EVALUATION OF *TOONA SINENSIS* ETHANOL EXTRACT AGAINST SELECTED MICROORGANISMS

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Introduction

Due to health concerns, consumers tend to accept "natural" antimicrobial agents rather than the artificial ones. Recently, antimicrobial properties of plant and spice extracts have been widely reported. *Toona sinensis* is a perennial tree that has become widely grown in Taiwan and China (Edmonds and Staniforth, 1998). Its tender buds have a special aroma and they are often consumed in Taiwan. Even though *Toona sinensis* was reported to have some antimicrobial activity (Shi, 2003), limited information regarding the antimicrobial effect of this plant was available. The objective of this study was to evaluate the antimicrobial effect of *Toona sinensis* ethanol extract against some selected common spoilage and pathogenic microorganisms in meat.

Materials and Methods

Fresh tender buds of *Toona sinensis* were purchased from a local farm in Pingtung, Taiwan, washed, dried in an oven at 45-50°C until totally dried (approximately 24 hrs), pulverized into powder, mixed with ethanol at a 1:20 (w/v) ratio, heated in a water bath of 50°C for 2 hrs, and filtered. The same procedure was conducted twice. The filtered residues of extract were then vacuum condensed at 50°C until dry and stored at 4°C. A 100µl of diluted bacterial suspension of *Staphylococcus aureus* CCRC 12657 (10^9 CFU/ml) or *Escherichia coli* CCRC 11509 (10^9 CFU/ml) was thoroughly mixed with approximately 15 ml TSA, which was pre-autoclaved and cooled to 50°C. When the agar was solidified, 30µl *Toona sinensis* ethanol extract of various concentrations from 0 to 5.0% was added into a well with a diameter of 7 mm on the solidified agar and incubated at 37°C for 24hr. Instead of TSA, a 100µl of diluted bacterial suspension of *Pseudomonas fragi* CCRC 10939 (10^7-10^9 CFU/ml) was mixed with pre-autoclaved NA (Nutrition agar), and incubated at 26°C for 24 hr. The inhibition zones (mm) were then determined.

Minimum inhibitory concentration (MIC)

The MIC of ethanol extract was determined, in triplicate, by an agar dilution method. Stock cultures of *S. aureus* CCRC 12657 and *E. coli* CCRC 11509 were grown in nutrient broth for 12-15 hr, and *P. fragi* CCRC 10939 for 24 hr, and then diluted to 10^5 CFU/ml with autoclaved water containing 0.1% peptone. A 100µl diluted bacteria culture and *Toona sinensis* ethanol extract of 0-4% were mixed thoroughly with approximately 15ml pre-autoclaved 50°C TSA (for *S. aureus* and *E. coli*) or NA (for *P. fragi*), solidified and incubated at 37°C for 24 hr for *S. aureus* and *E. coli* and at 26°C for 24 hr for *P. fragi*. Then the MIC was determined.

Results and Discussion

Concentration effect of Toona sinensis ethanol extract on the selected microorganisms

Table 1 shows that *Toona sinensis* ethanol extract had some antibacterial effects on the microorganisms tested in this study including S. *aureus* CCRC 12657, *E. coli* CCRC 11509, and *P. fragi* CCRC 10939. The higher concentration of the extract that was added, the larger the inhibition zone, which indicated a higher antimicrobial effect. When the concentration of extract was between 0.1 to 1.0%, the diameters of the inhibition zones of *S. aureus* CCRC 12657 were between 10.50 and 11.58 mm without significant ($P < 0.05$) difference in diameters. Adding extract up to 3 and 5%, increased the diameters of the inhibition zone significantly ($P < 0.05$) to 13.92 and 14.00 mm. Similarly, higher concentration of *Toona sinensis* ethanol extract had a larger antimicrobial effect on both *E. coli* CCRC 11509 and *P. fragi* CCRC 10939, which could be observed with larger inhibition zones. In addition, when higher than 0.5% of extract were added, S. *aureus* CCRC 12657 tended to have smaller diameters of inhibition zones when comparing with *E. coli* CCRC 11509 and *P. fragi* CCRC 10939 at the same levels of extract added.

Table 1: Antibacterial effects of *Toona sinensis* ethanol extract against the microorganism tested.

Extract conc. (%)	Inhibition zone diameter (mm)		
	S. aureus CCRC 12657	*E. coli* CCRC 11509	*P. fragi* CCRC 10939
0	–[1]	–	–
0.1	$10.50^{b}\pm1.32$[2]	$10.17^{f}\pm0.29$	$10.33^{e}\pm0.58$
0.3	$11.00^{b}\pm1.32$	$11.42^{e}\pm0.52$	$11.00^{be}\pm0.00$
0.5	$11.58^{b}\pm2.24$	$12.67^{d}\pm0.58$	$13.50^{ab}\pm0.87$
0.7	$11.08^{b}\pm0.63$	$14.00^{c}\pm0.00$	$14.17^{a}\pm0.29$
0.9	$10.67^{b}\pm1.18$	$14.00^{c}\pm0.00$	$15.17^{a}\pm2.02$
1.0	$10.75^{b}\pm0.43$	$14.08^{c}\pm0.14$	$15.17^{a}\pm2.02$
3.0	$13.92^{a}\pm0.52$	$15.33^{b}\pm0.29$	$16.17^{a}\pm2.25$
5.0	$14.00^{a}\pm0.50$	$16.67^{a}\pm0.29$	$15.83^{a}\pm1.60$

[1] '–': Inhibition zones cannot be detected.

[2] a-f: Means within a column that have different superscripts are significantly different ($P < 0.05$).

The minimum inhibitory concentration of Toona sinensis ethanol extract against the microorganisms tested

The minimum inhibitory concentration (MIC) values obtained for *Toona sinensis* ethanol extract against the three selected microorganisms are shown in Table 2. The MIC value of *Toona sinensis* ethanol extract against *S. aureus* CCRC 12657 was the lowest which was 1.2%, followed by 3.2% of *E. coli* CCRC 11509 and the highest of 4.0 % of *P. fragi* CCRC 10939.

Table 2: Minimum inhibitory concentration (MIC) of *Toona sinensis* ethanol extract against the microorganism tested.

Microorganism	MIC (%)
S. aureus CCRC 12657	1.2
E. coli CCRC 11509	3.2
P. fragi CCRC 10939	4.0

Conclusions

In conclusion, *Toona sinensis* ethanol extract had antibacterial effect against *S. aureus* CCRC 12657, *E. coli* CCRC 11509, and *P. fragi* CCRC 10939.

References

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