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Antimicrobial Efficacy of Garcinia Cambogia Fruit Extract Against Food Borne Bacterial Pathogens, In vitro and in Chicken Meat

K. S. Bhuvana, P. K. Mandal*, U. K. Pal, P. X. Antony[1] and S. Kasthuri

Department of Livestock Products Technology, [1]Department of Veterinary Microbiology

Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Pondicherry – 605009

ABSTRACT

The use of natural preservatives to increase the shelf-life of meat and meat products is a promising trend. The present study was done to evaluate the efficacy of the crude extract of *Garcinia cambogia* fruit as natural preservative in chicken meat. The in vitro antimicrobial efficacy of a crude extract of G. cambogia fruit against common food-borne bacterial pathogens was evaluated by agar diffusion assay. Minced chicken was inoculated with Escherichia coli and Salmonella Typhimurium and the effect of the extract on these pathogens was studied during storage under refrigeration. The extract was tested to evaluate its efficacy as a decontaminating agent in the form of a dip solution. The extract of G. cambogia fruit was found to have a high in vitro antimicrobial activity against the bacterial pathogens such as *Bacillus cereus, Staphylococcus aureus, Salmonella Typhimurium, Escherichia coli, Klebsiella pneumoniae, Shigella flexneri, Pseudomonas aeruginosa and Escherichia coli O157:H7*. Both 1 and 2% extract groups significantly ($P<0.01$) reduced the counts of Escherichia coli and Salmonella typhimurium inoculated into minced chicken meat under refrigeration storage but no significant ($P>0.05$) difference in antimicrobial activity was observed between levels. The evaluation of the extract as a decontaminating agent at levels of 0.5, 1 and 1.5% (v/v solution) revealed a significant ($P<0.01$) decrease in case of both SPC and coliform counts for selected cuts dipped in the solution of the extract. The results showed excellent antimicrobial efficacy of the crude extract of Garcinia cambogia fruit and found to be suitable decontaminating agent for chicken cuts.

Keywords: *Garcinia cambogia fruit extract, Minced chicken meat, Chicken cuts, Food borne pathogens, Decontaminating agent*

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INTRODUCTION

Although various foods can serve as sources of food borne illness, meat and meat products are important sources of human infections with Salmonella spp., Campylobacter jejuni/coli, Yersinia enterocolitica, and to some extent, L. monocytogenes. The most frequent chain of events leading to meat borne illness involves food animals as healthy carriers of the pathogens. These organisms are subsequently transferred to humans through production, handling and consumption of meat and meat products.

In spite of modern improvements in meat hygiene and production techniques, food safety is an increasingly important public health issue (WHO, 2002). In India, most of the chicken meat is produced from the birds slaughtered in the unorganized sector under improper hygienic conditions which offers ample scope for contamination. The safety of commercially processed poultry products is a major area of concern for producers, consumers and public health officials worldwide, for products excessively contaminated with microorganisms are undesirable from the standpoint of public health, storage quality and general aesthetics (Cunningham, 1982; Mead, 1989).

Studies have indicated that consumption of poultry meat has been associated with incidence of outbreaks of food borne infections including Salmonellosis (Prakash et al. 2005), Campylobacteriosis (Berrang and Dickens, 2000) and Listeriosis (Lunden et al. 2003). Reduction of initial bacterial load in meat is of prime importance in an attempt to improve the shelf-life of the product. Emergence of antimicrobial resistant pathogens and refusal of foods containing chemical preservatives by the consumers has compelled the exploration of natural preservatives that could ensure the safety of food products and in turn fulfill the demand for chemical free meat and meat products (Sudarshan et al. 2011).

Herbs and spices have been added to foods since ancient times, not only as flavoring agents, but also as preservatives (Buchart, 2001). They are generally recognized as safe (GRAS), either because of their traditional use without any documented detrimental impact (Smid and Gorris, 1999). Spices and herbs have inhibitory effect on pathogens like Salmonella Typhimurium (Roller, 2003) and Staphylococcus aureus (Paster et al. 1990), Listeria monocytogenes (Aureli et al. 1992), Clostridium botulinum (Ababouch et al. 1992) and E. coli (Yoon-Soon-Kim et al. 1996). As the popularity of natural preservatives is on the rise, consumers, regulatory agencies and meat processors require reliable information on their efficacy for use in reducing the bacterial load in chicken meat.

Garcinia cambogia fruit, a traditionally used culinary agent, has the potential as a natural preservative and antimicrobial agent. It is found in the tropical rain forests of Western Ghats, from Konkan southwards to Mysore, Coorg and Kerala. The G. cambogia fruit contains approximately 10–30% acid calculated as citric acid on a dry weight basis (Lewis et al. 1964). Hydroxy Citric Acid (HCA) is the principal organic acid in the fruit rinds of G. cambogia (Lewis, 1969). Small and complex molecules have been isolated from various species of Garcinia, which include xanthones and xanthone derivatives (Minami et al. 1994). The bioflavonones are the most dominant components in most Garcinia species (Waterman and Hussain, 1983). It contains significant amounts of vitamin C and has been used as a heart tonic (Geetha et al. 2011). The dried fruit rind of Garcinia cambogia is an important ingredient in fish curries. Though extensively used in herbal weight control products, there are no scientific reports available on Garcinia cambogia for use as an antimicrobial agent and preservative in meat. In Coorg

* Corresponding author Email address: mandalpkm@gmail.com
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district of Karnataka, this extract is added to the famous pork curry (pandhi curry) which has a unique flavor and a longer keeping quality. A preliminary study conducted in our laboratory revealed that pork fry with the extract of G. cambogia fruit at 1% level could be stored at room temperature up to six days (Bhuvana et al. 2012).

As the present scenario indicates steady increase of production and preference for chicken meat which is believed to remain so in the decades to follow, the present study is aimed at evaluating the antimicrobial efficacy of a crude extract of Garcinia cambogia fruit against common food-borne bacterial pathogens, in vitro and in chicken meat.

MATERIALS AND METHODS

The study was conducted in the Department of Livestock Products Technology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER). To evaluate the efficacy as an antimicrobial agent and natural preservative in chicken meat the crude extract of G. cambogia fruit rind procured from the market of Madikeri, Coorg district, Karnataka.

In vitro antimicrobial efficacy of crude extract of G. cambogia fruit against common food-borne bacterial pathogens by agar diffusion assay.

The antibiotic sensitivity test as per the method given by Bauer et al. (1966) was followed to determine the in vitro efficacy of the aqueous extract against the bacterial pathogens viz., *Bacillus cereus (MTCC 430), Staphylococcus aureus (MTCC 87), Escherichia coli (MTCC 40), Salmonella Typhimurium (MTCC 98), Shigella flexneri (MTCC 1457), Klebsiella pneumoniae (MTCC 39), Pseudomonas aeruginosa (MTCC 424), and E.coli O157:H7.*

Bacterial cultures

The reference strains used for this study were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and maintained in glycerol broth at -40ºC in the Department of Veterinary Microbiology, RIVER.

Procedure

Plate count agar (PCA) plates were prepared prior to the experiment. PCA (Hi-Media Laboratories Pvt. Ltd. Mumbai) of 23.5 Gram was suspended in 1000 ml of distilled water and boiled to dissolve the medium completely. It was sterilized at 15 lb pressure (121ºC) for 15 minutes using an autoclave and pour plate technique was followed for plating. The agar was allowed to solidify and was then checked for contamination by overnight incubation at 37°C.

Each test bacterial culture was separately streaked and the individual pure colonies obtained were transferred to liquid growth medium (Luria Broth). The test cultures of 0.1 ml containing approximately 109 cells /ml were transferred to the surface of Plate Count Agar (PCA) and spread uniformly on the entire surface of the agar medium using a sterile swab. Wells of 6 mm diameter were punched into the solidified agar into which 100 µl of the extract at 10, 20, 30 and 40 per cent (v/v) were placed, along with the antibiotic disc (selected for each organism, based on their respective sensitivities). A well with the same volume of sterile distilled water was also maintained. Ciprofloxacin discs (5µg) were used for all the test organisms except for Bacillus cereus where amoxicillin discs (10µg) were used. The plates were incubated at 35±1°C for 24 - 48 hours. After the incubation period, the zones of inhibition were measured in millimeter (using HiMedia scale).

Evaluation of antimicrobial efficacy of the extract on Escherichia coli and Salmonella Typhimurium in minced chicken meat under refrigeration (5±1^{0}C).

Challenge studies of the minced chicken meat treated with the aqueous extract of G. cambogia fruit at different levels (Control, 1% and 2%) separately were carried out with Escherichia coli (MTCC 40) and Salmonella Typhimurium (MTCC 98) as per the protocol of Fadia Naim et al. (2004).

Reference cultures

The reference strains of Escherichia coli (MTCC 40) and Salmonella Typhimurium (MTCC 98) used for the study were obtained from IMTECH, Chandigarh and maintained in glycerol broth at -40ºC at the Department of Veterinary Microbiology, RIVER. The stock cultures were prepared by streaking individual colonies of Escherichia coli and Salmonella Typhimurium on MacConkey agar and incubating at 37°C for 24 hours. Working cultures were prepared by growing Escherichia coli and Salmonella Typhimurium cells overnight (18 hrs) at 37°C in Luria broth (Hi-Media). The final concentration of the cells inoculated was enumerated by agar plate dilution using brilliant green bile agar.

Procedure

Escherichia coli and Salmonella Typhimurium cultures were diluted to a final concentration of 1010 cells/ml. The minced chicken meat was inoculated with pure cultures of these reference strains. One ml of each bacterial suspension was thoroughly mixed in 100 gram meat mince. The extract of G. cambogia fruit was added at the rate of one and two per cent (w/v). A control was maintained without the addition of the extract. The samples were packed in LDPE bags, sealed and stored at refrigeration temperature (5±1°C) up to 5 days. Evaluation of the survival of the pathogens was done daily, up to five days by serial dilution of the stored samples.

Preparation of serial dilutions

Ten Gram of samples were weighed aseptically and transferred to a sterile mortar containing 90 ml of sterile 0.1 % peptone water. The samples were homogenized for two minutes using a sterile pestle to make 10^{-1} dilution. To prepare 10^{-2} dilution, 1 ml from 10-1 dilution was mixed with 9ml of 0.1% peptone water and so on to obtain serial dilutions till 10-3. Preparation of samples and serial dilutions were made near flame in a horizontal laminar air flow (Model: Clean air systems) observing all possible aseptic conditions.

Brilliant Green Bile Agar (BGA) was used for the enumeration of Escherichia coli and Salmonella Typhimurium. The media was prepared and 1 ml of each dilution was placed in duplicate petri dishes. The sterile molten and cooled (45°C) agar was poured in about 15 ml quantities in each of the petri dishes separately and thoroughly mixed. After solidification of the media, the petri dishes were incubated at 37°C for 18 to 24 hours. Based on colony characters, the colonies were differentiated for counting and were subsequently expressed as log cfu /gram of sample.

Evaluation of efficacy of the extract as a decontaminating agent for selected chicken cuts

To evaluate the efficacy of the crude extract of Garcinia cambogia fruit as a decontamination agent for selected chicken cuts (breast, drumstick), the aqueous extract at concentrations of 0.5, 1 and 1.5 % were selected.

Procedure

Selected cuts of broiler chicken (breast and drumstick) were procured from a hygienic retail outlet. A 0.5, 1.0 and 1.5 % volume/ volume solution of Garcinia cambogia extract was prepared prior to the experiment, to serve as the dip solution. The selected chicken cut-up-parts were immersed in the prepared solutions of 0.5, 1 and 1.5 per cent. The control samples were dipped in the same volume of distilled water. A contact time of two minutes was allowed. The samples were drawn after 30 minutes by surface sampling technique (sterile swab method). A sterile template of 4cm2 was placed on the surface of the cuts. With a sterile swab, the sample was drawn from this area and transferred into 0.1% peptone water and subjected to standard plate count and coliform counts. The difference in log values between the control and dip treated (with extract) samples was used as a guide to assess the antimicrobial and decontamination ability of the extract at different concentrations.

Statistical Analysis

Each experiment was replicated thrice and each parameter was analyzed in duplicate. The data recorded were analyzed by one way and two way analysis of variance (ANOVA), using SPSS version 16.0 of windows (SPSS, Chicago, USA).

RESULT AND DISCUSSION

In vitro antimicrobial efficacy of a crude extract of G. cambogia fruit against common food-borne bacterial pathogens by agar diffusion assay

The extract of G. cambogia fruit was found to have a high in vitro antimicrobial activity against the bacterial pathogens. The zone of inhibition ranged between, *Bacillus cereus* (22.5 - 29.5 mm), *Staphylococcus aureus* (20.25 - 28.25 mm), *Salmonella Typhimurium* (17.0 - 25.25 mm), *Escherichia coli* (16.75 - 27.25 mm), *Klebsiella pneumonia* (16.0 - 26.5 mm), *Shigella flexneri* (20.25 - 30.25 mm), *Pseudomonas aeruginosa* (17.25 - 23.25 mm) and *Escherichia coli O157:H7* (15.25 - 24.25 mm) for the concentration of extract from 10 - 40% (table 1). The activity significantly ($P<0.001$) increased with the increasing extract concentration and all the pathogens tested were indicated to be sensitive to the extract. But no significant ($P>0.05$) difference was observed between 10 and 20% extracts for P. aeruginosa and between 20 and 30% in case of B. cereus, K. pneumoniae and P. aeruginosa. In case of Shigella flexneri and Salmonella Typhimurium, even 40% extract showed no significant ($P>0.05$) difference from that of the antibiotic. In case of Escherichia coli O157:H7, all the concentrations of extract showed a significantly lower activity than the antibiotic.

According to the results obtained, *B. cereus* was the most susceptible of all the test organisms used in the study, followed by *S. aureus* and *S. flexneri*. On the other hand, *E. coli O157:H7* showed the least sensitivity among all organisms, followed by that of *K. pneumoniae, E. coli, S. Typhimurium* and *P. aeruginosa*. The results obtained from the experiment are in accordance with that of Varalakshmi et al. (2010) who studied the antibacterial properties of the aqueous extract of G. indica fruit rind. They reported that the extract inhibited the growth of Escherichia coli, Bacillus subtilis, Enterobacter aerogenes and Staphylococcus aureus. The highest effect was observed in B. subtilis and the least on S. aureus which was also the case in the present study where Bacillus cereus was the most susceptible. Iinuma et al. (1996) concluded that the strong antibacterial activity of the extract maybe due to the presence of xanthones and related metabolites that have been implicated for the potent antibacterial activity in the various species of Garcinia. The results of the experiment revealed that the extract of G. cambogia has a significant antimicrobial activity and were more effective against Gram-positive bacteria. In general, Gram-positive bacteria are more sensitive to spice and herb extracts or plant essential oils than Gram-negative bacteria (Zaika, 1988). It has been hypothesized that this is due to the differences in the cell envelope structure and antibacterial substances that can penetrate through Gram-positive bacterial cell wall and attack the cytoplasmic membrane, leading to leakage of the cytoplasm and/or cytoplasm coagulation (Kalemba and Kunicka, 2003).

Similar to the findings of the present study, comparison of the susceptibility of bacteria to the various extracts (roots, leaves, fruits) of G. atroviridis by Mackeen et al. (2000) indicated that E. coli was the most resistant and that its growth was inhibited by the extracts and they exhibited broad-spectrum antibacterial activity, i.e. to both Gram-positive (B. subtilis B28 and B29, MRSA, S. aureus) and Gram-negative bacteria (E. coli and P. aeruginosa). Nimsha et al. (2010) reported the antimicrobial activity of Garcinia quasita (fruit) aqueous extract which in spite of possessing a very low phenolic content exhibited a high activity against some of the pathogenic bacterial strains. They further concluded that the antimicrobial activity in some herbs and spices may be due to the presence of substances other than phenolic compounds. The antimicrobial activity of Garcinia cambogia against the selected pathogens might be due to the presence of xanthones and xanthone derivatives, bioflavonones, benzophenone, lactones and phenolic acids.

Table 1: Antimicrobial activity (zone of inhibition in mm) of Garcinia cambogia fruit extract against selected pathogens by agar diffusion assay (Mean±SD)

Pathogens	Antibiotic	Concentration of the extract			
		10%	20%	30%	40%
Bacillus cereus	10^{A}	22.5 ± 1.29^{B}	25.5 ± 1.73^{C}	26.25 ± 1.70^{C}	29.5 ± 0.57^{D}
Staphylococcus aureus	10^{A}	20.25 ± 1.89^{B}	21.75 ± 4.03^{BC}	25.25 ± 1.25^{CD}	28.25 ± 1.70^{D}
Salmonella Typhimurium	25^{C}	17 ± 1.41^{A}	20.25 ± 1.70^{AB}	22.75 ± 2.06^{BC}	25.25 ± 2.50^{C}
Escherichia coli	24^{C}	16.75^{A}	18.5 ± 1.0^{B}	23^{C}	27.25 ± 0.95^{D}
Klebsiella pneumonia	10^{A}	16 ± 1.75^{B}	19.5 ± 1.91^{C}	21.75 ± 0.95^{C}	26.5 ± 2.38^{D}
Shigella flexneri	30^{D}	20.25 ± 0.50^{A}	$22.75\pm$٠,٥٠^{B}	26.5 ± 0.57^{C}	30.25 ± 0.50^{D}
Pseudomonas aeruginosa	10^{A}	17.25 ± 0.95^{B}	$191.15\pm^{B}$	$21.51.29\pm^{C}$	$23.25\pm$٠,٥$0^{C}$
E.coli O157:H7	31^{E}	15.25 ± 0.95^{A}	18.5 ± 1.91^{B}	21 ± 1.15^{C}	24.25 ± 0.50^{D}

Means with different superscripts in the same row differ significantly ($p<0.01$)

Evaluation of the antimicrobial efficacy of the extract on Salmonella Typhimurium and Escherichia coli in minced chicken meat under refrigeration storage (5±1⁰C)

The counts of Salmonella Typhimurium for control, 1 and 2% extract groups from day 0 to day 5 ranged from 6.06 - 7.59, 6.06 - 6.01 and 6.06 - 5.76, respectively (table 2). The counts of Escherichia coli for control, 1 and 2% groups from day 0 to day 5 ranged from 6.96 - 7.75, 6.96 - 6.58 and 6.96 - 6.55, respectively (table 2). Both 1 and 2% extract groups significantly reduced the counts of Escherichia coli and Salmonella Typhimurium inoculated into minced chicken meat under refrigeration storage. The antimicrobial activity was evident by log reduction which lasted until the initial 24 hours for S. Typhimurium and until 48 hours for E. coli. However, there was a gradual significant ($P<0.001$) increase in count after day 1 in case of S. Typhimurium and after day 2 in case of E. coli. A significant log reduction of 1.6 and 1.8 was observed for S. Typhimurium at the end of the study for 1 and 2% extracts, respectively. In case of E. coli, a significant log reduction (1.2) was observed on day 5 for the same levels of extract. E. coli was comparatively more resistant than S. Typhimurium to the extract of G. cambogia fruit as observed from this study. This is in accordance with the study by Mackeen et al. (2000) who reported that E. coli was the most resistant to the fruit extract of G. atroviridis. It also is in close conformity with the results of the in vitro antibacterial efficacy, where the shiga toxigenic serotype, E. coli O157:H7 was the most resistant among all the test pathogens used. Nimsha et al. (2010) reported that Garcinia quasita (fruit) aqueous extract exhibited a high antimicrobial activity against some of the pathogenic bacterial strains such as E. coli, S. Typhimurium, L. monocytogenes and S. aureus. Varalakshmi et al. (2010) reported the antibacterial properties of the aqueous extract of the G. indica fruit rind where the extract inhibited the growth of Escherichia coli, Bacillus subtilis, Enterobacter aerogenes and Staphylococcus aureus.

Table 2: Antimicrobial activity of Garcinia cambogia fruit extract against Salmonella Typhimurium and Escherichia coli in minced chicken meat at 5±1°C (Mean ±SD of log cfu/g).

	***Salmonella* Typhimurium**		
Days	**Control**	**1.0% extract**	**2% extract**
0	6.06 ± 0.19^{aA}	6.06 ± 0.19^{cA}	6.06 ± 0.19^{dA}
1	5.92 ± 0.26^{aB}	5.25 ± 0.18^{aA}	4.91 ± 0.15^{aA}
2	6.55 ± 0.21^{bB}	5.46 ± 0.15^{abA}	5.07 ± 0.15^{abA}
3	7.11 ± 0.07^{cB}	$5.67\pm0.16ab^{abcA}$	5.34 ± 0.20^{bcA}
4	7.29 ± 0.03^{cdB}	5.83 ± 0.20^{bcA}	5.52 ± 0.14^{cA}
5	7.59 ± 0.08^{dB}	6.01 ± 0.20^{cA}	5.76 ± 0.06^{cdA}

Escherichia coli

Days	Control	1.0% extract	2% extract
0	6.96±0.06^{aA}	6.96±0.06^{dA}	6.96±0.06^{bA}
1	7.46±0.03^{bB}	6.65±0.06^{cA}	6.29±0.10^{aA}
2	7.54±0.04^{bcB}	6.37±0.02^{aA}	6.16±0.15^{aA}
3	7.62±0.06^{cdB}	6.44±0.05^{abA}	6.31±0.19^{aA}
4	7.67±0.05^{cdB}	6.53±0.04^{bcA}	6.40±0.26^{bA}
5	7.75±0.05^{dB}	6.58±0.03^{cA}	6.55±0.21^{abA}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly (P<0.001)

Evaluation of efficacy of Garcinia cambogia fruit extract as a decontaminating agent for selected chicken cuts.

The standard plate count and coliform counts for selected chicken meat cuts dipped in 0.5, 1 and 1.5% extract solutions were significantly (P<0.001) lower than control (table 3). The SPC counts for chicken meat cuts dipped in 0.5, 1 and 1.5% extract solutions significantly (P<0.001) reduced to 4.81, 3.24 and 2.59, respectively. A significant (P<0.001) reduction (log) was observed in case of the extract groups as compared to control which was 1.73, 3.3 and 3.95 for 0.5, 1 and 1.5% extract solutions, respectively. The coliform counts for control, 0.5, 1 and 1.5% extract solution treated groups were 5.36, 3.07, 2.40 and 1.48, respectively. A significant (P<0.001) reduction (log) was observed as compared to control which was 2.29, 2.96 and 3.88 for 0.5, 1 and 1.5% extract solution treated groups, respectively. This antimicrobial effect might be due to the acidic pH and the presence of xanthones and its metabolites (Iinuma et al. 1996), bioflavonones, benzophenone, lactones and phenolic acids in the extract which might have had a bactericidal effect on the surface bacteria in the chicken cuts. Further, the effect was higher with the increasing concentration of the extract in the solution. This shows that its activity is concentration dependent.

Table 3: Efficacy of Garcinia cambogia fruit extract as a decontaminating agent for chicken meat cuts (Mean ±SD of log cfu/g).

Level of the extract	SPC	Coliforms
Control	6.54±0.19^{D}	5.36±0.14^{D}
0.5%	4.81±0.16^{C}	3.07±0.06^{C}
1.0%	3.24±0.06^{B}	2.40±0.15^{B}
1.5%	2.59±0.10^{A}	1.48±0.04^{A}

Means with different superscripts in the same column differ significantly (P<0.001)

CONCLUSION

Based on the findings of the present study it may be concluded that, the crude extract of G. cambogia fruit was found to possess a high in vitro antimicrobial activity against common food-borne bacterial pathogens such as Bacillus cereus, Staphylococcus aureus, Salmonella Typhimurium, Escherichia coli, Klebsiella pneumoniae, Shigella flexneri, Pseudomonas aeruginosa and Escherichia coli O157:H7. Both 1 and 2% extracts of G. cambogia fruit were found to exert a significant inhibitory effect on Salmonella Typhimurium and Escherichia coli in minced chicken meat under refrigeration and since there was no significant difference between the two levels, 1% of the extract can be used for this purpose. All the concentrations of the extract (0.5, 1 and 1.5%) were observed to be highly effective in reducing the microbial load of selected chicken cuts. Though the bacterial load reduced with increasing extract concentration, 0.5% extract solution can effectively be used as a decontaminating agent for chicken cuts.

COMPETING INTERESTS: the authors have no known competing interests either financial or personal between themselves and others that might bias the work.

ETHICS STATEMENT: Not applicable.

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