DOI: 10.21088/jafst.2321.1628.14126.3

Journal of Animal Feed Science and Technology
Volume 14 Number 1 January - June 2026

ORIGINAL ARTICLE

Antimicrobial Effectiveness of (*Cleome viscosa* and *Angelica glauca)* in preserving the quality of Chicken nuggets

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HOW TO CITE THIS ARTICLE:

Brijmohan Singh Rawat, Anita Arya, Maansi et. al, Antimicrobial Effectiveness of (*Cleome viscosa* and *Angelica glauca*) in preserving the quality of Chicken nuggets. Jrl of Ani Feed Sci and Tech 2026; 14(1): 21-29.

ABSTRACT

Experiment was undertaken to determine the antimicrobial effectiveness of *Cleome viscosa* and *Angelica glauca* commonly in chicken nuggets. Minimum inhibitory concentration (MIC) of the *Cleome viscosa* and *Angelica glauca* against Pseudomonas. *aeruginosa* and *Salmonella enterican typhimurium* was determined by tube dilution method. Jakhiya at 0.175% concentration was able to inhibit the growth of *P. aeruginosa* while S. Typhimurium was inhibited at 0.25%. Aqueous extract of Gandreni inhibited the growth of *Pseudomonas. aeruginosa* at 0.25% while *Salmonella enterican typhimurium* was inhibited at 0.375%. These Spices were incorporated in breaded chicken nuggets based on various optimization trails. Chicken nuggets incorporated with optimum level of these spices jakhiya(CV), gandreni(AG), jakhiya and gandreni(CVAG) were subjected to microbial storage stability analysis at refrigeration temperature for Total plate counb, Proteolytic count andYeast and

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➢ **Received:** 17-03-2026 ➢ **Accepted:** 20-04-2026

mold count The general decreasing trend of microbial count with incorporation of J, G and its combinations:wasCVAG<CV<AG<C. Treatments showed significant antimicrobial activity against *Pseudomonas aeruginosa* and *Salmonella enterica* Typhimurium in chicken mince and alsoconsiderably enhanced the microbial quality of chicken nuggets.

KEYWORDS

• *Cleome Viscosa* • *Angelica Glauca* • Antimicrobial Activity • Chicken Nuggets

INTRODUCTION

Spices and herb are potentially important source natural safe and effective food preservatives **(Martínez-Graciá *et al.*, 2015)**. Extract of spices and herbs used to improve the sensory characteristics of the food, as preservatives, for their nutritional characteristics, safe and also for their antimicrobial effect **(Gyawali and Ibrhahim, 2014)**. of herbs *Cleome viscosa* aqueous extract were used for the antipyretic analgesic and a hypoglycemic treatment in Israel **(Z.Yaniv *et al.*, 1997)**. Some of the natural compounds found in various spices possess antimicrobial **(Indu *et al.*, 2006)**. *Cleome viscosa* were used in a no. of therapeutic purposes like carminative, diuretic, emmenagogue, galactogogue, nervine, stimulant and tonic. The shelf life of the food is largely dependent on the no. of microbes invasion in the food over the period of the time as well as the type of microbes initially present and subsequent growth **(Borch *et al.*, 1996)**. Meat and meat products are excellent source of the growth and proliferation of the microbial **(Jay *et al.*, 2005)**. Different Grams-negative psychotropic member of the family. Enterobacteriaceae family is predominantly found in refrigeration storage in meat and meat products. Different type of microbial spoilage bacteria are found on the storage in meat and meat products **(Cerveny *et al.*, 2009)**. In refrigeration storage (4±1°c) the shelf life of beef was only 3-5 day **(Oussalah *et al.*, 2004)**. *Cleome viscosa* extract have antibacterial and antifungal activity the 14-member ring Cembranoid is potent antimicrobial **(Williams *et al.*, 2003)**.

The Uttarakhand state of India is richness with the diversity of flora and fauna. The Garhwal division and kumaon of Uttarakhand have a vast varieties of flora such as *Cleome viscosa* (CV) and *Angelica glauca* (AG) commonly known as Jakhiya and Gandreni in hilly region of Uttarakhand both of these plants are reared from thousands of years back in India and others tropical region of world. Jakhiya belongs to family Capparidaceae and Gandreni belongs to family Apiaceae family. Both plants beings having no of medicinal uses and is an aromatic bitter tonic herb that reduces blood pressure, relieves indigestion, stimulates the uterus and is anti-inflammatory., *CV* belongs to Cappracdae family and *AG* belongs to Apiaceae family.

Present investigation has been planned with objectives to determine the minimum inhibitory concentration of the CVand AG against *Pseudomonas aeruginosa* and *Salmonella enteric typhimurium* bacteria and to determine the antimicrobial and antioxidant effect in chicken nuggets during storage at refrigeration tempetrtaure.

MATERIALS AND METHODS

Seed of plants *Cleome viscose* and dried root of *Angelica glauca* were purchased from the local market of Pantnagar. All the chemicals reagents for laboratory analysis to be used were of analytical grade were obtained from standard firms (Qualigen, Hi-Media, Sdefine etc.).

Preparation of aqueous extract of the spices: Spices were dried at 50) C for 24 hr, grinded in the form of fine powder and dissolved in freshly prepared distilled water (Yield *C.viscosa* 9g/100ml and *A. glauca* 11g/100ml). After sieving through muslin cloth filtrate was again filtered through whatman filter paper no 1 and kept in incubator for making moisture free extract. Aqueous Extract were Stored in desiccator for further use.

Minimum inhibitory concentration: MIC was determined by tube dilution method. **(Eloff, 1998)**. BHI (Brain Heart infusion) broth

was taken in 4 sets of 9 test-tube, each test-tube having 9ml broth in it. 1ml of diluted standard bacterial culture of *Pseudomonas aeruginosa* and *Salmonella enterica* Typhimurium were mixed in each respective test tube with an increasing concentration of aqueous extract of *CV* and *AG* in each respective test tube.

RESULT AND DISCUSSION

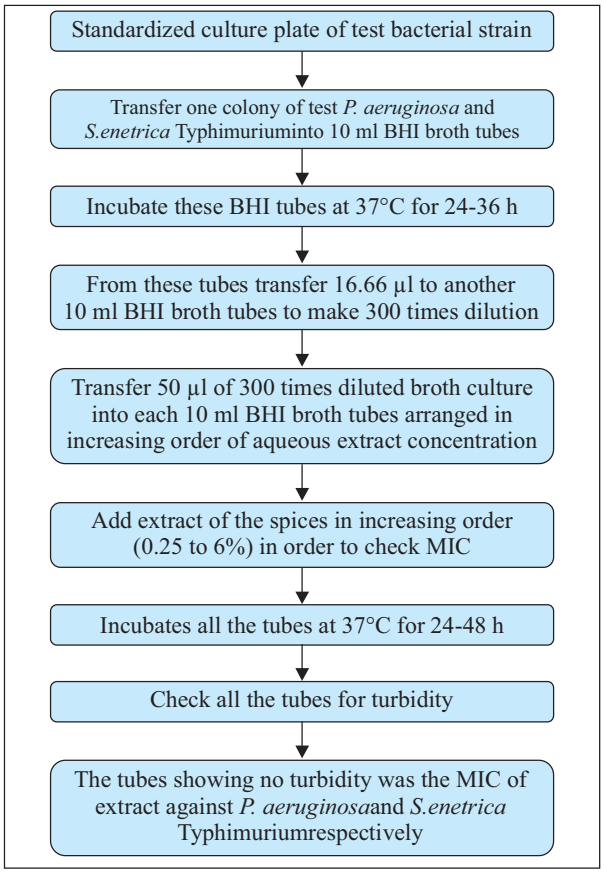

Table 1: Optimization of of C. viscosa and A. glauca in level of for the nuggets Formulation of chicken nuggets:

Ingredients	C	CV	AG	CVAG
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%
CV	-	1.5	-	-
AG	-	-	1.5	-
CV+AG	-	-	-	0.75+0.75

C. viscosa (cv) A. glauca AG) and its combination (CVAG)

The Control and treatment product containing optimum level of Jakhiya and Gandreni were subjected to storage analysis at refrigeration temperature (4±1°C) till spoilage.

C. viscosa (cv) ***A.glauca*** AG) and its combination (CVAG) were also incorporated in the chicken nuggets and kept for refrigeration storage (4±1°c) for 0, 5th, 10th and 15th day of storage. TPC,

Microbial quality evaluation:

Total Plate Count, Yeast and mold count and Proteolytic count were determined by the standard method described by AOAC (1992).

EVALUATION OF ANTIOXIDANT EFFECT

Thiobarbituric acid reacting substances (TBARS) value

TBARS value was determined according to the method described by the Tarladgis *et al.* (1960). The O.D. was measured at 538nm against blank. The TBA value as mg of malonaldehyde per 1000 g of sample was calculated using fallowing formula.

TBA value (mg of malonaldehyde/1000g of sample) = O.D of sample x 7.8

Free fatty acid value was determined by the method described in AOAC (1995) with little modification. Peroxide value of the sample was determined by the method described by Koniecko (1979).

DPPH radical scavenging activity

DPPH assay were performed according to the method of **Tepeet *al.* (2005)** with little modifications. 0.1g of the samples were taken in a clean test tube and 5ml of 0.004% DPPH in methanol were added to the same test tube. The samples were subjected to homogenization for 30sec by using clean pestle mortar. The samples were kept at room temperature for 30min with constant mixing. Absorbance was measured using spectrophotometer at 517nm, using methanol as a blank. Measurements were expressed as absorbance.

Total Phenolics by F-C method: Total phenolic content in chicken nuggets was determined using the Folin Ciocalteu.

Statistical analysis: Each trial was replicated thrice in duplicate (n=6). The statistical analysis of the data was done through analysis of variance (ANOVA one way analysis technique

using SPSS-16 Statistics Software. Differences between means were considered significant when P<0.05. Duncan's multiple range tests were used to detect differences among mean values.

RESULTS AND DISCUSSION

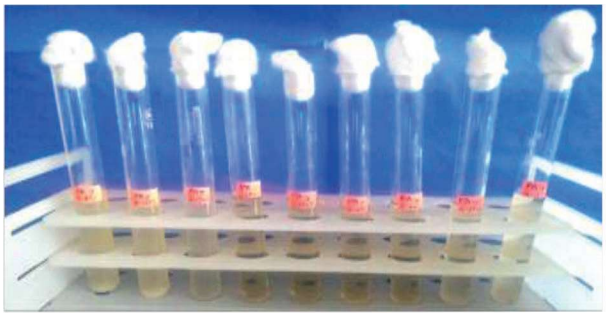

Figure A: MIC of Jakhiya against *Pseudomonas aeruginosa* showing no turbidit at 0.175%

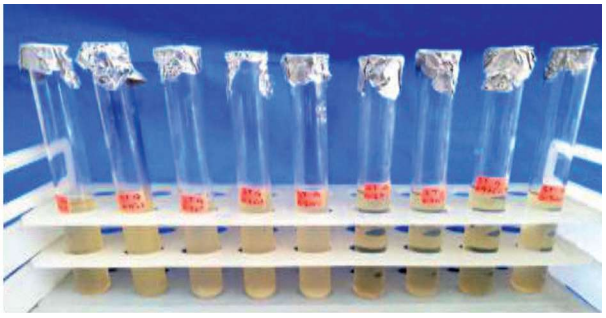

Figure B: MIC of Gandreni against *Salmonella enterica Typhimurium* showing no turbidity at 0.375%

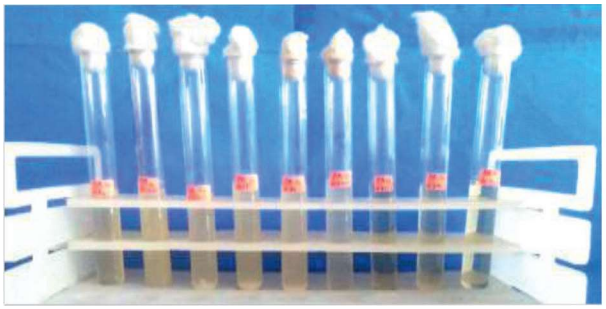

Figure C: MIC of Gandreni against *Pseudomonas aeruginosa* showing no turbidit at 0.25%

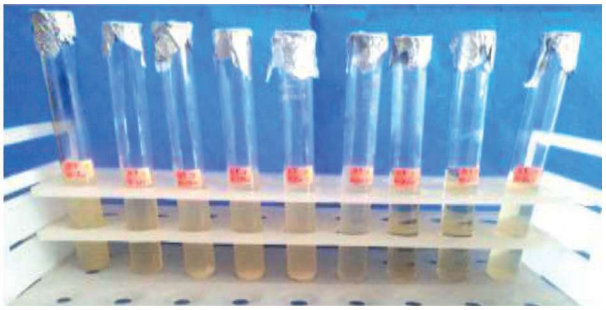

Figure D: MIC of Jakhiya against *Salmonella enterica* Typhimurium showing no turbidity at 0.25%

Figure 1: The MIC of the aqueous extract of the CV against *Pseudomonas aeruginosa* and *Salmonella enterica Typhimurium*

The MIC of the aqueous extract of the CV against *Pseudomonas aeruginosa and Salmonella enterica Typhimurium* were 0.175% and 0.25% respectively while MIC of the aqueous extract of AGagainst *Pseudomonas aeruginosa* and *Salmonella enterica Typhimurium* was 0.25% and 0.375% respectively. (Figure 1). **Wake *et al.* (2011)** observed the various extracts of seeds of Cleome viscosa Linn plant was reported to be significantly effective against broad spectrum of microbeswhereas, **Sudhakar *et al.* (2016)** 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*.

Antimicrobial effect of Cleome *viscose, Angelica glauca* and their combination on chicken nuggets at refrigeration temperature (4±1°c).

Total plate count value significantly increased (P<0.05) in control as well as in treatment products. Control had significantly (P<0.05) higher value compared to storage. Among treatment CVAG had least (P<0.05) TPC throughout the storage period (Table3). Different extract of seed of Cleome viscosa had strong antibacterial activity against *P. aeruginosa, S. Typhimurium, E.coli, B. cereus. As also investigated by* **Wake *et al.*, (2011)**.

Table 2: Effect of incorporation of Cleome viscose, Angelica glauca and their combination on the total Plate count (log_{10} cfu/g)

Treatment	0 day	5 day	10 day	15 day
CV	1.34±0.02BCd	1.84±0.10Cc	2.30±0.03Bb	2.96±0.4BCa
AG	1.43±0.03Ac	2.30±0.01Bb	2.38±0.04Bb	3.15±0.31Ba
CVAG	1.30±0.01Cd	1.56±0.08Dc	1.96±0.05Cb	2.38±0.33Da
C	1.39±0.02ABa	2.94±0.41Aa	3.56±0.59Aa	4.86±0.48Aa

n=6 Mean ± S.E. bearing different superscripts row wise and column wise differ significantly (P<0.05). ***C. viscosa*** (cv) ***A.glauca*** AG), combination (CVAG)

Y&M count significantly (P<005) higher in control than the treatment throughout the storage period (Table 3). Among the treatments lowest count were observed for CVAG treatment. Samelis and Metaxopoulos, (1998) also reported thsat Y&M are potent spoilage organisms in Greek sausages at 8th – 10th day of storage reaching its value at 3 $\log_{10}$ cfu/g and 5 $\log_{10}$ cfu/g at 12th -15th days of storage.

Table 3: Effect of incorporation of Cleome viscose, Angelica glauca and their combination on the Yeast and mold count ($\log_{10}$ cfu/g)

Treatment	0 day	5 day	10 day	15 day
CV	0.13±0.07Cd	0.86±0.07Cc	1.57±0.01Cb	2.17±0.02Ca
AG	0.35±0.01Bd	1.17±0.06Bc	1.88±0.02Bb	2.54±0.03Ba
CVAG	0.75±0.06Dd	0.78±0.02Dc	1.47±0.01Db	1.97±0.60Da
C	0.54±0.51Ad	1.95±0.45Ac	2.46±0.25Ab	3.18±0.49Aa

n=6 Mean ± S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) differ significantly (P<0.05)

The extract of the Jakhiya had significantly effect on the Aspergillus niger, Aspergillus flavous, Candida albicans and Saccharomyces cerviceae(Wake *et al,* 2011). The leave of C. viscosa had significan antifungal activity on the growth of *Rhizopus oligosporus* (Sudhakar *et al.,* 2016).

During the storage period the Proteolytic count significantly increased in control as well as in treatment products. Control had significantly (P<0.05) higher value compared to storage among treatment CVAG had least proteolytic count throughout the storage period (Table 4).

Table 4: Effect of Jhakiya *(Cleome viscose)* and Gandreni *(Angelica glauca)* and its combination on proteolytic count ($\log_{10}$cfu/g)

Treatment	0 day	5 day	10 day	15 day
CV	1.53±0.04Bd	2.20±0.12Cc	2.48±0.04Bb	3.20±0.02BCa
AG	1.66±0.04Ac	2.46±0.02Bb	2.53±0.04Bb	3.30±0.05Ba
CVAG	1.51±0.03Bd	1.70±0.05Dc	2.20±0.03Cb	2.55±0.02Ca
C	1.71±0.5Ad	3.28±0.04Ac	3.88±0.06Ab	5.13±0.47Aa

n=6 Mean ± S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) differ significantly (P<0.05).

Nayak *et al. (*2004) found increased proteolytic count in chicken patties in refrigeration storage (4±1°c). Pork muscle was inoculated with the *P. fragi* bacteria for 20day incubation period at 10°c storage temperature. *P.fragi* produced large amount of extract cellular proteolytic activity in ground pork (Tarant 1971).

Antioxidant effect on chicken nuggets at refrigeration temperature (4±1°c).

Thiobarbituric acid reactive substance (TBARS)

Control had significantly (P<0.05) higher TBARS value than CV, AG and CVAG throughout the storage period. Among treatments the AG had significantly (P<0.05) higher TBARS value as compared to CV and CVAG. CVAG had significantly (P<0.05) lower values as compared to CV, AG and C throughout the storage period (Table 5). Lowest TBARS value reported in CVAG and was in accordance with that of **Lopez-Bote *et al.* (1998)** who stated that the dietary administration of the extract of the spices slightly increases the oxidative stability of the chicken meat by reducing the microsomal and cholesterol oxidation, stability increases when spices used in the combination by employing the synergistic effect.

Table 5: Effect of incorporation of Cleome viscose, Angelica glauca and their combination on TBARS values (mgMDA/Kg of sample) of chicken nuggets (Mean ±SE) during refrigerated storage (4±1°C)

Treatment	0 day	5 day	10 day	15 day
CV	0.28±0.01Ac	0.14±0.01Bc	0.25±0.17Bb	0.47±0.17Ca
AG	0.57±0.02Ac	0.16±0.06ABc	0.28±0.02ABb	0.54±0.03Ba
CVAG	0.20±0.02Ac	0.17±0.05Ac	0.25±0.01Bb	0.48±0.05BCa
C	0.14±0.01Ac	0.11±0.04Cc	0.32±0.04Ab	0.68±0.04Aa

n=6 Mean ± S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) differ significantly (P<0.05).

Wellwood and Cole (2004) reported that phenols present in the spices stabilize hydroperoxides which prevents further degradation to more active oxidizing forms, such as malonaldehyde.

Free Fatty Acid value

Control had significantly higher (P<0.05) FFA value than CV, AG and CVAG throughout the storage period. Initially, at 0th day nonsignificant (P>0.05) difference between the treatments and control were observed. During storage period, significant increase (P<0.05) in FFA was observed in control and treatments from 5th day onwards. Among treatments the AG had significantly (P<0.05) higher FFA value as compare to CV and CVAG. CV and C showed nonsignificant (P>0.05) difference up to 15th days of storage. CVAG had significantly (P<0.05) lower values as compared to CV, AG and C throughout the storage period (Table 6).

Table 6: Effect of Jakhiya, Gandreni and their combinations on FFA values (% of oleic acid) of chicken nuggets (Mean ±SE) during refrigerated storage (4±1°C)

Treatment	0 day	5 day	10 day	15 day
CV	0.18±0.02Bd	0.24±0.09Dc	0.57±0.03Cb	0.64±0.01Ca
AG	0.19±0.01Bd	0.35±0.08Bc	0.55±0.04Bb	0.79±0.03Ba
CVAG	0.17±0.01Bd	0.28±0.01Cb	0.44±0.04Db	0.63±0.01Da
C	0.23±0.01Ad	0.44±0.08Ac	0.64±0.09Ab	0.87±0.07Aa

n=6 Mean ± S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) differ significantly (P<0.05).

Kin *et al.* (2011) reported that there was little or no FFA produced in the smoked dry catfish on storing upto 7 days when uziza *(Piper guinensis)* an indigenous spice used for the preservation. **Ninan *et al.* (2011)** reported that the incorporation of spice in freeze drying fish balls extended the shelf life upto 98 days as compare to 70 days in control by delaying the formation of FFA content. **Zhao *et al.* (2011)** reported that the adding of black pepper in the dry fermented mutton sausage advances the product with lower SFA and higher MUFA, PUFA and MUFA + PUFA/SFA as compared to control.

Peroxide value

Mean ± SE values of PV during refrigeration storage of chevon patties are given in Table 4.21, Fig 4.18 and ANOVA in Table 4.25. Analysis of variance revealed a highly significant difference (P<0.01) between treatments and storage days. Interaction of treatment and storage days was also found to be significant (P<0.01).

Table 7: Effect of Jakhiya, Gandreni and their combinations on Peroxide values (mEq/Kg of fat) of chicken nugggets (Mean ±SE) during refrigerated storage (4±1°C)

Treatment	0 day	5 day	10 day	15 day
CV	0.11±0.01Cd	0.19±0.01Cc	0.26±0.02Cb	0.66±0.09Ba
AG	0.13±0.03Bd	0.26±0.09Bc	0.55±0.03Bb	0.75±0.02Ba
CVAG	0.11±0.01Cc	0.16±0.05Db	0.17±0.08Db	0.55±0.01Ca
C	0.14±0.01Ad	0.37±0.08Ac	0.77±0.01Ab	1.22±0.05Aa

n=6 Mean ±S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) significantly (P<0.05)

upto the 27 days of storage. These finding confirms to the observation of **Özcan *et al.* (2009)** in turkey products who suggested that the extracts and essential oils of the spices, herbs and condiments have the low peroxide value and high phenolic content and thus have good antioxidant property. Similar finding were reported by **Das *et al.* (2011)** who stated that on adding curry leaf powder significantly reduced the peroxide value of the ground goat meat.

Control had significantly higher (P<0.05) PV value than CV, AG and CVAG. Among treatments the AG had significantly (P<0.05) higher PV value as compared to CV and CVAG. CV and CVAG showed nonsignificant (P>0.05) difference up to 10th days of storage. CVAG had significantly (P<0.05) lower values as compared to CV, AG and C throughout the storage period (Table 7). **Aguirrezábal *et al.* (2000)** stated that the treatment containing paprika in dry sausage tends to lower the PV

DPPH activity (% inhibition)

DPPH activity significantly decreased (P<0.05) in control as well as in treatments at each interval of storage period. Critical difference analysis indicated that there was significant difference (P<0.05) between DPPH value of all the treatments and control. The DPPH activity significantly decreased in control followed by CV then AG and then AVAG.

Table 8: Effect of Jakhiya, Gandreni and their combinations on DPPH (% inhibition) of chicken nuggets (Mean±SE) during refrigerated storage (4±1°C)

Treatment	0 day	5 day	10 day	15 day
CV	19.03±0.14Ba	19.05±0.02Aa	15.30±1.25ABb	13.16±0.03Bc
AG	18.71±0.29Ba	18.21±0.86Ba	14.42±0.29Bb	12.6±0.19Cc
CVAG	20.55±0.18Aa	18.34±0.25Ab	16.74±0.14Ac	14.94±0.06Ad
C	10.86±0.10Ca	9.35±0.45Cb	7.17±0.21Cc	4.83±0.11Dd

n=6 Mean ± S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) significantly (P<0.05).

Factors like release of free and bound phenols on increasing the storage period is responsible for the decreasing the DPPH content of the samples **(Shan *et al.*, 2005)**. CV and AG showed nonsignificant (P>0.05) at the 15th day. Overall mean revealed C and all treatments are significantly (P<0.05) higher DPPH activity at 0 day. CVaG had significantly (P<0.05) higher DPPH activity among all the treatments and Control. DPPH activity was lowest in Control on 15th day followed by the CVAG, CV and AG. Difference in the polyphenols, flavonoids and other antioxidant properties might be responsible for the difference in the DPPH radical scavenging activity of the treated sample (Table 8).

Similar findings were reported by **Malav *et al.* (2015)** who reported that the mutton patties containing cabbage powder had significantly (P<0.05) higher DPPH radial scavenging activity as compare to control the reason might be due to the powder containing high bioactive compounds and thus had high antioxidant potential. **Kodal *et al.* (2014)** reported that the essential oil of the oregano had higher DPPH radical scavenging activity than thyme essential oil.

Total phenolic content (mg/g)

Total phenolic content decreased significantly (P<0.05) at each interval of storage period. However, the rate of decrease of total phenolic content was slower in case of treatment products (Table 9)throughout the storage period indicating that treatment products had better oxidative stability than control. De crease in phenolic compounds during storage might be due to oxidation of phenolic compounds in the presence of oxygen. **Russo *et al.* (2000)** reported that decrease in phenolic content in treatments is due to the presence of some bioactive compounds in the spices which interferes with the oxidation.

Table 9: Total phenolic (mg/g) of chicken nuggets (Mean ± SE) during refrigerated storage (4±1°C)

Treatment	0 day	5 day	10 day	15 day
CV	1.95±0.02Ba	1.85±0.03Ba	1.58±0.02Aa	1.25±0.03Ba
AG	1.23±0.07Ca	1.14±0.01Cb	0.77±0.01Bc	0.65±0.13Cd
CVAG	2.04±0.01Aa	1.93±0.02Ab	1.77±0.02Bd	1.65±0.02Ac
C	0.73±0.04Da	0.59±0.02Db	0.45±0.01Cc	0.36±0.01Dd

n=6 Mean ±S.E. bearing different superscripts row wise (small alphabet) and column wise (capital alphabet) differ significantly (P<0.05)

Total phenolic content was highest in CVAG might be due to the reason of combination of spices antimicrobial compound & their synergistic contributed to increase in the total phenolic content. Aparadh et al. (2012) reported that the Cleome viscosa contain the highest amount of polyphenols in compare to other Cleome species and has the highest antioxidant activity.

CONCLUSION

There is a vast opportunity of using extract from culinary herbs and spices as antimicrobial additives in food without any negative impact on health as in case of synthetic additives.. aqueous extract of Cleome viscosa and Angelica glauca from uttarakhand hilly region in the study showed significant antimicrobial and antioxidant activity for preserving the

quality of chicken nuggets without any added chemical preservatives. Advanced studies for use in oher processed and functional meat food products need to be done to explore further potential of these herbs against different microbial and fungal food poisoning pathogen and to prevent food spoilage. Aqueous extract of these spices can be effectively used as natural antimicrobials in chicken mince and nuggets.

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