against dairy mastitis pathogens and reference ATCC strains. The targeted field strains were genetically homologous to gene sequences in GenBank database and literatures for

S. aureus and *E. coli* found in Asia and Europe. The extract had potentially antibacterial spectrum against both gram-negative and gram-positive bacteria possessing different bacterial structure, commonly known as clinical strains─major contagious (*S. aureus*) and environmental (*E. coli*) mastitis pathogens in dairy cows. However, there are limitations associated with the antibacterial properties of the extract that may lead to erroneous inferences. The current study did not cover all other phytochemicals of mulberry leaf and diverse mastitis-causing pathogens. Further research is warranted to clearly understand the antibacterial properties of the mulberry leaf extract.

CONCLUSION

Extraction of *M. alba* L. leaves with 95% ethanol yielded considerable amounts and an average quality of quercetin, a major active flavonoid in mulberry leaves. The ethanolic *M. alba* L. leaf extract showed antibacterial efficacy against selected strains of *E. coli* ECCM62 and *S. aureus* SACM62 isolated from mastitis in dairy cows, as well as *E. coli* ATCC 25922 and

S. aureus ATCC 25923, and gave MIC values of 10 mg/mL and MBC values of 20 mg/mL. The mulberry leaf extract had a stronger antibacterial effect on *S. aureus* than on *E. coli* based on extract concentration and time-killing assays.

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AUTHOR CONTRIBUTIONS

Conceptualization and study design, XP, CT, PS JA and RM; data acquisition, analysis and interpretation, XP, CT, PS, JA and RM; supervision, CT, JA and PS; funding acquisition and project administration, CT; writing—original draft preparation, XP, JA and RM; writing—review, editing and approval, CT. All authors have read carefully read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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