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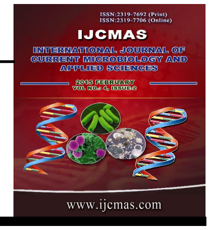

Original Research Article

Effects of *Cosmos caudatus* Kunth. (*Ulam raja*) Extract on Microflora in Raw Chicken Meat

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Keywords

Cosmos caudatus, chicken meat, foodborne pathogens, natural sanitizer, *Ulam raja* extract

A B S T R A C T

Recently, there has been a great interest in the use of plant extract to eliminate microorganism contamination in food materials. The aim of this study was to investigate the effect of *Cosmos caudatus* Kunth (*Ulam raja*) extract on the number of microflora in chicken meat. Raw chicken meat was obtained from wet market and supermarket. *C. caudatus* extract at different concentrations (0.00%, 0.05%, 0.50%, 5.00%) and exposure times (0, 5, 10, 15 min) used to treat chicken meat by using dilution method. Results showed that the number of microorganisms in chicken meat from both locations has no significant difference; Total Plate Count, *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* from wet market were 6.17 ± 0.02, 6.16 ± 0.02, 5.90 ± 0.05 and 6.46 ± 0.09, respectively, meanwhile for supermarket was 6.12 ± 0.01, 6.04 ± 0.06, 5.97 ± 0.04 and 6.46 ± 0.00, respectively. Reduction number of microflora was dependent both on the concentrations and exposure times. The reduction number of microflora was starting to reduce significantly at 0.05% concentration of *C. caudatus* extract for 10 min of exposure time. In conclusion, *C. caudatus* extract can be developed as natural sanitizer for rinsing raw food materials such as chicken meat.

Introduction

Nowadays, issues on the human health caused by the contamination of bacteria in food product are on the rise even in developed countries. It has been estimated that as many as 30% of people in industrialized countries suffer from a food borne disease each year and at least two million people died from diarrheal disease worldwide (WHO, 2002). Even though a variety of methods had been used in preservation of foods, but is still required to inspire the new methods to overcome these challenges.

The preservation technique such as hurdle technology which involve simultaneous multiple preservation is one of the technique that success in controlling pathogens and maintaining the food quality during storage, but it still has the food safety issues

(Leistner, 2000). Besides, a wide range of chemical preservatives have been added during the handling and manufacturing of foods to inhibit and prevent the growth of microorganisms in food products, but the use of chemical preservatives in food become a major concern for consumer due to negatively side effects (Marino *et al*., 2001). Therefore, this has led the researchers and food processors to look on the natural preservatives that have ability as the antimicrobial activity to replace or improve the existence preservation method.

Natural antimicrobial agents have potential or current value for use in foods as "secondary preservatives" were recently reviewed (Naidu, 2000). Furthermore, the use of natural additives as the food preservative has become popular among the consumer due to their awareness and concern regarding on the negative side effects exist from synthetic chemical additives in food products. Gutierrez *et al.* (2009) had reported the presence of bioactive compounds in plant possesses an antimicrobial activity have an ability against wide range of spoilage and pathogenic bacteria in raw food materials. A number of compounds from plant sources were reported to have antimicrobial activity such as terpenoids, alkaloids, flavonoids, tannins and phenols (Ababutain *et al.,* 2011).

For instance, cinnamic aldehyde and eugenol found in cinnamon extract have an antimicrobial activity both on Gram negative and Gram positive bacteria (Yadav and Singh, 2004). Other researcher also reported the effectiveness of garlic extract to reduce microbial growth on poultry meat such as *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Bacillus cereus* (Yadav *et al.,* 2002). Clove extract was found to inhibit the growth of *S. typhimurium, Aeromonas hydrophila* and toxin production by *E. coli* in chicken meat during refrigerated storage (Singh *et al.*, 2004).

Cosmos caudatus is one of the traditional vegetables in Malaysia which also known as *Ulam raja*. *C. caudatus* belong to the family of Asteraceae and can grows up about 1 to 8 feet tall, contain shoots, leaves of 10 - 20 cm long and florets with different colours like purple, pink, or white ray (Patil and Nikam, 2013). Traditionally, *C. caudatus* has been used for medical purposes such as to improve blood circulation, as an anti-aging agent, reducing body heat, strengthening bone narrow, promote fresh breath and antimicrobial activity (Shui *et al.,* 2005). A number of biological active compounds in *C. caudatus* extract were reported including quercetin 3-o-rhamnoside, quercetin 3-o-β-arabinofuranoside, quercetin3-o-β-glucoside, quercetin, proanthocyanidin, crypto-chlorogenic acid, neo-chlorogenic acid, chlorogenic acid, catechin, epicatechin, myricetin and naringenin (Mediani *et al.*, 2012).

However, no study has been reported on the treatment of *C. caudatus* extract against the microorganisms in chicken meat. Hence, in this study the effect of *C. caudatus* extract on the microflora in chicken meat from wet market and supermarket at different concentrations of *C. caudatus* extract and exposure time was analysed. Therefore, this study is important to know the ability of plant extract in inhibiting microbial activity in chicken meat. Results from this study also can be used as information in order to apply the *C. caudatus* plant as food preservative.

Materials and Methods

Sampling of *C. caudatus* and chicken meat

The sample of *C. caudatus* was purchased from Pasar Borong Selangor (Seri

Kembangan, Selangor, Malaysia). Leaves were separated from stem and thoroughly washed under tap water. Sample was left to dry at room temperature (27°C) for 2-3 days until dried properly. Then, dried sample was grinded, put in sealed plastic bag and kept in fridge (-80°C) for further use.

While, the sample of chicken meat was purchased from two different places; wet market (Pasar Borong Selangor, Seri Kembangan, Selangor, Malaysia) and supermarket (Jusco Supermarket, Equin Selangor, Malaysia). Both samples were bought in raw material forms in order to investigate the effect of *C. caudatus* extract against the natural microflora in chicken meat and sample is from broiler types. Chicken meat was cut into cube size of approximately about 10 gram each for treatment.

Extraction of *C. caudatus*

The extraction of *C. caudatus* was conducted according to the method described by Rukayadi *et al* (2008) with slight modification. A 300 gram of grinded *C. caudatus* was soaked in 1500 ml of absolute methanol (99.8%) (Sigma-Aldrich, Saint Louis, MO, USA) and left for 2 days with occasionally shaken. Then, the mixture was filtered and further concentrated using rotary evaporator (Heidolph Instruments, Germany) to get the gummy-like crude extract. Crude extract was stored in fridge until further analysis.

Preparation of *C. caudatus* extract for treatment

Briefly, 5 g of *C. caudatus* extract was dissolved in 50 ml of 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Saint Louis, MO, USA) to make it 10% concentration (100 mg/ml). Then, 12.5 ml from 10% concentration were pipetted into 12.5 ml sterile deionized water (DIW) (B Braun Medical Industries, Penang, Malaysia) to make 5.00% concentration. 0.50% and 0.05% concentrations were prepared by diluting 2.5 ml (5.00% concentration) into 22.5 ml of DIW and 2.5 ml (0.50% concentration) into 22.5 ml of DIW, respectively. The DIW was bought from Nasra Pharmacy Sdn. Bhd. at Bandar Baru Bangi, Selangor. 10% of DMSO used in this study was found not to kill most microorganisms.

Preparation of selective media for different grow of microorganism in chicken meat

Four types of selective media used for the enumeration of bacteria in chicken meat; Total Plate Count (TPC) agar (MERCK, Darmstadt, Germany), Polymyxin-Mannitol-Egg Yolk-Phenol (PMYPA) agar (Difco, Becton Dickinson and Company, Spark, MD, USA) for *Bacillus cereus*, Mac-Conkey agar (MERCK, Darmstadt, Germany) for *Escherichia coli* and Tryptic Soy agar (TSA) agar (MERCK, Darmstadt, Germany) for *Staphylococcus aureus*. Those agars were obtained from Microbiology Laboratory, Faculty of Food Science and Technology, UPM. Quantities (grams) of agar needed for each preparation were followed accordingly to the label attached on the bottles. Then, mixture was properly stirred before it was sterilized at 121^{o}C for 15 min. The sterilized solution was left to be cooled down before being poured into sterile petri dish.

Treatment of chicken meat with *C. caudatus* extract

The samples of chicken meat (10 g each) were treated with tap water and 0.00% (DIW) as positive control and negative control, respectively. As for natural sanitizer, 0.05%, 0.50% and 5.00% concentration of *C. caudatus* extract were applied. All treatments including both positive and negative control were soaked separately at different time interval, 0, 5, 10 and 15 min to determine their microflora growth viability. For bacteria growth determination, 1 ml from each treatment was diluted into 10^{-1}, 10^{-2}, and 10^{-3} dilution. Then, 0.1 ml from each dilutions series was spread on the different types of agar; TPC, PMYP, TSA and Mac-Conkey using sterile cotton swabs and incubated overnight at temperature of 37ºC. The presence of colonies was counted and data was analyzed.

Statistical analysis

The means value for microbial population (Log CFU/g) from each treatment was calculated from the duplicates on each treatment. The application of MINITAB software was used to analyse the data for the analysis of variance (ANOVA). During this analysis, the Turkey's test was used in order to identified the significance of difference ($P = 0.05$) between those treatments. Then, the results were interpreted as means ± standard deviation (SD) of duplicate analysis.

Results and Discussion

There are variety of sanitizer had been developed for a removal and destruction of pathogenic microorganisms from fresh produce products. However, there are some of organisms which exist in foods are resistant with certain treatments and differ in their susceptibility. Hence, the antimicrobial effects of various sanitizing compounds act differ in different types of foods.

Potential contamination in chicken meat by the foodborne pathogens can be caused through the unhygienic practices in handling, cooking or post cooking storage of products. During washing process of raw foods material is also one of the causes for the foodborne pathogens to growth with rapidly. However, those numbers of microorganisms in raw foods can be reduced by using the antimicrobial compounds in the sanitizers. As the best of our knowledge, there was none of sanitizer used *C. caudatus* extract to treat microflora in raw food products like chicken meat. Thus, this study was set up to determine the effect of *C. caudatus* extract against the microflora in chicken meat which was referred to the number of microorganisms in total plate count, *B. cereus, E. coli* and *S. aureus*.

Determination of Microflora in Chicken Meat

In this experiment, *C. caudatus* extract was obtained through the extraction process as reported by Rukayadi *et al.* (2008). The absolute methanol was used instead of other solvents due to its capability to produce high yield of extract containing high chemical compounds inside (Caunii *et al.,* 2012). The higher yield of plant extract will affect the bioactivity of the compounds.

As stated by Moure *et al.* (2001), both extraction yield and biological activity of the plant extracts are strongly dependent on the solvent used. Table 1 shown the number of microorganisms detected in chicken meat from different location; wet market and supermarket. Analysis was found that there was no significant difference between the numbers (Log CFU/g) of detected micro-organisms between these two different locations. However, *S. aureus* showed the highest number of microbes that can be detected in the sample of chicken meat. This finding was correlated with Akbar and Anal (2013) where they investigated that most of *S. aureus* species easily be found in poultry meat.

Effects of *C. caudatus* Crude Extract on Microflora in Chicken Meat

The application of *C. caudatus* extracts on the number of microflora (TPC) in chicken meat was shown in Table 2. Results showed that the total of surviving bacteria population (TPC) on chicken meat was reduced after treated with tap water, 0.00%, 0.05%, 0.50% and 5.00% concentrations of *C. caudatus* extract. However, the number of surviving TPC (Log CFU/g) was start to reduce significantly at concentration 0.05% of *C. caudatus* extract during 10 min of exposure time; 3.66 ± 0.07 (supermarket). Other significant reduction also was found at concentration 0.50% during 5 min of exposure time; 5.74 ± 1.22 (wet market) and 3.76 ± 0.02 (supermarket). On the contrary, chicken meat sample immersed in tap water and DIW (0.00% extract) showed no significant difference even though at different exposure time. It was observed that the relative influence in terms of inactivation of TPC was: tap water ~ DIW < 0.05% < 0.50% < 5.00% of *C. caudatus* extract. Results indicated that the germicidal efficacy of *C. caudatus* extract on naturally microflora on chicken meat was stronger than both filtered tap water and DIW used in this study, while 5.00% concentration of *C. caudatus* extract gave the maximum significant germicidal efficacy among the treatments.

Disinfection effect of *C. caudatus* extract at different concentration and exposure time of *B. cereus*, *E. coli* and *S. aureus* were stated in Table 3, Table 4 and Table 5, respectively. Table 4 listed the survival of microbial population of *B. cereus* after being treated with tap water, DIW, 0.05%, 0.50% and 5.00% of *C. caudatus* extract. The untreated sample of chicken meat contained *B. cereus* in 6.16 ± 0.02 (wet market) and 6.04 ± 0.06 (supermarket), respectively. The effect of *C. caudatus* extract on the microflora of *B. cereus* was started to reduce significantly at concentration 0.05% during 10 min of exposure time in supermarket; 1.70 ± 0.00. While, the significant reduction for wet market was start to reduced significantly at concentration 0.50% of *C. caudatus* extract after treated for 15 min of exposure time; 1.63 ± 0.05.

The number of survival microbes (Log CFU/g) of *E. coli* in treated chicken meat was shown in Table 5. The inhibition action was started at concentration 0.05% of *C. caudatus* extract for 5 min of exposure time. Based on Table 5, the number of *E. coli* was significantly reduced at concentration of 5.00% for 10 min exposure time (wet market: 5.90 ± 0.05 reduced until 0.00 ± 0.00; supermarket: 5.97 ± 0.04 reduced until 0.00 ± 0.00). While, for *S. aureus*, the significant reduction was start at concentration 0.50% of *C. caudatus* extract for 10 min of exposure time; 5.38 ± 0.02 (supermarket). The next significant reduction was shown at concentration 5.00% of *C. caudatus* extract for 10 min of exposure time; 3.81 ± 0.04 (wet market) and 3.27 ± 0.06 (supermarket). *S. aureus* is one of most infections in poultry meat caused by coagulase positive staphylococci. The disease condition by the *S. aureus* are most seen in the heavier breeds and also associated with poor management conditions resulting in moist or wet litter that facilitates skin entry (Aerestrup *et al*., 2000).

All of the results on numbers of microorganisms that survived when treated the samples of chicken meat with *C. caudatus* extract became decreasing with the increasing of concentrations of *C. caudatus* extract used. It showed that the reduction number of microorganisms in chicken meat was strongly correlated with the concentration of *C. caudatus* extract and exposure time. According to Wariska *et al.* (2014), plant extracts contain of different active compounds where each of them functions differently for inhibition of pathogens and a mixture of them will cause synergism activity which sometimes makes compounds became less optimum. Nazihah *et al.* (2013) has reported that the leaves extract of *C. caudatus* contains phytochemical such as terpenoids, fatty acids, flavonoids, alkaloids, tannins and saponins which possess antimicrobial activity. Different types of phytochemicals in *C. caudatus* extract had their own ability to inhibit and reducing the growth of foodborne pathogens in food products. The antimicrobial properties of those compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth. The presence of terpenoids compound in *C. caudatus* extract can disturb the form of membrane or cell of the microbes through the partitioned of protein, lipid or carbohydrates of the bacterial cell membrane or cell wall (Wariska *et al.*, 2014). Apparently, the concentration of them will increase when applied in real food system (Tajkarimi *et al.*, 2010).

Although the higher concentrations of *C. caudatus* extract is more powerful to inhibit the growth of microorganisms, but the usage with a lower concentration with less exposure time would be the best choice for economics. Application of plant extract with high concentration is not recommended because it gives less good effect towards human. It was recommended to use the application of *C. caudatus* extract at 0.05% concentration level in order to achieve a better reducing number of microorganisms in chicken meat and the most important thing it is safe.

In conclusion, the effect of *C. caudatus* extract against microflora in chicken meat both from wet market and supermarket were highly dependent on the concentration of *C. caudatus* extract and exposure time. The significant reduction was started at concentration 0.05% of *C. caudatus* extract for 10 min exposure time. Further study on the effect of sensory acceptability on treated chicken meat with the *C. caudatus* extract would be more interesting to determine people acceptance on this *C. caudatus* sanitizer. Besides, the effect of *C. caudatus* as a sanitizer on other raw food materials can also be analysed.

Table.1 Number of microorganisms in chicken meat

Total Plate Count (TPC) or Microorganisms	**Wet market (Log CFU/g)**	**Supermarket (Log CFU/g)**
Total Plate Count (TPC)	6.17 ± 0.02□	6.12 ± 0.01□
Bacillus cereus	6.16 ± 0.02□	6.04 ± 0.06□
Escherichia coli	5.90 ± 0.05□	5.97 ± 0.04□
Staphylococcus aureus	6.46 ± 0.09□	6.46 ± 0.00□

□) Showing there is no significant different between results at P > 0.05

Table.2 Comparison of different treatment of chicken meat from wet market and supermarket with *C. caudatus* extract at different concentrations and exposure time on Total Plate Count (Log CFU/g)

Concentration / Exposure Time	Tap Water		0.00%		0.05%		0.50%		5.00%	
Sample Origin	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
0 min	6.17 ± 0.02□	6.12 ± 0.01□	6.09 ± 0.02□	6.08 ± 0.15□	5.95 ± 0.01□	5.93 ± 0.12□	5.89 ± 0.05□	5.56 ± 0.13□□	4.95 ± 1.64 □□	3.69 ± 0.03□
5 min	6.15 ± 0.04□	6.11 ± 0.01□	6.04 ± 0.01□	5.99 ± 0.09□	5.94 ± 0.12□	5.92 ± 0.06 □	5.74 ± 1.22□	3.76 ± 0.02□	3.72 ± 0.01□	2.62 ± 1.40□
10 min	6.09 ± 0.08□	6.09 ± 0.05□	6.00 ± 0.05□	5.98 ± 0.07□	5.81 ± 0.06□□	3.66 ± 0.07□	3.47 ± 0.00□	3.32 ± 0.04□	1.73 ± 0.03□	1.65 ± 0.01□
15 min	6.06 ± 0.04□	6.01 ± 0.08□	5.97 ± 0.07□	5.97 ± 0.01□	3.55 ± 0.04□	3.30 ± 0.01□□	2.56 ± 1.31□	1.67 ± 0.06□□	1.65 ± 0.05□□	1.52 ± 0.09□

Means that do not share a letter are significantly different
[W] = Wet market; [S] = Supermarket

Table.3 Comparison of different treatment of chicken meat from wet market and supermarket with *C. caudatus* extract at different concentrations and exposure time on *Bacillus cereus* (Log CFU/g)

Concentration / Exposure Time	Tap Water		0.00%		0.05%		0.50%		5.00%	
Sample Origin	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
0 min	6.16 ± 0.02[a]	6.04 ± 0.06□	5.99 ± 0.08□	5.95 ± 0.01□	5.82 ± 0.02□□	4.94 ± 1.54□	3.84 ± 3.11□□	3.64 ± 0.08□□	3.54 ± 0.00 □□	2.74 ± 1.35□
5 min	5.96 ± 0.09□	5.89 ± 0.01□	4.85 ± 1.66□	5.46 ± 0.11□	3.77 ± 0.16□	3.68 ± 0.02□	2.66 ± 1.34□□	2.64 ± 1.40□□	1.79 ± 0.14□	1.64 ± 0.02□
10 min	6.08 ± 0.15□	6.00 ± 0.05□	5.75 ± 0.02□	5.46 ± 0.11□	2.51 ± 1.25□	1.70 ± 0.00□	1.68 ± 0.00□	1.67 ± 0.02□	0.00 ± 0.00□	0.00 ± 0.00□
15 min	5.96 ± 0.09□	5.89 ± 0.01□	5.59 ± 0.18□	5.42 ± 0.16[a]	1.67 ± 0.04□	1.64 ± 0.04□	1.63 ± 0.05□	1.60 ± 0.01□	0.00 ±	0.00 ± 0.00□

										0.00□

Means that do not share a letter are significantly different
[W] = Wet market; [S] = Supermarket

Table.4 Comparison of different treatment of chicken meat from wet market and supermarket with *C. caudatus* extract at different concentrations and exposure time on *Escherichia coli* (Log CFU/g)

Concentration / Exposure Time	Tap Water		0.00%		0.05%		0.50%		5.00%	
Sample Origin	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
0 min	5.90 ± 0.05□	5.97 ± 0.04□	5.75 ± 0.02□	5.59 ± 0.04□	4.84 ± 0.01□□	4.43 ± 1.42□	3.21 ± 0.11□	3.09 ± 0.04^{cd}	1.66 ± 0.01^{d}	1.60 ± 0.02^{d}
5 min	5.96 ± 0.01□	5.76 ± 0.09□	4.59 ± 0.18□	5.46 ± 0.11□	4.23 ± 1.30□	4.18 ± 1.34□^{c}	3.16 ± 0.04□	2.37 ± 1.20□□	1.64 ± 0.01^{d}	1.59 ± 0.02^{d}
10 min	5.82 ± 0.02□	5.75 ± 0.02□	5.46 ± 0.11□	5.42 ± 0.16□	3.28 ± 0.02□	3.16 ± 0.13^{b}	3.07 ± 0.21^{bc}	1.46 ± 0.04^{bc}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
15 min	5.60 ± 0.04□	5.59 ± 0.18□	5.42 ± 0.15□	5.39 ± 0.10□	3.13 ± 0.03□	3.12 ± 0.04□	1.59 ± 0.01^{bc}	1.46 ± 0.04^{bc}	0.00 ± 0.00□	0.00 ± 0.00□

Means that do not share a letter are significantly different
[W] = Wet market; [S] = Supermarket

Table.5 Comparison of different treatment of chicken meat from wet market and supermarket with *C. caudatus* extract at different concentrations and exposure time on *Staphylococcus aureus* (Log CFU/g)

Concentration / Exposure Time	Tap Water		0.00%		0.05%		0.50%		5.00%	
Sample Origin	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
0 min	6.46 ± 0.09□	6.46 ± 0.00□	6.39 ± 0.03□	6.29 ± 0.01□	6.25 ± 0.13□	6.17 ± 0.02□	5.97 ± 0.04□□	5.56 ± 0.05□□	5.39 ± 0.10□	3.49 ± 0.04□
5 min	6.42 ± 0.06□	6.37 ± 0.04□	6.17 ± 0.04□	6.15 ± 0.06□	6.11 ± 0.11□□	6.11 ± 0.03□□	5.96 ± 0.01□	5.94 ± 0.16□	5.01 ± 1.63□□	3.39 ± 0.02□
10 min	6.41 ± 0.04□	6.29 ± 0.00□	6.16 ± 0.16□	6.15 ± 0.08□	6.11 ± 0.01□□	6.05 ± 0.02□	5.63 ± 0.08□□	5.38 ± 0.02□	3.81 ± 0.04□	3.27 ± 0.06□
15 min	6.28 ±	6.15 ±	6.09 ±	6.05 ±	5.42 ±	5.34 ±	4.87 ±	3.85 ±	2.74 ±	0.00 ±

	0.11□	0.08□	0.00□	0.07□	0.10□	0.00□	1.69□□	0.28□	1.46□	0.00□

Means that do not share a letter are significantly different
[W] = Wet market; [S] = Supermarket

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