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Effect of incorporation of Jakhiya (*Cleome viscosa*) and Gandreni (*Angelica glauca*) against *Pseudomonas aeruginosa and Salmonella enteric* typhimurium in chicken mince meat and their sensory characteristics in breaded chicken nuggets

Brijmohan Singh Rawat, Bedika Bora and Anita Arya

Abstract
Present study was undertaken to determine the MIC (Minimum Inhibitory Concentration) of Jakhiya and Gandreni against *P. aeruginosa* and *S. enteric* Typhimurium. Antibacterial effect of spices in chicken mince against these bacteria was also studies. Spices were incorporated in breaded chicken nuggets based on various optimization trails. The antibacterial zone of inhibiton of aqueous extracts were determined by disc diffusion assay method in which the aqueous extract of Jakhiya produced 10±0.3mm, 11±0.4mm and 13±0.5mm inhibitory zone against *P. aeruginosa* at 2%, 4% and 6% respectively while against *S.* Typhimurium it produced 8±0.4mm, 9±0.5mm and 12±0.3mm at 2%, 4% and 6% respectively. The aqueous extract of Gandreni produced 9±0.2mm, 10±0.7mm and 13±0.2mm zone of inhibition against *P. aeruginosa* at 2%, 4% and 6% respectively. While against *S. typhimurium* it produced 6±0.3mm, 7±0.4mm and 9±0.5mm at 2%, 4% and 6% respectively. MIC of the Jakhiya and Gandreni were calculated by conventional tube dilution method using Brain Heart Infusion (BHI) as cultured media. Extracts of the spices were prepared in different concentrations (0.25%-6%) in BHI broth which were inoculated with loopful standard cultures of the *P. aeruginosa* and *S.*Typhimurium and incubated for 24 hrs at 37 ºC. Different levels of Jakhiya and Gandreni (2%, 4% and 6%) were incoculated in raw chicken mince meat and incubated with standard cultures of the Pseudomonas *aeruginosa* and *Salmonella enteric* Typhimurium and mince meat was analyzed for count of upto 15th day at refrigeration temperature (4±1 ºC). Among treatments, mince having higher level of Gandreni i.e. 6% showed maximum response against *P. aeruginosa* count. Chicken mince containing Jakhiya at 6% level were safe even upto the 10th day of storage. Various preliminary trials were incorporated & finally compared for final incorporation in the products. On the basis of sensory scores, 1.5% Jakhiya was selected as optimum level for incorporation in chicken nuggets. Treatment having 1% Gandreni scored highest in flavor, juiciness and overall acceptability. (1+0.5%) Jakhiya+Gandreni were selected as optimum level for incorporation in chicken nuggets. Therefore, Jakhiya and Gandreni have potential to be used as natural preservative in processed chicken products.

Keywords: Jakhiya, Gandreni, chicken nuggets, natural preservatives, antimicrobial

Introduction
Meat is considered as an integral component of human diet. Meat from poultry such as chicken, contain good digestible protein, unsaturated fatty acids as well as other nutrients that make it a valuable food. Poultry is one of the fastest growing segments of the agricultural sector in India with around 8% growth rate per annum. However, food safety remains a significant public health issue for the poultry industry.

Food-borne pathogens can come in contact during any phase of poultry production. *Salmonella* and *Pseudomonas* have been considered as some of the major food-borne pathogens associated with poultry and are major sources of human food-borne illness **[1].** *Pseudomonas aeruginosa* is a zoonotic pathogen that infects commercial poultry with great losses. In humans, infection occurs through contaminated chicken carcasses and some related poultry retail products [2]. Similarly, *Salmonella* is one of the most common pathogens associated with food-borne illness in poultry. In 2012, a study reported that despite several efforts to improve hygienic poultry processing, *Salmonella* spp. are still prevalent at a high rate in raw chicken [3]. *S. typhimurium* is the main causative agents of Human Salmonellosis.

In the recent years, spices and herbs have slowly gained importance as potential sources of

natural, effective and safe food preservatives [4]. Since ancient times, the extracts of the spices and herbs were used to improve the sensory characteristics of the food, as preservatives, for their nutritional characteristics, as well as for their antimicrobial effect [5].

The Uttarakhand state of India is rich in a diverse flora and fauna. The Garhwal division and Kumaon of Uttarakhand have a vast variety of flora such as Jakhiya (*Cleome viscosa*) and Gandreni (*Angelica glauca*) which are used by the local people for their medicinal values.

Jakhiya belongs to family Capparidaceae (Caper family). The plant has been traditionally used for its medicinal values. They are reported to possess various beneficial properties such as antiseptic, carminative, anthelmintic and as a cardiac stimulant [6].

Gandreni belongs to family Apiaceae. The whole plant has been reported to be useful as a stimulant, appetizer, carminative, etc. They are also used to treat stomach troubles, infantile atropy, constipation, etc. [7].

In view of the facts presented, present investigation was carried with the following objectives:

-To determine the minimum inhibitory concentration of the spices Jakhiya and Gandreni against *Pseudomonas aeruginosa and Salmonella enteric* Typhimurium bacteria.

-To determine the antimicrobial effect of spices Jakhiya and Gandreni in Chicken nuggets against *Pseudomonas aeruginosa and Salmonella enteric* Typhimurium bacteria.

Materials and Method

Location of experiment

The experiment was conducted in the Department of Livestock Products Technology and Department of Veterinary Public health & Epidemiology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar.

Sources of Material

Source of Chicken meat

Fresh chicken was procured from Instructional poultry farm Pantnagar and slaughtered within 1-2 hours of purchasing. Meat was inspected, washed, cleaned properly in running water and deboned. The boneless meat was packaged airtight in low density poly-ethylene bags (LDPE) and stored at -20 °C till further use.

Spice and condiment mix

Spice mix & condiments (garlic, black pepper, chili and ginger) were purchased from local market. The condiment (black pepper, garlic and ginger) mix was used in ratio of 1:2:1 by removing their external covering and ground in the mixer grinder to the consistency of fine paste.

Test ingredients (Jakhiya and Gandreni)

Jakhiya and Gandreni were procured from local markets of Haldwani, grinded in Philips Mixture grinder to the consistency of fine powder and stored in PET jar at room temperature for further use.

Chemicals and media

All the chemicals and media used in the study were of analytical grade and obtained from Hi media® Mumbai and Merck® Mumbai.

Preparation of standard bacterial inoculum

Preparation of stock inoculum

Under the laminar flow, unopened vial were carefully removed from the storage and allowed to equilibrate in the room temperature for 30-60 min. Then the forceps were sterilized and the pellets were carefully taken out from the vial without removing the desiccant. The pellets were dissolved in 0.5 ml of sterilized NSS. Pellets were crushed with the help of the swab and the same swab was evenly saturated in the hydrated suspension.

The primary culture plates were inoculated by using pressure and the swab were rolled within a circular area approximately 25mm in diameter. Streaking was done by the sterilized loop through the inoculated area of approximately 10-20 times and streaking was done to facilitate colony isolation. Under the properly biohazard disposal, the remaining hydrated material were discarded and immediately all the primary culture plates were incubated at 37 °C for 24-36 hours.

Preparation of serial dilutions of stock inoculum

Six clean, sterile test tubes were taken in duplicates and marked as 1 to 6 for *Pseudomonas aeruginosa* and *Salmonella enteric* Typhimurium separately. 9 ml of normal saline solution was added to each of the test tubes aseptically. One ml of stock inoculum was transferred to the test tubes no.1 and mixed thoroughly. One ml of properly mixed suspension from the test tubes no.1 was transferred to test tube no.2 and same procedure was repeated to get the serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Lastly, one ml of suspension from the test tube no.6 was discarded of properly. Under the Laminar Air Flow, one ml of each stock inoculum and its dilution were transferred to the previously marked and sterilized petri plates followed by the immediate pouring of molten Cetrimideagar (40-45°) and Xylose Lysine Deoxycholate (without autoclave) for *Pseudomonas aeruginosa* and *Salmonella enetrica* Typhimurium respectively. The petri plates were stirred slowly to mix the content thoroughly and left undisturbed until the nutrient agar gets solidify. Thereafter, the plates were incubated for 24-36 h at 4 °C for *Pseudomonas aeruginosa* growth and at 37 °C for *Salmonella enteric* Typhimurium bacterial colonies. The bacterial colonies ranging from 30-300 on the petri plates were counted under the colony counter. Number of colonies for each duplicate was averaged and the suspension containing on an average 10^5 bacterial colonies per ml were selected and used as standard bacterial inoculum.

Determination of antibacterial activity and zone of inhibition of Jakhiya and Gandreni against *Pseudomonas aeruginosa and Salmonella enteric* Typhimurium by disk diffusion assay

The antibacterial zone of Jakhiya and Gandreni were performed by disk diffusion method as described by Natta *et al*. [8] with slight modifications. The diameter of antibacterial zones was measured in millimeters with the help of MIC scale.

Determination of minimum inhibitory concentration (i.e., MIC) of the Jakhiya and Gandreni against *Pseudomonas aeruginosa* and *Salmonella enteric* Typhimurium

MIC was determined using Tube dilution method by using brain heart infusion (BHI) broth as a culture media. Each tube containing 10ml BHI broth was inoculated with a loopful of *Pseudomonas aeruginosa* and *Salmonella enetrica*

Typhimurium *separately* and incubated in a shaker incubator at 35 ºC for 24-36 h to enable enough growth of bacteria. Next day, 16.66 µl of cultured broth was transferred into another 10ml BHI broth tubes to make 300 times dilution. Transferred 50 µl of cultured broth from 300 times dilution into each 10ml BHI broth was arranged with increasing order of concentration of the aqueous extract of the Jakhiya and Gandreni. All the tubes were incubated for 24-48 h at 37 °C to check MIC. A control tube of 10ml BHI broth was also maintained for result comparison.

Evaluation of antibacterial effect of spices Jakhiya and Gandreni against *P. aeruginosa and S. enterica* Typhimurium in meat mince

1000g of boneless chicken was defrosted before use. All the food contact surfaces of meat mincer were sanitized before mincing. The deboned meat was minced by passing twice through 4mm plates of meat mincer. Then, the boneless chicken was subdivided accurately in 100g portions and then 2%, 4% & 6% of the spices were directly added in the different portions of the mince and at the same time dilutions of the culture which were previously analyzed (1:1000) were inoculated and mixed properly.

Samples were prepared according to APHA [9]. Ten grams of the mince which were incorporated with different concentrations of the spices and 1ml of appropriate dilution i.e. of 1:1000 of the test culture respective to the microbiological analysis were taken in a pestle mortar to which 90ml of NSS was added and homogenized thoroughly. Then, 1ml of this sample was taken and serial dilutions were prepared in test tubes.

***Pseudomonas aeruginosa* count**

*P. aeruginosa*count was determined by the APHA [9] method. Using Cetrimide agar media, one ml of appropriate dilution of sample was transferred aseptically to sterile petri plates in duplicate then 20-25 ml melted Cetrimide agar medium was poured at 45 °C. After solidification the petri-plates were incubated at 4-7 °C for 24-48 hrs. After incubation, the petri-plates containing 30-300 colonies were selected. They produced pin-point, white, small, rounded colonies with uniform green color background along with grape-like odour. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per gm of the sample. This count was then converted to *P. aeruginosa* count of log CFU/g of sample.

***Salmonella enterica* Typhimurium count**

S. enterica Typhimurium count was determined by the APHA [9] method using Xylose Lysine Deoxycholate agar. One ml of appropriate dilution of sample was transferred aseptically to sterile petri plates in duplicate and then 20-25 ml of Xylose Lysine Deoxycholate agar was poured. After solidification the petri-plates were incubated at 37 °C for 24-48 hrs. After incubation, the petri-plates having 30-300 colonies were selected. Small round black concave colonies were counted by using colony counter chamber. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per gm of the sample. This count was then converted to *S.* Typhimurium count of log CFU/g of sample.

The minced meat assigned for this study were sealed in the LDPE bags and kept for storage at refrigeration temperature (4 ±1 °C) for further analysis. The antimicrobial assay were carried out at 0, 5th 10th and 15th day of storage. All the analysis was done in duplicates.

Optimization of the level of incorporation of Jakhiya, Gandreni and their combination in chicken nuggets.

Experiment was conducted to incorporate J, G and JG separately on the basic formulation developed and product was evaluated on the basis of sensory trials. Jakhiya (1-2%), Gandreni (1-2%) and Jakhiya + Gandreni in combination were added.

Table 1: The Level of Jakhiya(J 1-2%), Gandreni(G 1-2%) and combination (JG 0.75+0.75% to 0.5+1%)

Product	Jakhiya (J)%	Gandreni%	Jakhiya + Gandreni (JG)
Control	-	-	-
J-T1	1	-	-
J-T2	1.5	-	-
J-T3	2	-	-
G-T1	-	1.0	-
G-T2	-	1.5	-
G-T3	-	2.0	-
JG-T1	-	-	0.75%+0.75%
JG-T2	-	-	1.0%+0.5%
JG-T3	-	-	0.5%+1.0%

Formulation of nuggets containing all the ingredients and optimized level of Jakhiya and gandreni

Various preliminary sensory trials were conducted to formulate composition of chicken nuggets and proportion of chicken and nonmeat additive (salt spices, condiments, gram flour, sodium tripolyphosphate) salt, spices, condiments and test ingredients were standardized. The level selected were above the MIC of the spices against *P. aeruginosa* and *S. enteric* Typhimurium and previous sensory trails.

Table 2: Selection of ingredient level for the nuggets

Ingredients	C	T_J	T_G	T_{JG}
Boneless meat	1000gm	1000gm	1000gm	1000gm
Gram dal	10%	10%	10%	10%
Salt	2%	2%	2%	2%
Polyphosphate	0.3%	0.3%	0.3%	0.3%
Garlic	1%	1%	1%	1%
Ginger	1%	1%	1%	1%
Spice mix	3%	3%	3%	3%
Black pepper	0.5%	0.5%	0.5%	0.5%
Jhakiya	-	(1/1.5/2)%*	-	-
Gandreni	-	-	(1/1.5/2)%*	-
Jhakiya+ Gandreni	-	-	-	(1+0.5)%/(0.75+0.75)%/ (0.5+0.75)%*

*****concentrations were optimized

Deboned chicken meat
↓
Cutting into small pieces
↓
Mincing by using 8 mm plate followed by 4 mm plate
↓
Addition of salt, spices, condiments and other additives and mixed properly
↓
Mixing the test ingredients (Jakhiya, Gandreni and their combination at optimized concentration in the mince)
↓
Formulation of nuggets and baked till internal temperature reached at 82 ºC for 15 min on both sides
↓
Take out nuggets from the pan and allowed it to cool
↓
Packaging in air tight polyethylene bags

Flow chart for the preparation of chicken nuggets using test ingredients jakhiya and gandreni

Results and Discussion

Determination of antibacterial activity of the Jakhiya and Gandreni extract against *Pseudomonas aeruginosa and Salmonella enteric* Typhimurium on nutrient agar plate

The antibacterial zone of inhibition was produced by the aqueous extract at different concentration of Jakhiya and Gandreni spices. The clear antibacterial zone of inhibition wasproduced when the disc diffusion assay method was performed against the *Pseudomonas aeruginosa* and *Salmonella enteric* Typhimurium bacteria. The diameter of antibacterial zone of inhibition was measured with scale.

Table 3: Mean ± S.E. for the antibacterial zone of the aqueous extract of the Jakhiya spice against the *Pseudomonas aeruginosa* and *Salmonella enteric* Typhimurium.

Test Organisms	Concentration of the spice		
	T_{J2}	T_{J4}	T_{J6}
Pseudomonas aeruginosa	10±0.3mm	11±0.4mm	13±0.5mm
Salmonella Typhimurium	8±0.4mm	9±0.5mm	12±0.3mm

Table 4: Mean ± S.E. for the antibacterial zone of the aqueous extract of the Gandreni spice against the *Pseudomonas aeruginosa* and *Salmonella enterica Typhimurium*

Test Organisms	Concentration of the spice		
	T_{G2}	T_{G4}	T_{G6}
Pseudomonas aeruginosa	9±0.2mm	10±0.7mm	13±0.2mm
Salmonella Typhimurium	6±0.3mm	7±0.4mm	9±0.5mm

It had been observed that the aqueous extract of the spice Jakhiya (*Cleome viscosa*) plant produces a wide range of antibacterial zone of inhibition against *Pseudomonas aeruginosa* when the concentration of the spice was increased. A comparatively narrow zone of inhibition was observed against *Salmonella enteric* Typhimurium compared to *P. aeruginosa* by aqueous extract of jakhiya.

Sudhakar *et al.* [10] observed that the different zones of inhibition produced by the leafs of *C. viscosa* against *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* were 34, 26, 22, 23 and 22 mm respectively at 0.100 mg/ml concentration. The MIC of various extracts of *Cleome viscosa* against different bacterial strains *E. coli- enteropathogen*, *P. vulgaris*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *Streptococcus faecalis* were from 0.10 to 0.45mg/ml.

Some other finding obtained by the Williams *et al.* [11] suggested that the hexane extract derived from the stem and leaves of *C. viscose* revealed significant antibacterial property against *Bacillus subtilis* (Gram-positive) and *Pseudomonas fluorescence* (Gram-negative). The antimicrobial property of the plant maybe due to cembranoid diterpene present in the plant. Bhamarapravati *et al.* [12] reported that *Cleome viscosa* were highly significant against *Helicobacter pylori*.

Determination of the minimum inhibitory concentration (i.e., MIC) of the spices Jakhiya and Gandreni against *Pseudomonas aeruginosa* and *Salmonella enteric* Typhimurium using tube dilution method

The zone of inhibition and MIC was performed according to the disc diffusion assay and conventional tube dilution method respectively. Increasing concentrations of the aqueous extract of spices were inoculated with bacteria and the tube where turbidity did not occur was the MIC of the extract. The MIC of the aqueous extract of the Jakhiya against *Pseudomonas aeruginosa and Salmonella enteric Typhimurium* were 0.175% and 0.25% respectively while MIC of the aqueous extract of Gandreni against *Pseudomonas aeruginosa* and *Salmonella enteric Typhimurium* was 0.25% and 0.375% respectively. Sudhakar *et al.* [10] reported that extracts derived from the leafs and flowers of *Cleome viscosa* plant was determined for the antimicrobial activity and showed that there is significant resistant against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, and less intense activity against *Bacillus subtilis*. The extract of the leaves *C. viscosa* showed mild activity against fungi named as Rhizopusoligosporus. Wake *et al.* [13] observed the various extracts of seeds of *Cleome viscosa Linn* plant was reported to be significantly effective against broad spectrum of microbes.

Antimicrobial effect of spices Jakhiya and Gandreni in raw chicken mince against *P.aeruginosa* and *S. enterica* Typhimurium

Antibacterial activity against *Pseudomonas aeruginosa* (log $_{10}$ cfu/gm) count on adding Jakhiya in chicken mince at refrigeration temperature

A highly significant difference (P<0.01) between treatments and storage days was observed (Table 5). Interaction of storage days and treatments was also found to be highly significant (P<0.01).

During storage, *P. aeruginosa* increased significantly (P<0.05) in control as compared to that of treatment. Treated products always showed slower rate of increase in *P. aeruginosa* count than control. Critical difference analysis indicated that control had significantly higher (P<0.05) TPC than treatments during entire storage period. Among treatments, significantly higher (P<0.05) *P. aeruginosa* count were observed in mince incorporated with 2% Jakhiya. Antimicrobial effect of Jakhiya was comparable for reducing the *S. aureus* count. Lowest count observed for mince having 6% Jakhiya the reason due to high level of spice which acts as an antimicrobial agent. The general decreasing trend of *P. aeruginosa* count with incorporation of Jakhiya was: control> TJ2>TJ4>TJ6.

P. aeruginosa count was 7.87±0.03, 6.84±0.02, 6.42±0.02 and 5.93± 0.01 $\log_{10}$cfu for control, TJ2, TJ4 and TJ6 respectively. Among treatments, TJ4 and TJ6 showed significant difference (P<0.05) from 5th days onwards. Control started to spoil at the 5th day, while treatment containing 6% Jakhiya was safe upto 10th day of storage.

Table 5: Effect of different concentration of Jakhiya on *pseudomonas aeruginosa* count values (log $_{10}$cfu /gm) of chicken mince (Mean ± S.E) during refrigerated storage (4±1 °C)

Treatment	0 day	5 day	10 day	15 day	Storage mean
TJ2%	5.2 ± 0.05^{Bd}	6.14 ± 0.01^{Bc}	7.47 ± 0.01^{Bb}	8.56 ± 0.02^{Ba}	6.84 ± 0.02^{B}
TJ4%	5.141 ± 0.03^{BCd}	5.60 ± 0.02^{Cc}	6.82 ± 0.03^{Cb}	8.13 ± 0.03^{Ca}	6.42 ± 0.02^{C}
TJ6%	5.03 ± 0.014^{Cd}	5.29 ± 0.01^{Dc}	6.18 ± 0.02^{Db}	7.24 ± 0.01^{Da}	5.93 ± 0.01^{D}
C	5.59 ± 0.04^{Ad}	7.10 ± 0.02^{Ac}	8.60 ± 0.03^{Ab}	10.2 ± 0.03^{Aa}	7.87 ± 0.03^{A}
Treatment mean	5.2404 ± 0.04^{d}	6.03 ± 0.14^{c}	7.27 ± 0.18^{b}	8.53 ± 0.22^{a}	

n=6, Mean±S.E. overall means bearing different superscript differ significantly, in each row by small alphabet and in each column by capital alphabet.

The results were found to be in accordance with that of Sudhakar *et al.* [10] who reported that the all the extracts of the Jakhiya showed high activity against *Pseudomonas aeruginosa* and produced a zone of inhibition of 22mm of the leaves extract and 14mm of the seed extract at the 0.150mg/ml and 0.500mg/ml concentration respectively. Wake *et al.* [13] stated that the compounds of the different extracts of the Jakhiya were highly effective against pathogenic bacteria; fungi and mixed cultures of the micro-flora derived from the common people and concluded that this spice can be used as a replacer of the synthetic antimicrobial agent.

Antibacterial activity against *Pseudomonas aeruginosa* (log$_{10}$ cfu/gm) count on adding Gandreni in chicken mince under refrigeration temperature

A highly significant difference (P<0.01) between treatments and storage days was observed (Table 6). Interaction of storage days and treatments was also found to be highly significant (P<0.01).

Table 6: Effect of different concentration of Gandreni on *Pseudomonas aeuginosa* count values (log $_{10}$ cfu /gm) of chicken mince (Mean ± S.E) during refrigerated storage (4±1 °C)

Treatment	0day	5day	10day	15day	Storage mean
TJ2%	5.85 ± 0.01^{Bd}	7.15 ± 0.04^{Bc}	8.15 ± 0.12^{Bb}	9.13 ± 0.01^{Ba}	7.57 ± 0.02^{B}
TJ4%	5.71 ± 0.02^{Cd}	6.363 ± 0.05^{Cc}	7.51 ± 0.03^{Cb}	8.94 ± 0.03^{Ca}	7.13 ± 0.03^{C}
TJG6%	5.15 ± 0.03^{Dd}	5.951 ± 0.04^{Dc}	6.85 ± 0.13^{Db}	7.95 ± 0.02^{Da}	6.47 ± 0.01^{D}
C	5.9 ± 0.03^{Ad}	7.525 ± 0.13^{Ac}	8.94 ± 0.01^{Ab}	10.14 ± 0.02^{Aa}	8.13 ± 0.01^{A}
Treatmentmean	5.66 ± 0.06^{d}	6.7475 ± 0.12^{c}	7.86 ± 0.16^{b}	9.04 ± 0.16^{a}	

n=6, Mean±S.E. overall means bearing different superscript differ significantly, in each row by small alphabet and in each column by capital alphabet.

Significant increase (P<0.05) in *Pseudomonas* count was observed with storage period in control as well as treatments, whereas in case of treatment products, significantly (P<0.05) lower count was observed throughout the storage period. Among treatments TG6 had lowest count. The general decreasing trend of *Pseudomonas* count with incorporation of Gandreni was: control>TJ2>TJ4>TJ6. The significantly (P<0.05) decreased in count of *Pseudomonas* with increased in the concentration of the spice in the mince might be due to the increased antibacterial activity of the Gandreni.

Antibacterial activity against *Salmonella enteric* Typhimurium (log$_{10}$ cfu /gm) count on adding Jakhiya in chicken mince under refrigeration storage

A highly significant difference (P<0.01) between treatments and storage days was observed (Table 7). Interaction of storage days and treatments was also found to be highly significant (P<0.01).

Table 7: Effect of different concentration of Jakhiya on *Salmonella* count values (log$_{10}$ cfu /gm) of chicken mince (Mean ± S.E) during refrigerated storage (4±1 °C)

Treatment	0 day	5 day	10 day	15 day	Storage mean
TJ2%	5.59 ± 0.03^{Bd}	6.22 ± 0.02^{Bc}	7.49 ± 0.02^{Bb}	8.98 ± 0.04^{Ba}	7.07 ± 0.03^{B}
TJ4%	5.01 ± 0.04^{Cd}	5.97 ± 0.05^{Cc}	6.59 ± 0.03^{Cb}	8.13 ± 0.03^{Ca}	6.42 ± 0.03^{C}
TJ6%	5.02 ± 0.03^{Cd}	5.51 ± 0.03^{Dc}	6.25 ± 0.01^{Db}	7.08 ± 0.02^{Da}	5.96 ± 0.02^{D}
C	6.12 ± 0.02^{Ad}	7.38 ± 0.02^{Ac}	8.95 ± 0.04^{Ac}	10.23 ± 0.09^{Aa}	8.16 ± 0.04^{A}
Treatment mean	5.43 ± 0.09^{d}	6.27 ± 0.14^{c}	7.32 ± 0.21^{b}	8.60 ± 0.24^{a}	

n=6, Mean ± S.E. overall means bearing different superscript differ significantly, in each row by small alphabet and in each column by capital alphabet.

Control had significantly higher (P<0.05) *Salmonella* count than treatment products from day 0th onwards. The general decreasing trend of *Salmonella* count with incorporation of Jakhiya was: control> TJ2>TJ4>TJ6. On increasing the concentration of the Jakhiya in the mince the *Salmonella* count had been significantly decreased.

Finding of Sudhakar *et al.* [10] suggested same results that extracts of the leaves and flowers of *Cleome viscosa* exhibited antimicrobial activity, particularly significant against *Salmonella* and pathogenic fungi. Williams *et al.* [11] examined that hexane extract of the stems and leaves of *Cleome viscosa* exhibited wide range of antimicrobial activity.

Antibacterial activity against *Salmoenlla enterica* Typhimurium (log$_{10}$ cfu/gm) count on adding Gandreni in mince

A highly significant difference (P<0.01) between treatments and storage days (Table 8) was observed. Interaction of storage days and treatments was also found to be highly significant (P<0.01).

Table 8: Effect of different concentration of Gandreni on *Salmonella* count values (log_{10} cfu /gm) of chicken mince (Mean±S.E) during refrigerated storage (4±1 °C)

Treatment	0 day	5 day	10 days	15 days	Storage mean
TG2%	6.16 ± 0.03^{Dd}	7.53 ± 0.02^{Bc}	9.25 ± 0.02^{Bb}	10.57 ± 0.04^{Aa}	8.38 ± 0.03^{B}
TG4%	6.29 ± 0.04^{Cd}	7.44 ± 0.05^{Cc}	9.11 ± 0.03^{Cb}	10.26 ± 0.03^{Ba}	8.27 ± 0.03^{C}
TG6%	6.36 ± 0.03^{Bd}	7.23 ± 0.03^{Dc}	8.92 ± 0.01^{Db}	10.22 ± 0.02^{Ba}	8.18 ± 0.02^{D}
C	6.37 ± 0.02^{Ad}	7.73 ± 0.02^{Ac}	9.53 ± 0.04^{Ab}	10.66 ± 0.09^{Aa}	8.57 ± 0.04^{A}
Treatment mean	6.29 ± 0.09^{d}	7.48 ± 0.14^{c}	9.20 ± 0.21^{b}	10.43 ± 0.24^{a}	

n=6, Mean ± S.E. overall means bearing different superscript differ significantly, in each row by small alphabet and in each column by capital alphabet.

The *Salmonella* count was significantly ($P<0.01$) increased from 0 to 15 days but there is no significant ($P>0.01$) difference in the control and treatments (TG2%). On 15th day, significant difference ($P<0.05$) was observed in *Salmonella* count of Control, T4% & T6%. Gandreni showed very less antibacterial activity against *Salmonella* on increasing its concentration at each level. This may due to the reason that the Gandreni was not completely able to penetrate the cell membrane of the bacteria. Gandreni was less effective against *Pseudomonas* and *Salmonella*, which might be due to the difference in the outer layer of both the Gram – bacteria. Gram – bacteria possess an extra outer membrane as well as periplasmic space which is absent in Gram + bacteria. The periplasmic space consist lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules and the enzyme which is highly effective against outer antibacterial substances by breaking the complex molecules [14].

Standardization of level of Incorporation of Jakhiya, Gandreni and their combination in the breaded chicken nuggets.

Optimization of the level of the Jakhiya in the nuggets.

On the basis of various preliminary sensory trials 1%, 1.5% and 2% level of Jakhiya were selected for final optimization in the product (Fig 1).

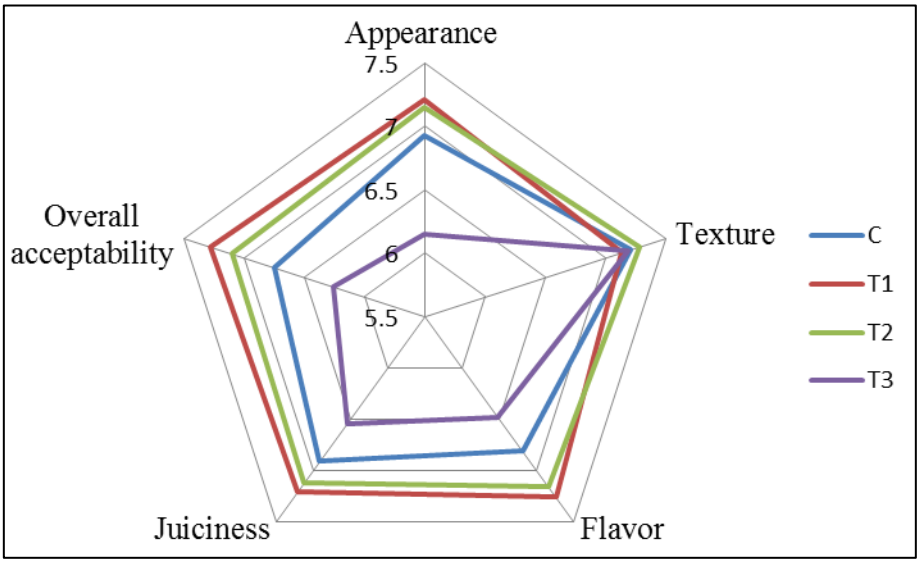

Fig 1: Radar chart on effect of different levels of Jakhiya on sensory attributes of chicken nuggets.

T1: Chicken nuggets having 1% Jakhiya
T2: Chicken nuggets having 1.5% Jakhiya
T3: Chicken nuggets having 2% Jakhiya
C: Chicken nuggets having no Jakhiya

A significant difference ($P<0.05$) in appearance was observed. Whereas, highly significant difference ($P<0.01$) in flavor, juiciness and overall acceptability was seen. Control product scored highest in appearance while T2 scored highest in flavor, juiciness and overall acceptability than all other treatments and control.

These results were in accordance with that of Ibrahium *et al.* [15] who observed that odor and taste score of control and treatment cake samples containing 400 ppm clove essential oil did not differ significantly with control.

Optimization of the level of the Gandreni in the nuggets

On the basis of various preliminary sensory trials 1%, 1.5% and 2% level of Gandreni were selected for final optimization in the product (Fig 2).

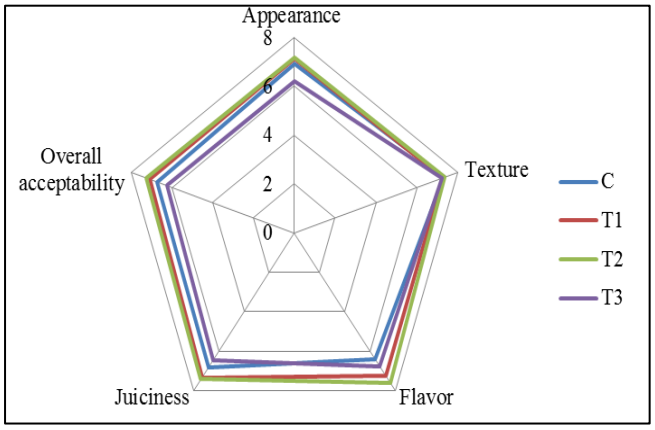

Fig 2: Radar chart on effect of different levels of Gandreni on sensory attributes of chicken nuggets

T1: Chicken nuggets having 1% Gandreni
T2: Chicken nuggets having 1.5% Gandreni
T3: Chicken nuggets having 2% Gandreni
C: Chicken nuggets having no Gandreni

A highly significant difference (P<0.01) in appearance, flavor, texture and overall acceptability was observed. Whereas, non-significant difference (P>0.01) in juiciness of control and treatments were observed. There was non-significant difference (P>0.01) in flavor and juiciness among T1 and T2 treatments whereas C scored more than T3 in appearance, flavor and juiciness. T1 product scored highest in appearance while T2scored highest in flavor, juiciness and overall acceptability than all other treatments and control.

Optimization of the level of the Jakhiya + Gandreni in the nuggets

On the basis of various preliminary sensory trials three levels of Jakhiya + Gandreni were selected for final optimization in the product (Fig 3).

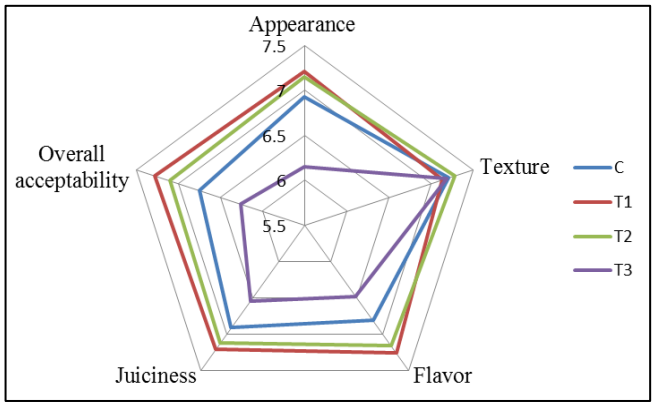

Fig 3: Spider chart on effect of different levels of combination of Jakhiya + Gandreni on sensory attributes of chicken nuggets.

C: Chicken nuggets having no Jakhiya + Gandreni
T1: Chicken nuggets having 0.75%Jakhiya+0.75% Gandreni
T2: Chicken nuggets having 1%Jakhiya+0.5% Gandreni
T3: Chicken nuggets having 0.5%Jakhiya+1% Gandreni

A significant difference (P<0.01) in appearance, flavor and overall acceptability was observed. Whereas, non significant difference (P>0.01) was reported in texture and juiciness of control and treatments. T2 product scored highest in flavor, juiciness, overall acceptability and overall mean than all other treatments and control.

The results is in accordance with Antony *et al.* [16] who reported that spices imparts slightly darker color to chicken slices with lower lightness values without affecting of redness and yellowness values and thus on higher concentration decreases acceptability. On the basis of sensory trials and scores patties containing Jakhiya at 1.5%, Gandreni at 1% and J+G at 1%+0.5% scored highest amongst all.

Conclusion

Jakhiya and Gandreni both were effective against *P. aeruginosa and S. enteric* Typhimurium when added in chicken mince. Antimicrobial activity increased with increasing the concentration of Jakhiya in the mince. The sensory attributes of the chicken nuggets prepared by incorporating these spices on the mince were also quite satisfactory. Hence, they have the potential in future to be used as natural preservative or antimicrobial agents in poultry meat products.

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