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Research Article
In vitro Antibacterial Activity of *Capparis sepiaria* L. Against Human Pathogenic Bacteria

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Abstract

Background and Objective: The rise of antibiotic-resistant bacteria is a major medical problem. The finding of new source of antibiotic substance is required. The present study was aimed to determine the antibacterial activity of extracts from *Capparis sepiaria* L. collected from Roi Et Rajabhat University forest, Thailand. **Materials and Methods:** The stalk, fruit and leaves of *C. sepiaria* L. were extracted using four different solvents including hexane, ethyl acetate, dichloromethane and methanol. The *C. sepiaria* L. extracts and grinded fresh fruit were screened for their antibacterial activity against six pathogenic bacteria (*Staphylococcus aureus* TISTR 1466, *Staphylococcus epidermidis* TISTR 518, *Bacillus subtilis* TISTR 008, *Pseudomonas aeruginosa* TISTR 2370, *Escherichia coli* TISTR 780 and *Klebsiella pneumoniae* TISTR 1383) using disc diffusion method. **Results:** The result indicated fruit extracts and grinded fresh fruit can be inhibited the growth of Gram-positive and Gram-negative bacteria. The Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of each extract were evaluated using iodonitrotetrazolium chloride (INT) colorimetric assay. The results indicated that the lowest MIC value of 0.31 mg mL^{-1} against *Staphylococcus aureus* TISTR 1466, *Klebsiella pneumoniae* TISTR 1383 and *Escherichia coli* TISTR 780 was obtained from *C. sepiaria* L. fruit extracts. The lowest MBC value at 0.62 mg mL^{-1} was presented in methanolic extract from *C. sepiaria* L. fruit against *B. subtilis* TISTR 008. **Conclusion:** This was the first report to demonstrate the antibacterial substance was presented in *C. sepiaria* L. fruit which can be developed for new natural drug production.

Key words: *Capparis sepiaria* L., anti-pathogenic bacteria activity, antibacterial substance, grinded fruit, drug production

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to the rapid global and rising of antibiotic-resistant bacteria, the need for the discovery of newer and alternative drug agents for the remediation of drug-resistant diseases. The pathogenic bacteria frequently develop to improve antimicrobial-tolerance before antimicrobial resistance development[1]. This problem has become a significant public health threat as there are fewer or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria[2].

Plants are source of antibiotic substances and have been used to treat infectious diseases for at least 2000 years[3]. Many plants have been used to study the antibacterial activity of extracts such as *Oxalis corniculate*, *Cinnamomum tamala*, *Ageratina adenophora*, *Artemesiavulgaris*[2], *Cuminum cyminum*, *Punica granatum*, *Syzygium aromaticum*, *Thymus vulgaris*, *Zingiber officinale*[4], *Allium sativum*, *Bunium persicum* (Boiss.) B. Fedtsch, *Oryza sativa* L., *Triticum aestivum* L.[5] and *Capparis* plant[6], etc. A few reports of antibacterial activity from Capparis plant extracts were presented such as *Capparis brevispina* DC has been report about antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*[6]. *Capparis spinosa* and *Capparis decidua* were presented antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis* and *Pasteurella multocida*[7]. *Capparis sepiaria* was reported their antibacterial activity against *E. coli*, *P. mirabilis* and *E. aerogenes*[8].

Capparis L. or Caper is a large natural distribution shrub plant which used to cure various illnesses in traditional medicines. Many active phytochemical substances were found in this plant including spermidine, rutin, quercetin, kaempferol, stigmasterol, campestrol, tocopherols and carotenoids[9]. *Capparis* species has been reported for their medicinal activity such as hepatoprotective activity, analgesic activity[10], antibacterial activity[11], antidiabetic activity[12], anti-hyperlipidemic[13], anti-inflammatory[14], treatment of stomach problems, cough, cold, asthma, ulcers, vomiting, diabetes, fever, gout, jaundice, dysentery, smallpox, cholera and diarrhea[15].

Capparis sepiaria L. is a local plant located at Roi Et Rajabhat University forest, Thailand. Only little information was reported about antibacterial activity presented in *C. sepiaria* L. extracts and fresh fruit. Therefore, the aim of this research was to evaluate the anti-pathogenic bacterial activity of *C. sepiaria* L. extracts and fresh fruit against six human pathogenic bacteria. The finding of this research is important for drug development to treat bacterial infectious disease.

MATERIALS AND METHODS

Study area: All the experiments were performed during October, 2018 to April, 2019 in the Microbiology Laboratory, Major of General Science, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Chemicals and reagents: Hexane, Dichloromethane, Ethyl acetate, Methanol were purchased from QRëC™ (Republic of New Zealand), Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich Co. (St. Louis, Missouri, U.S.A.), Nutrient Broth (NB) and Agar powder were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd, India).

Human pathogenic bacteria: Three strains of Gram positive (*Staphylococcus aureus* TISTR 1466, *Staphylococcus epidermidis* TISTR 518, *Bacillus subtilis* TISTR 008) and three strains of Gram negative (*Pseudomonas aeruginosa* TISTR 2370, *Escherichia coli* TISTR 780 and *Klebsiella pneumoniae* TISTR 1383) bacteria were purchased from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. All pathogenic bacteria were cultured in Nutrient Agar (NA) and stored at 4°C until used.

***Capparis sepiaria* extracts preparation:** The fruits, stalks and leaves of *Capparis sepiaria* L. which belonging to the Family, Capparaceae were collected from Roi Et Rajabhat University forest, Thailand (Fig. 1). The plant identification was confirmed by Forest Botany Division (Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation), Thailand (BKF No. 196970). For plant extraction, fruits, stalks and leaves of *C. sepiaria* L. were dried by using hot air oven (POL-EKO-APARATURA Company, WodzisławŚląski, Poland) at 50°C for 3 days. Dried plant samples were powdered using a mixer grinder. Plant powders were stored in an auto desiccator cabinet (PATRON, Taiwan) until used. For grinded fresh *C. sepiaria* L. fruits, the fruits were washed 3 times and then grinded using a sterile mortar before the experiment.

Ten grams of each plant powder was taken in 250 mL Erlenmeyer flask and 100 mL of each extraction solvent including hexane, ethyl acetate, dichloromethane and methanol were added individually. The mixtures were extracted at room temperature with shaking at 150 rpm for 48 hrs. The extract of each plant part was filtered through Hyundai Micro No. 10 filter paper. Each filtrate was evaporated and dried at 40°C under reduced pressure using rotary

Fig. 1: *Capparis sepiaria* L.

vacuum evaporator (BÜCHI Labortechnik AG, Switzerland). Each crude extract was mixed with Dimethyl sulfoxide (DMSO, Sigma) to the final concentration at 50 mg mL^{-1} before used. Screening of antibacterial activity of *Capparis sepiaria* extracts: Six pathogenic bacteria (*S. aureus* TISTR 1466, *S. epidermidis* TISTR 518, *B. subtilis* TISTR 008, *P. aeruginosa* TISTR 2370, *E. coli* TISTR 780 and *K. pneumoniae* TISTR 1383) were cultured with shaking at 37°C using Nutrient Broth (NB) for 18 hrs and the bacterial concentration was adjusted at OD600 to 0.1 using a spectrophotometer. The disc diffusion method was used to screen the antibacterial activity of plant extracts and grinded fresh fruits. For plant extracts, 100 mL of each pathogenic bacteria were spread on NA and the sterile filter paper disc with a diameter of 6.0 mm was placed onto agar. Twenty micro liters of each plant extract were loaded onto sterile filter paper disc. The DMSO and kanamycin were used as control. For grinded fresh fruit, the ground fresh fruits were transferred using aseptic technique onto agar medium containing pathogens[16]. Plate was incubated at 37°C for 24 hrs in bacterial incubator (JSR, Korea). The presence of inhibition zone was recorded and considered as indication for an antibacterial activity.

***In vitro* antibacterial activity of *Capparis sepiaria*. extracts:** The plant extract that presented inhibition zone against human pathogenic bacteria was determined their MIC and MBC using micro broth dilution method in 96-well microtiter plate. Two-fold serial dilutions of plant extracts were done in 96-well plate containing NB to obtain various concentrations (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 and 0.048 mg mL^{-1}). The pathogenic bacteria inoculum (OD_{600} = 0.1) was added in each well. Kanamycin was used as positive control and cell free NB was used as negative control. The 96-well microtiter plate was incubated at 37°C for 24 hrs. Iodonitrotetrazolium chloride (INT) (GTI Laboratories Supplies, Texas) was added in each well of 96-well microtiter plate and was incubated at 37°C for 30 min. The wells containing the pathogenic bacterial growth turned to purple color whereas the well without pathogenic bacterial growth remained yellow. The MIC value was considered as the lowest concentration of the plant extract that completely inhibits the bacterial growth[2]. MBC was defined as the lowest concentration of plant extract that did not exhibit any bacterial growth, which did not produce a color change after addition of INT[17,18].

Data analysis: The inhibition zone was measured and expressed as the length of the diameter (mm). The MIC and MBC values were determined and presented as the concentration of plant extract (mg mL^{-1}).

RESULTS AND DISCUSSION

Disc diffusion assay of *C. sepiaria* L. extracts: The fruits, stalks and leaves of *C. sepiaria* L. were extracted using 4 different solvents including hexane, ethyl acetate, dichloromethane and methanol. The plant extracts and fresh fruit were evaluated for their antibacterial activity against six pathogenic bacteria using the disc diffusion method. The results indicated that the highest of inhibition zone at 12 mm was obtained from fruit extracted using hexane, ethyl acetate and dichloromethane against *E. coli* TISTR 780 and *B. subtilis* TISTR 008. The *C. sepiaria* L. stalks extracts were potentially effective in inhibiting only *B. subtilis* TISTR 008 (7-8 mm) and leaves extracts were no potentially effective in inhibiting pathogenic bacterial growth (Table 1). The result of this research was similar to Satyanarayana *et al.*[19] which reported 62.5-500 mg mL^{-1} of ethanol soluble extract was inhibited the bacterial growth (*E. faecalis, S. aureus*, *P. aeruginosa* and *E. coli*) with 8-20 mm zone of inhibition. Kalpana and Prakash[10] have presented that the ethanolic leaf extracts of *C. sepiaria* L. were inhibited the tested bacterial growth at 0.8-2.1 cm of zone of inhibition. Ethanolic Fruit Extracts of *C. sepiaria* L. were showed the inhibition zone at 1.0-2.4 cm against 5 tested bacteria. Abdalrahman *et al.*[20] reported that the most effective antimicrobial activity of twigs extracts of *C. decidua* at 21 cm of inhibition zone was found in ethyl acetate extract. Some result of extracts from this study were no effective inhibiting pathogenic bacterial growth might be from low

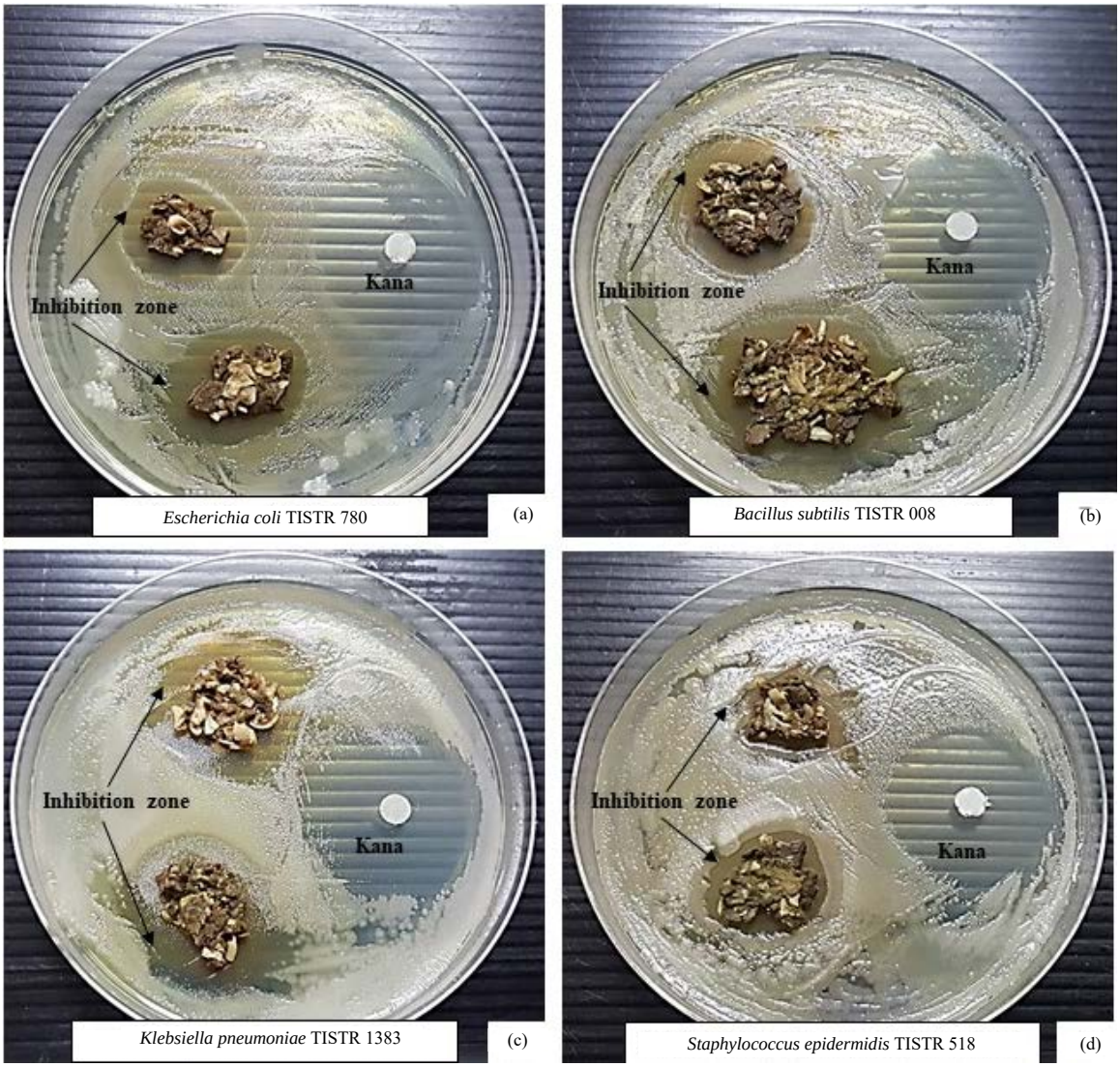

Fig. 2(a-d): Zone of inhibition of grinded fresh fruit of *C. sepiaria* L. against (a) *E. coli* TISTR 780, (b) *B. subtilis* TISTR 008, (c) *K. pneumoniae* TISTR 1383 and (d) *S. epidermidis* TISTR 518

concentration of extract or that extracts did not contain any active antibacterial substances. The results demonstrated that the grinded fresh *C. sepiaria* L. fruit inhibited all tested human pathogenic bacteria including *E. coli* TISTR 780 (Fig. 2a), *B. subtilis* TISTR 008(Fig. 2b), *K. pneumoniae* TISTR 1383 (Fig. 2c) and *S. epidermidis* TISTR 518 (Fig. 2d). This result was similar with previous report that demonstrated that fresh *C. sepiaria* L. fruit had both of antibacterial and antifungal activity[16].

MIC and MBC values of *C. sepiaria* L. extracts: The result revealed that the lowest MIC value of stalk extracted using ethyl acetate against *E. coli* TISTR 780 was at 0.31 mg mL^{-1}. The lowest MIC values of fruit extract using hexane, dichloromethane and methanol against *S. aureus* TISTR 1466, *K. pneumoniae* TISTR 1383 and *E. coli* TISTR 780, were at 0.31 mg mL^{-1}. The lowest MIC values of leaves extracted using hexane, ethyl acetate, dichloromethane and methanol against *S. aureus* TISTR 1466, *S. epidermidis* TISTR 518, *B. subtilis* TISTR 008, *K. pneumoniae* TISTR 1383 and *E. coli* TISTR 780 were at 0.62 mg mL^{-1} (Table 2). The MIC values of *Capparis sepiaria* L. extracted using methanol were lower than previous report from Moharram *et al.*[21] which reported that MIC values of 5.0 mg mL^{-1} against *S. aureus* were obtained from *C. sepiaria* L. leaves and stem extracted using methanol and 11.25 mg mL^{-1} against *S. aureus* was obtained *Capparis*

Table 1: Inhibition zone diameter of plant extracts using the disc diffusion method

		Inhibition zone (mm)					
Parts of plant	Solvent	*S. aureus* TISTR 1466	*S. epidermidis* TISTR 518	*B. subtilis* TISTR 008	*P. aeruginosa* TISTR 2370	*K. pneumoniae* TISTR 1383	*E. coli* TISTR 780
Stalk	H	NA	NA	8±0.23	NA	NA	NA
	E	NA	NA	7±0.12	NA	NA	NA
	D	NA	NA	0.0±00	NA	NA	NA
	M	NA	NA	0.0±00	NA	NA	NA
Fruit	H	10±0.12	10±0.11	12±0.22	NA	0.0±00	12±0.12
	E	10±0.12	8±0.22	12±0.21	NA	0.7±0.11	12±0.12
	D	11±0.23	8±0.23	12±0.13	NA	10±0.08	12±0.12
	M	6±0.14	7±0.12	6±0.16	NA	10±0.02	6±0.22
Leaves	H	NA	NA	NA	NA	NA	NA
	E	NA	NA	NA	NA	NA	NA
	D	NA	NA	NA	NA	NA	NA
	M	NA	NA	NA	NA	NA	NA
Grinded fresh fruit		13±0.00	5±0.00	12±0.00	5±0.00	20±0.00	5±0.00

H: Hexane, E: Ethyl acetate, D: Dichloromethane, M: Methanol, NA: No activity

Table 2: MICs and MBCs values in mg mL^{-1} of *Capparis sepiaria* L. extracts and kanamycin

	MIC and MBC (in bracket) values (mg mL^{-1})												
	Stalk				Fruit				Leaves				
Pathogenic bacterial strains	H	E	D	M	H	E	D	M	H	E	D	M	Kana
S. aureus TISTR 1466	1.25	1.25	-	1.25	0.31	1.25	0.31	0.31	0.62	1.25	1.25	1.25	0.039
	>2.5	>2.5		>2.5	1.25	>2.5	1.25	>2.5	>2.5	>2.5	>2.5	>2.5	(0.078)
S. epidermidis TISTR 518	0.62	0.62	-	1.25	2.5	2.5	2.5	0.62	-	1.25	-	0.62	0.039
	2.5	>2.5		>2.5	>2.5	>2.5	>2.5	>2.5		>2.5		>2.5	(0.078)
B. subtilis TISTR 008	0.62	0.62	-	-	0.62	0.62	0.62	0.15	1.25	0.62	0.62	0.62	0.039
	>2.5	>2.5			>2.5	>2.5	>2.5	0.62	>2.5	>2.5	2.5	>2.5	(0.078)
P. aeruginosa TISTR 2370	-	-	-	-	2.5	2.5	-	2.5	-	-	2.5	-	0.62
					>2.5	>2.5		>2.5			>2.5		(2.5)
K. pneumoniae TISTR 1383	1.25	0.62	-	0.62	2.5	1.25	0.31	0.31	1.25	1.25	0.62	1.25	0.039
	>2.5	>2.5		>2.5	>2.5	2.5	2.5	2.5	>2.5	>2.5	>2.5	>2.5	(0.31)
E. coli TISTR 780	0.62	0.31	1.25	1.25	1.25	1.25	0.31	0.31	1.25	1.25	0.62	0.62	0.039
	2.5	2.5	>2.5	2.5	2.5	>2.5	1.25	2.5	2.5	>2.5	2.5	2.5	(0.078)

H: Hexane, E:Ethyl acetate, D: Dichloromethane, M: Methanol, Kana: kanamycin

zeylanica root extracted using methanol[22]. Rahimifard *et al.*[23]. were reported methanolic fraction of *C. cartilaginea* was the most effective fraction with MIC of 10.42 μg mL^{-1} against *Salmonella enterica*. The highest antibacterial activity of *C. mucronifolia* was against *Staphylococcus epidermidis* with MIC of 7.8 μg mL^{-1}.

The lowest MBC value at 0.62 mg mL^{-1} was presented in *C. sepiaria* L. fruit extracted using methanol against *B. subtilis* TISTR 008. Follow by at 1.25 mg mL^{-1} were obtained from hexane and dichloromethane extracts against *S. aureus* TISTR 1466 and *E. coli* TISTR 780, respectively (Table 2). The result of this research was similar to Al-Bayati and Al-Jarjry[24] that reported the lowest MBC values from *C. spinosa* root extracts using ethanol and chloroform extraction were at 1 mg mL^{-1} against *S. aureus*, *B. subtilis* and *Proteus vulgaris*. Upadhyay *et al.*[25] also reported about the chloroform extract has shown lowest MBC value for *Lactobacillus acidophilus* 0.125 μg mL^{-1} followed by intermediate MBC values against *K. pneumoniae* and *E. coli* (0.25 μg mL^{-1}). This is the first report about *C. sepiaria* L. fruit extract was the high potential antibacterial activity against *B. subtilis* TISTR 008, *S. aureus* TISTR 1466 and *E. coli* TISTR 780. It will be useful for development of drug production. This report was presented the potential *C. sepiaria* L. extracts of antibacterial activity. The result was indicated that *C. sepiaria* L. extracts and fresh fruit can be eliminated the tested pathogenic bacteria that useful for drug development.

CONCLUSION

The extracts of fruits, stalks, leaves and fresh fruit of *C. sepiaria* L. were determined their antibacterial activity against 6 human pathogenic bacteria. The results demonstrated that the lowest MIC value of 0.31 mg mL^{-1} was

obtained from *C. sepiaria* L. fruit extracts and the fresh fruit of *C. sepiaria* L. was presented antibacterial activity against all tested pathogenic bacteria by showing the inhibition zone on plate.

SIGNIFICANCE STATEMENT

This study discovers the novel antibacterial activity from *Capparis sepiaria* L that can be beneficial for the new drug development from natural plant. This study will help the researcher to uncover the critical areas of the evaluation of antibacterial activity of *Capparis sepiaria* L. extracts that many researchers were not able to explore. Thus, a new application using the antibacterial activity obtained from *Capparis sepiaria* L. extracts may be arrived at.

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