



Antibacterial Activity of Commercially Available Plant Extracts on Selected *Campylobacter jejuni* Strains

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MFS and GSKP designed the study, performed the statistical analysis and wrote the protocol. Authors GSKP and DOS wrote the first draft of the manuscript and managed literature searches. All the authors managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Campylobacter jejuni causes over 99% of the foodborne infections associated with *Campylobacter* in the United States. This study involves evaluation of commercially available plant extracts of oregano, green tea, hawthorn and curcumin against four isolates of *C. jejuni*. Initial studies were first carried out in broth cultures to determine the general effectiveness of the extracts. The study then was carried out on chicken breast meat to determine the effect of the plant extracts in marinades. Cell counts were determined at intervals of 0, 2, 6 and 24 hours and bacterial viability was determined using different concentrations of the above extracts. Both oregano and green tea extracts were found to be antibacterial even at low concentrations in broth cultures and killed all bacteria in 24 hours. But as marinades on chicken breast meat, the extracts were found to be effective only at higher concentrations. No significant differences were found in the antibacterial

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effects of the extracts on different *C. jejuni* strains. These results demonstrate that commercially available plant extracts such as oregano and green tea have potential to reduce and/or eliminate *C. jejuni* in chicken meat.

Keywords: *C. jejuni*; green tea; oregano; hawthorn; curcumin; plant extracts.

1. INTRODUCTION

Food contaminated with bacterial pathogens is still a problem worldwide. One method to reduce / eliminate foodborne pathogens is to add antimicrobials to food marinades [1]. To reduce bacterial loads on meat is especially challenging because of the rich nutritional availability, pH and water activity level [2]. *Campylobacter* is one of the major causes of foodborne bacterial illnesses in the world. Although symptoms of this condition are usually not severe, the total annual cost as a result of these infections is millions of dollars in the United States. Investigations have revealed significant links between human *Campylobacter jejuni* infections and handling raw poultry meat as well as the consumption of undercooked poultry [3]. Poultry products are an important component of the human diet and the safety of poultry products is very important [4]. In an opinion expressed by the European Food Safety Authority (EFSA), the European Center for Disease Control and Prevention (ECDC), the European Medicines Agency (EMA) and European Commissions Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the importance of *Campylobacter* and chicken meat production was highlighted [5]. Thus, better methods to reduce *Campylobacter* in poultry are still needed.

Natural antimicrobials can be found in plant or animal sources with plant sources the more significant which can be used in the form of pure compounds or extracts including phenolics, phenolic acids, quinones, flavonoids, thiosulfonates. Glucosinolates and tannins that exist in these plant products are responsible for antibacterial properties of the plants [6,7]. These compounds are found in different parts of the plant from the leaves and fruit to the root, i.e., all parts of the plant and are responsible for their natural ability to protect against insects and harmful pathogens [1]. The major mechanism of action of essential oils is their ability to significantly weaken the cytoplasmic membrane resulting in leakage of cell contents and bacterial cell destruction [8].

Oregano (*Origanum vulgare* L.) has components that have shown varying degrees of antimicrobial activity which is dependent on the species, geographical location, age and component of the plant from which the extract is separated [8]. The major components of oregano were compiled along with their identified range of percentage [2]. Thymol and carvacrol are the major components having inhibitory action against a wide range of bacteria with *p*-cymene being a minor component of oregano. *In vitro* model study using cecal contents, carvacrol at 0.75% and thymol at 1% resulted in significant reduction of *C. jejuni* populations after 24 hours [4].

The antibacterial activity of tea against *C. jejuni* and *C. coli* has been reported with black and green tea extracts achieving total inhibition at 4 hours [9]. There have been conflicting reports detailing the effectiveness of tea which could be because the researchers used different types of tea for experiments along with different sources and strengths of tea in the study [10]. Flavonoids are important secondary organic compounds [11] of which flavanols and flavonols are important subgroups found in tea [12]. Important constituents of green tea are catechins [12] which have shown significant antibacterial activity and constitutes 15% of the dry weight of green tea [13] with epigallocatechin gallate (EGCg) being the major antimicrobial agent [10]. Theaflavins and thearubigins are other groups present in tea leaves [14]. An important study [15], evaluated the antimicrobial activities of tea catechins against *B. cereus* and documented the ratio in antibacterial activity between the most active catechin and the least active catechin.

Hawthorn has been used for centuries as part of medicines across Europe and Asia and is mainly the *Crataegus* species [16]. Hawthorn extracts are normally consumed as tea or tinctures [17]. Hawthorn extracts have shown antibacterial activity against *Micrococcus flavus*, *Bacillus subtilis* and *Listeria monocytogenes* with no effect against *Candida albicans* [18]. In studies on the effect of *Crataegus monogyna* on *C. jejuni*, the extract did not totally inhibit the growth of the bacterium, but less than 25% growth was observed and the results were

consistent for both water and methanol extracts [19].

Curcumin is responsible for the yellow color of turmeric and is composed of 94% curcumin I, 6% of curcumin II and 0.3% of curcumin III [20]. *Curcuma longa* has shown antibacterial activity against *E. coli*, *S. aureus*, *K. pneumoniae* and *S. epidermidis* at low concentrations [21]. In studies, even microencapsulation of curcumin was found to have antibacterial effects comparable to free curcumin against *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli* and *Y. enterocolitica* with maximum inhibition towards *S. aureus* and lowest with *E. coli* [22].

Taking into view the antibacterial effect of different commercial plant extracts, the main objective of our study was to investigate the effects of these different extracts on *Campylobacter jejuni* in culture as well as on chicken breast meat.

2. MATERIALS AND METHODS

2.1 Bacterial Strains and Growth Media

In this study, four different *C. jejuni* strains were used: one human strain (81176) and three poultry strains isolated from different stages of poultry processing including a pre-chilled chicken carcass (PRCC), a post-chilled chicken carcass (POCC) and a retail chicken carcass (RECC). The three chicken isolates were obtained and isolated in our lab using procedures as detailed in [23]. The human strain 81176, was provided by Dr. Michael Johnson, University of Arkansas, Fayetteville, AR [24]. Media used for initial culturing of *C. jejuni* strains was *Campylobacter* Enrichment (CE) broth (Acumedia®). Frozen stock cultures of 81176, PRCC, POCC and RECC were passed twice onto *Campylobacter* blood agar plates and were then inoculated into CE broth which was incubated at 42°C under microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂) for 24 hours. These conditions were created with the use of GazPak™ EZ Campy Container System (260680, BD) in gas pack jars. *Campylobacter* Enrichment Agar (CEA) supplemented with 5% horse blood was used for plating the cultures after serial dilution.

2.2 Plant Extracts

The four plant extracts used in this study were green tea, oregano, hawthorn and curcumin. All

were commercially purchased from Gaia® herbs except for curcumin. Curcumin in powder form was purchased from Alfa Aesar®. Gaia® herbs use a two-step method for the production of extracts from the plants, wherein, the constituents of the plants are extracted using either water or a combination of water and alcohol in a ratio to maximize constituent content [25] followed by a step of concentrating the extract using 24 millibar pressure at 60°C via a closed loop system maintaining integrity of all components [26]. The organic green tea liquid herb extract was in water with 500 mg/ml herb equivalency, whereas the oregano leaf extract was from the herb *Origanum vulgare* with a 333 mg/ml herb equivalency. The hawthorn extract purchased was of the hawthorn berry, flower and leaf of *Crataegus* species with 667 mg/ml herb equivalency and the curcumin extract derived from turmeric rhizome had a total of 95% curcuminoid content. All these plant extracts were diluted in 1X phosphate buffered saline (PBS) to obtain concentrations of 0.25%, 0.5% and 1% of the purchased extracts for the broth culture studies as well as 1%, 5%, 10%, 20%, 30%, 50% and 100% of the purchased extracts for chicken meat model studies.

2.3 Effect of Plant Extracts on *C. jejuni* Strains in Broth Cultures

All the plant extracts green tea, oregano, hawthorn and curcumin were tested at 3 different concentrations of 0.25%, 0.5% and 1% in PBS of the purchased plant extracts. The different concentrations of the extracts were prepared in 1X phosphate buffered saline (PBS) solution. To each 1 mL of the different concentrations of the extracts, 100 µL of each of the *C. jejuni* strains was added to form the different treatment groups. A volume of 1.1 mL of each of the four *C. jejuni* strains in CE broth was used as positive control. The treatment culture samples along with the controls were incubated at 42°C under microaerophilic conditions. Samples were taken at time intervals of 0 h, 1 h, 2 h, 4 h and 24 h and subsequently serial dilutions from 1 to 7 were performed in 1X PBS. 100 µL of each of the treated cultures from all the dilutions were plated onto *Campylobacter* Enrichment agar plates supplemented with 5% horse blood. All these plates were then incubated at 42°C under microaerophilic conditions for 48 hours and viable *C. jejuni* colonies were counted using a colony counter. The results were recorded and expressed in terms of log CFU/mL vs time. The experiments were performed in triplicates.

2.4 Effect of Plant Extracts as Marinades on Chicken Meat Inoculated with *C. jejuni*

Marinades used were prepared using one of the three plant extracts of oregano, green tea and hawthorn. Marinades of green tea and hawthorn extracts were used at concentrations of 50% and 100% of the purchased plant extracts whereas marinade with oregano was prepared at 30% and 50% of the purchased extract. All the marinades were prepared on the same day of the experiment by diluting the extracts with 1X phosphate buffered saline solution (PBS). The inoculum for the meat study was also prepared the same way as for the broth study. Frozen stock cultures of 81176, PRCC, POCC and RECC were passed twice onto *Campylobacter* blood agar plates and were then inoculated into *Campylobacter* Enrichment (CE) broth which was incubated at 42°C under microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂) for 24 hours.

A chicken meat model was used in this study using uncooked boneless chicken breasts fillets purchased from a local supermarket in Fayetteville, Arkansas. The breast fillets were cut into approximately 1 inch square pieces using a sterile knife under a laminar flow hood to prevent external contamination. These meat pieces were placed in a sterile beaker and washed 10 times first with distilled water and then with sterile water twice. After thorough washing, these meat pieces were placed in sterile open petri plates under UV light (at 254 nm) for 30 minutes on each side and then stored at 4°C for 24 hours in order to reduce bacterial counts [27]. The chicken meat pieces were transferred into individual stomacher® bags after 24 hours. Individual pieces were inoculated on the surface with 100µL of each bacterial suspensions of *C. jejuni*, i.e., 81176, PRCC, POCC and RECC strains in their respective bags. For the negative control, one piece of chicken which was not inoculated with *C. jejuni* was marinated with 5 mL PBS (1X). The bags containing the inoculated chicken pieces were held at room temperature for 30 minutes to allow bacterial attachment. A volume of 5 mL of each prepared marinade at the above mentioned concentrations were added to the individual bags containing the chicken pieces. The marinated chicken pieces were kept in the refrigerator at 4°C and samples were taken at 2 h, 6 h and 24 h. Stomacher bags containing the chicken pieces were taken out at these time points and were stomached for 2 minutes using a

Stomacher® 400 (Seward) to remove the attached bacteria. Bacterial cell counts in suspension were then enumerated by serial dilution and plating onto CE agar plates. All the plates were incubated at 42°C under microaerophilic conditions for 48 hours after which the plates were read using a colony counter and viable cell counts recorded.

2.5 Statistical Analysis

Both experiments to study the effect of plant extracts against *C. jejuni* in broth culture and on chicken meat were repeated three times to establish statistical significance. Statistical analysis was performed using JMP 11.0 provided by the University of Arkansas, Fayetteville.

3. RESULTS

The effectiveness of commercially available plant extracts was investigated in this study against four *C. jejuni* strains including 81176, PRCC, POCC and RECC. The plant extracts selected for the analysis included green tea, oregano, hawthorn and curcumin. The initial set of experiments on the four isolates were done *in vitro* in broth culture at three different concentrations of the extracts in PBS which were 0.25%, 0.5% and 1% of the purchased plant extracts. Of the four extracts, green tea (Fig. 1) and oregano (Fig. 2) showed 7-log reduction and killed all the bacteria within 24 hours of incubation at all the three concentrations. Hawthorn showed no significant log reduction ($P>0.05$) with respect to control after 24 hours of incubation for all the concentrations and isolates tested (Figs. 3 – 4). Curcumin at the highest concentration of 1% of the purchased extract was found to produce a 2-3 log reduction from the control at 24 hour time point, whereas, the concentrations of 0.25% and 0.5% were found to be not that effective in reducing *C. jejuni* (Figs. 5 – 6). In order to narrow down the effective concentrations of the extracts in the meat model study, two of the isolates, 81176 and PRCC, were selected to represent the human strain and poultry strain. Concentrations of 1%, 5%, 10% and 20% of the purchased plant extracts in PBS were tested at time points of 2 hours and 24 hours to narrow down the range of effective extract concentrations (Table 1). Based on these initial results, chicken breast pieces then were inoculated with all the four isolates, 81176, PRCC, POCC and RECC, separately and were marinated with 50% and 100% concentrations of the purchased green tea

extract, 30% and 50% concentrations of the purchased oregano extract and 50% and 100% concentrations of the purchased hawthorn extract. Marinades prepared using 50% and 100% concentrations of the purchased hawthorn extract as well as using 50% and 100% concentrations of the purchased green tea extract did not kill all the bacteria within 24 hours

for all four strains of *C. jejuni*. Oregano at a concentration of 50% of the purchased extract showed 6-log reduction of 81176, PRCC, POCC and RECC *C. jejuni* strains. Oregano at 30% concentration of the purchased extract showed 6-log reduction against POCC and RECC compared to 2-log reduction against 81176 and PRCC strains (Figs. 7 – 10).

Table 1. Log₁₀CFU / ml at 2 h & 24 h time point for oregano and green tea extracts to determine concentrations that reduced bacteria over a 2 hour period in chicken breast fillet compared to *in vitro* studies

Plant extracts	<i>C. jejuni</i> 81176 - Log ₁₀ CFU		<i>C. jejuni</i> PRCC - Log ₁₀ CFU	
	2 hour	24 hour	2 hour	24 hour
Control	7	7	7	7
Green Tea - 1%	6	5	6	6
Green Tea - 5%	7	7	7	7
Green Tea - 10%	7	7	7	7
Green Tea - 20%	7	7	7	7
Oregano - 1%	6	5	6	5
Oregano - 5%	7	7	7	7
Oregano - 10%	7	7	7	7
Oregano - 20%	7	7	6	7

The maximum concentration that effectively killed bacteria within 24 hours in all strains tested was the starting point for meat model study

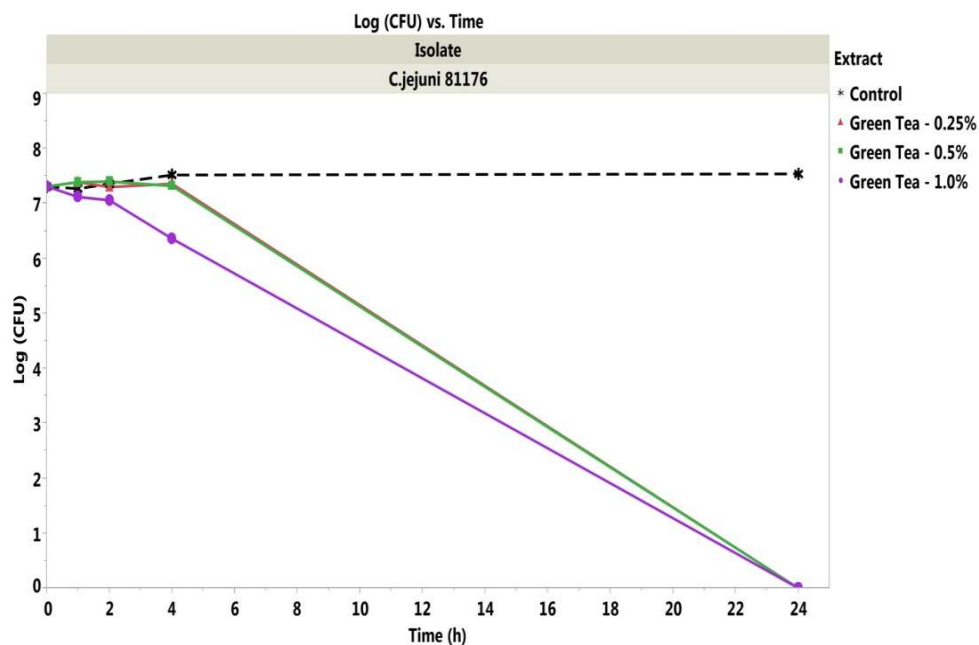


Fig. 1. Log₁₀CFU / ml vs Time of green tea extract at 3 different concentrations against *C. jejuni* 81176

Green tea was effective at killing bacteria within 24 hours of incubation. There was a 7-log decrease in growth in 24 hours for all 3 concentration levels ($P < 0.05$). The action of green tea was the same for all the other 3 isolates PRCC, POCC and RECC

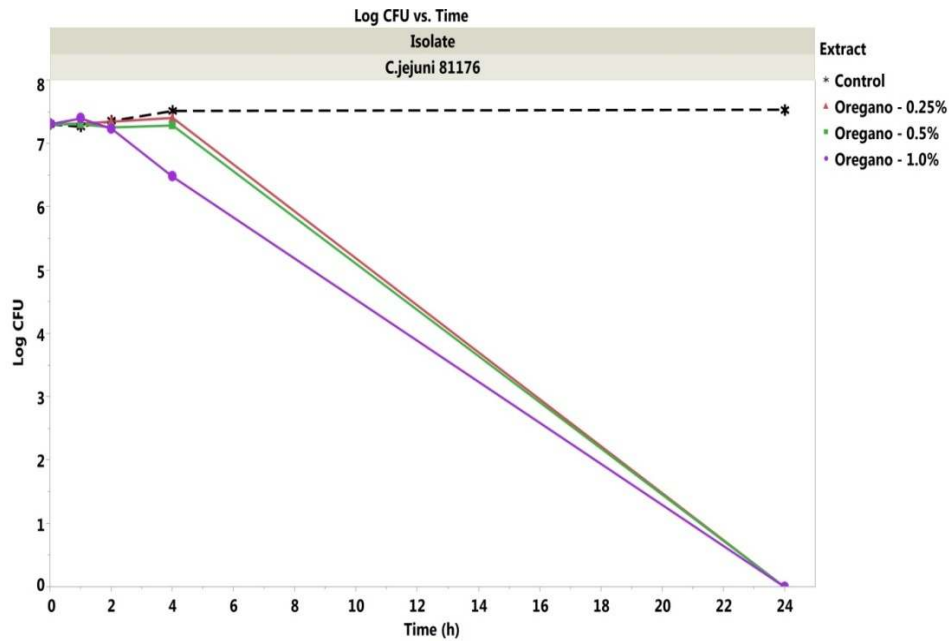


Fig. 2. Log₁₀CFU / ml vs Time of oregano extract at 3 different concentrations against *C. jejuni* 81176

Oregano was effective at killing bacteria within 24 hours, showing a 7- log reduction ($P < 0.05$). Although oregano 1% showed a 1- log reduction in 4 hours, there was a steep decline in growth after that ($P < 0.05$). The trend was the same for all the other 3 isolates PRCC, POCC and RECC

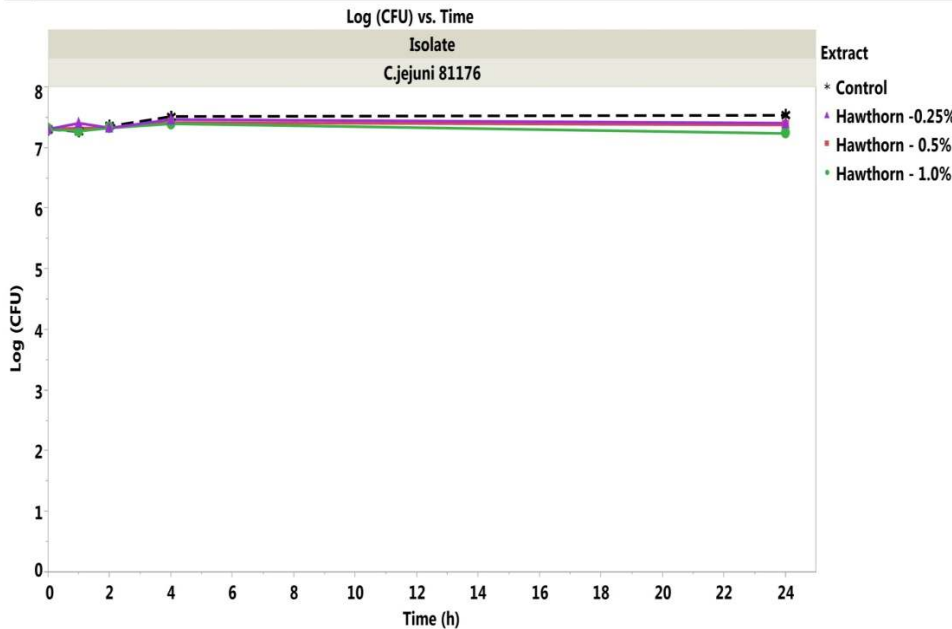


Fig. 3. Log₁₀CFU / ml vs Time of hawthorn extract at 3 different concentrations against *C. jejuni* 81176

All the 3 concentrations did not show significant difference in their action against this isolate for all time points. Also, hawthorn at all concentrations failed to kill the bacteria within 24 hours of incubation. All the 3 different concentrations of hawthorn had a similar pattern of action against isolate RECC

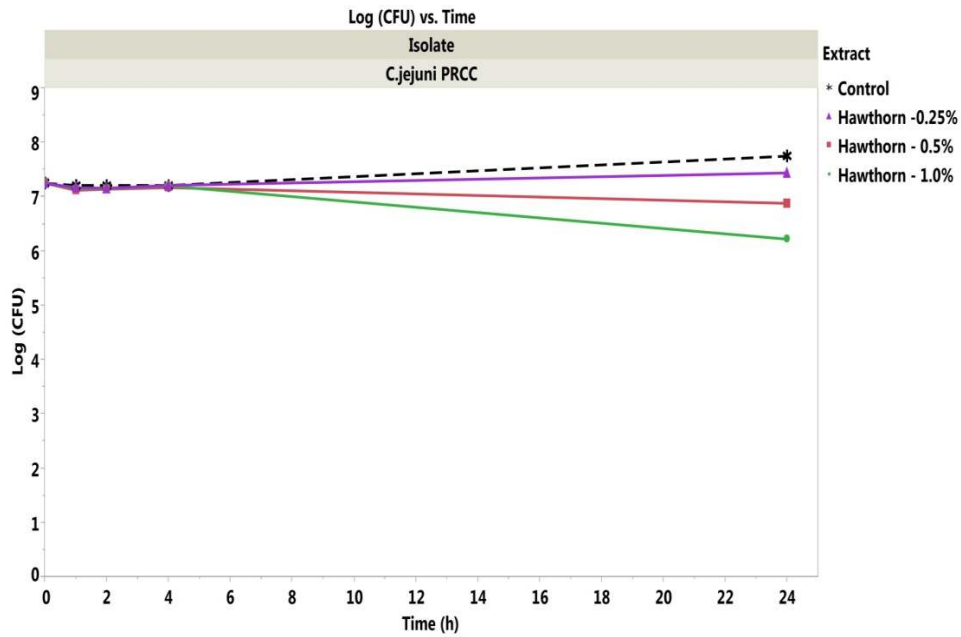


Fig. 4. Log₁₀CFU / ml vs Time of hawthorn extract at 3 different concentrations against C. jejuni PRCC

Hawthorn 1% showed more than 1- log reduction in growth at 24 hours. Hawthorn showed a similar trend with isolate POCC

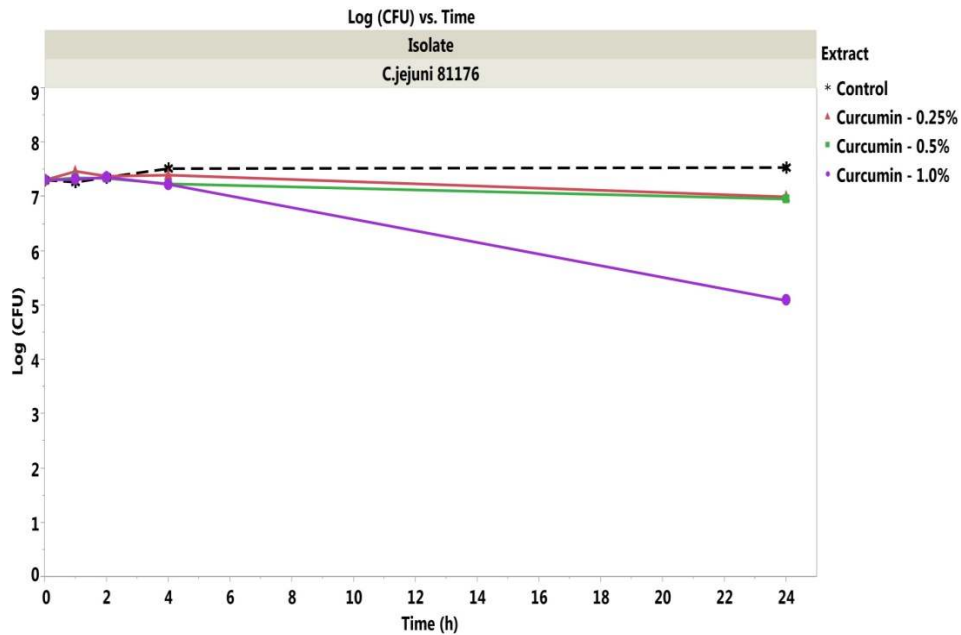


Fig. 5. Log₁₀CFU / ml vs Time of curcumin extract at 3 different concentrations against C. jejuni 81176

Curcumin 1% showed more than 2- log reduction in 24 hours whereas 0.25% and 0.5% showed less than 1- log reduction with no significant difference in its action between the two concentrations. Curcumin was found to show a similar pattern of action towards isolate POCC

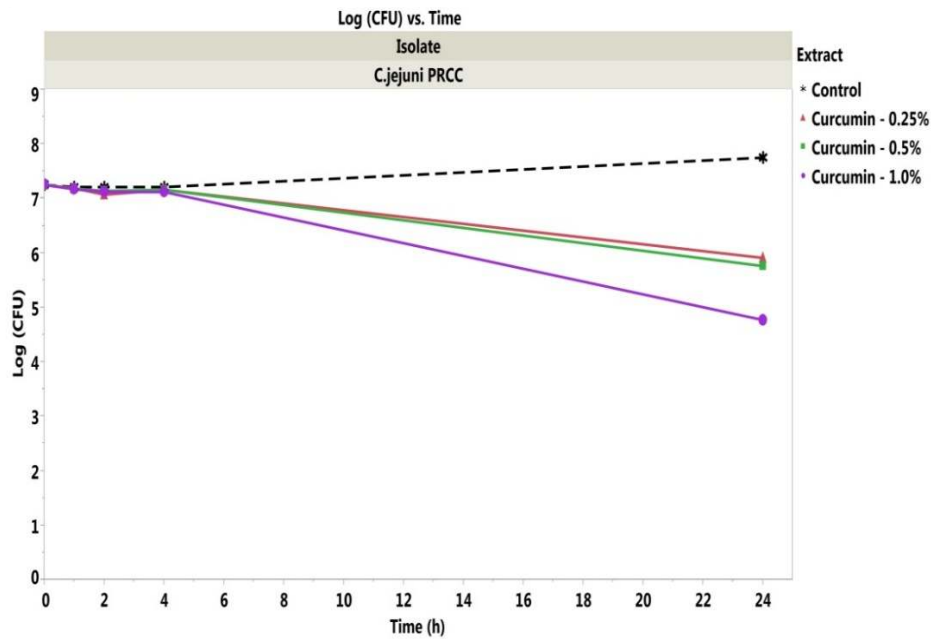


Fig. 6. Log₁₀CFU / ml vs Time of curcumin extract at 3 different concentrations against C. jejuni PRCC

Curcumin 1% was found to be effective among the three concentrations and showed a 3-log reduction in 24 hours of incubation whereas 0.25% and 0.5% concentrations showed a 2 – log decrease in growth. This action of curcumin was found to be the same towards isolate RECC

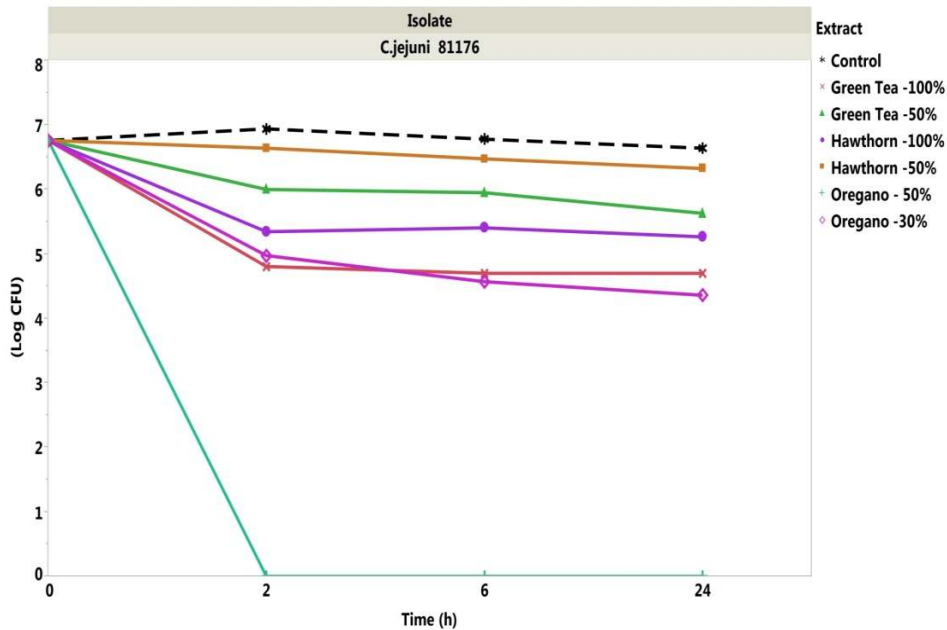


Fig. 7. Log₁₀CFU / ml vs Time of oregano, green tea and hawthorn extract marinated on chicken breast pieces at 2 different concentration levels against C. jejuni 81176

Oregano at 50% succeeded in killing all bacteria within 2 hours of incubation resulting in a 6- log reduction ($P < 0.05$) whereas oregano 30% showed a 2 – log decrease in 24 hours. Green tea 100% showed 2- log reduction whereas green tea 50% and hawthorn 100% showed a 1 – log reduction in 2 hours of incubation

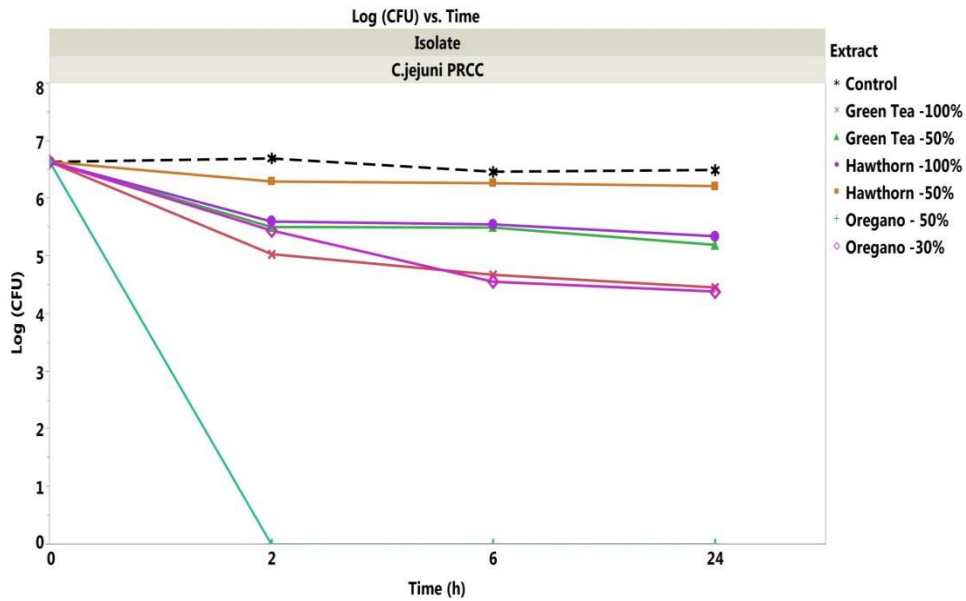


Fig. 8. Log₁₀CFU / ml vs Time of oregano, green tea and hawthorn extract on chicken breast pieces at 2 different concentration levels against C. jejuni PRCC

Oregano at 50% was the most effective of the extracts which succeeded in killing all bacteria within 2 hours of incubation ($P < 0.05$)

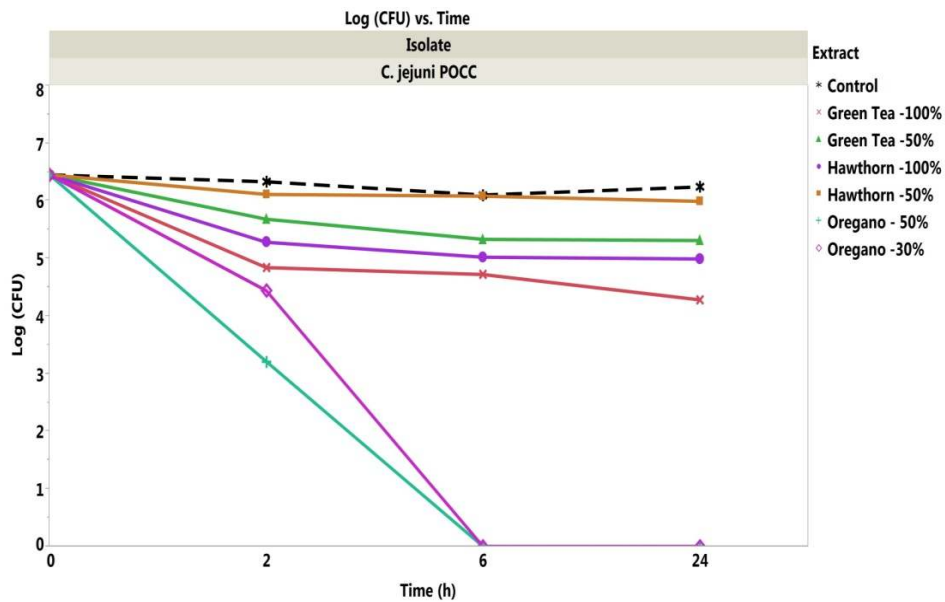


Fig. 9. Log₁₀CFU / ml vs Time of oregano, green tea and hawthorn extract on chicken breast pieces at 2 different concentrations against C. jejuni POCC

Oregano at 30% and 50% succeeded in killing all bacteria within 6 hours of incubation ($P < 0.05$)

4. DISCUSSION

Extract of tea was found to have an effect on *C. jejuni* in broth culture and was reported as killing all the bacteria in 4 hours [9]. No reference

is given for the bacterial strains used or the concentration of the extract which makes it difficult to compare results. In our present study, green tea was found to successfully kill all the *C. jejuni* in broth culture in 24 hours. Another

study also had reported the effects of green tea on *C. jejuni* strains 81176 and RECC wherein green tea was able to kill the bacteria with a longer period (36 hours) of incubation [27]. The method of tea extraction was found to be similar in those studies [9,27] but the concentration of extracts may be dependent on the source and variety of the tea leaves used. In our study, extracts were purchased commercially from Gaia® herbs where it is claimed that the extracts have “a consistent, measurable concentration of a recognized phyto-constituent” [28] to account for the variability of plant product. Initial testing in the meat model, at the highest concentration used in broth studies (1% concentration of the purchased extract in PBS), did not show any reduction in viable counts of *C. jejuni* which required testing at higher concentrations all the way up to full concentration of the commercial extracts. At 100% of the purchased concentration of green tea in meat model, a 2- log reduction in bacteria was observed at the 2 hour time point and no further decrease was observed until the 24 hour time point. The growth inhibition of tea polyphenols against *B. stearothersophilus* [29] shows a similar steady antibacterial activity *in vitro* at 20 hour time point for different concentrations of the tea polyphenols.

Oregano extracts, in our broth culture study, demonstrated a 7-log reduction in bacterial counts for all the four strains of *C. jejuni* after 24 hours. Thymol and carvacrol, two major components of oregano have shown a reduction of *C. jejuni* counts to $< 1 \log_{10}$ CFU/ml from the original value of $4.5 \pm 1 \log_{10}$ CFU/ml at time 0, at concentrations of 10, 20 and 30 mM by 8 hours of incubation and maintained these lower levels of bacteria at 24 hours post treatment [30]. This trend matches the results observed in our study with the 0.25%, 0.5% and 1.0% concentrations of the purchased oregano extract. When testing in the meat model, the minimum concentration of oregano that showed reduction in bacterial count was 30% of the purchased extract with the POC and RECC isolates of *C. jejuni*. The bacteria were completely killed using the 50% of the purchased concentration in all *C. jejuni* strains indicating that the 30% of the purchased concentration of oregano is very close to the minimum inhibitory concentration of the extract in the meat model. Testing carried out to determine the effect of oregano origanum oil on RM1221, RM1230, RM1274 & RM1046 strains of *C. jejuni* revealed increased sensitivity to this essential oil [31]. Oregano oil did not show any variation in the antibacterial activity between the different

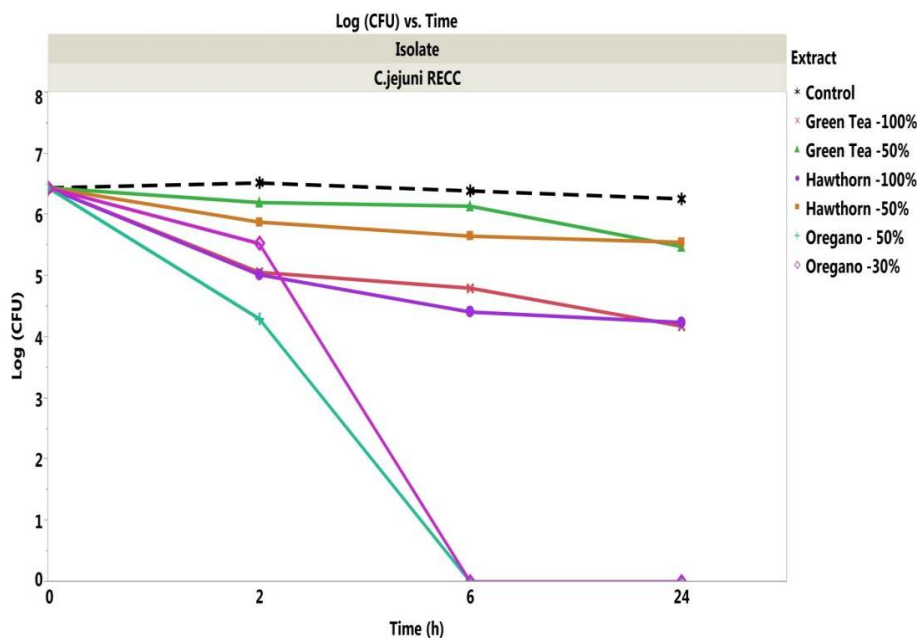


Fig. 10. Log₁₀CFU / ml vs Time of oregano, green tea and hawthorn extract on chicken breast pieces at 2 different concentrations against *C. jejuni* RECC

Oregano at 30% and 50% succeeded in killing all bacteria within 6 hours of incubation ($P < 0.05$)

C. jejuni strains used in this *in vitro* study [31]. This is very similar to the results observed in our broth culture study. In our meat model study, differences were observed in the antibacterial activity of oregano on POCC (post-chilled) and RECC (retail chicken carcass) isolates as compared to 81176 (human strain) and PRCC (pre-chilled) isolates. One possible explanation could be that exposure to low temperatures might have increased the sensitivity of *C. jejuni* toward oregano as the strains POCC and RECC were isolated after the chilling stage. Studies have shown that there is a difference in the death rate in some *C. jejuni* strains when the cultures were exposed to 6°C for 24 hours [32].

Hawthorn extract did not exhibit log reduction similar to that observed with green tea and oregano extracts in broth culture against all four isolates of *C. jejuni* all throughout 24 hours of incubation. In meat model studies hawthorn extract at 100% concentration of the purchased extract exhibited only a 1-log reduction at the 24 hour time point. This result is in agreement with the study conducted using extracts of *Crataegus monogyna* on *C. jejuni* [19]. *Crataegus monogyna*, commonly known as hawthorn was found to produce only less than 25% growth inhibition with *C. jejuni*. Unfortunately, no reference is made in the study as to the concentration of the extract or the isolate of *C. jejuni* used.

Curcumin experiments were conducted only in broth culture to see the effect of the extract at low concentrations. At the 24 hour time point a minimum of 2 log-reduction was observed for all strains of *C. jejuni* at 1% concentration. The broth culture studies of curcumin were primarily part of the study to determine suitable extracts to test on meat model. The initial tests in meat model indicated the requirement of higher concentrations for eliminating bacteria at 24 hour time point for all strains and the cost of curcumin was higher than other extracts which limited the range of testing.

There were major differences in concentrations of extracts required to inhibit the bacteria between broth culture studies and meat model studies. This has been recognized as a potential drawback of using plant extracts in food to reduce / eliminate bacteria [1,7,33]. This difference may be due to interaction of plant extracts with components present in food model such as high fat and protein content [8], lipids

[34] and water activity. The pH levels in meat may also influence the hydrophobic effect of extracts and in turn their antibacterial effect [7]. The higher concentration requirements of plant extracts in meat models can be resolved by determining combinations of lower concentration plant extracts that can inhibit bacterial growth and avoid undesirable sensory changes [7]. Future tests will involve isolating individual components of each of the extracts and determining the dominant antibacterial component as well as their minimal inhibitory concentrations.

5. CONCLUSION

The present study on the effects of commercially available plant extracts on selected strains of *C. jejuni* revealed that some extracts were found to be effective in reducing / eliminating the bacteria. The extracts of oregano and green tea were found to be antibacterial even at low concentrations in broth cultures and killed all bacteria in 24 hours. But when they were used as marinades on chicken breast meat, the extracts were found to be effective only at higher concentrations. No significant differences were found in the antibacterial effects of the extracts on different *C. jejuni* strains. These results demonstrate that commercially available plant extracts such as oregano and green tea have potential to reduce and/or eliminate *C. jejuni* in chicken meat. However, combinations of plant extracts have to be explored to resolve the problem of high concentration required to inhibit *C. jejuni* strains in meat.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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