

FUNCTIONAL, ANTIOXIDANT, ANTIMICROBIAL POTENTIAL AND FOOD SAFETY APPLICATIONS OF *CURCUMA LONGA* AND *CUMINUM CYMINUM*

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Abstract

Turmeric (*Curcuma longa*) and Cumin (*Cuminum cyminum*) seeds are common spices used in foods and are necessary commodity of kitchens. They have known potential in health and pharmaceutical industries. In present study, we explored the antibacterial, antifungal, antioxidant and functional properties of turmeric and cumin seeds extracts. The extracts were also used against *Escherichia coli* in active packaging for pathogen control and food safety. It was found in the study that, phlobatannins, flavonoids, alkaloids and quinon were absent while the coumarin and terpenoids were present in cumin, whereas turmeric was found rich in phlobatannins, flavonoids, alkaloids, terpenoids and quinon except coumarin, which was found absent. The total phenolic contents of cumin and turmeric were estimated as 51.2 mg/g and 20 mg/g of dry weight equivalent to gallic acid. The DPPH radical scavenging activity of 44% and 46% were recorded for cumin and turmeric respectively. The FTIR analysis established the presence of different functional groups preliminary confirmed by chemical analysis. Both cumin and turmeric were found active against a group of pathogenic bacteria including, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Enhanced antibacterial activity of cumin was noted compared to turmeric extracts. Both extracts were found active against different fungal species, *Mucor mucedo*, *Aspergillus flavus*, *Aspergillus niger* and *Saccharomyces boulardii*. Cumin and Turmeric extracts incorporated in alginate- based film for packaging and foodborne pathogens control in meat were found reducing the number of pathogenic bacteria *E. coli*.

Key words: Packaging, Food safety, Phytochemicals, Pathogens, Antifungal, Antibacterial.

Introduction

Vegetables, plant-based medicines and food are the rich sources of natural healing compounds which can play vital role in the human health and wellbeing. The compounds present in these plants materials responsible for healing potential are usually called bioactive compounds, which can be utilized for new drug development. In food, they are termed as phytonutrients or functional food working as disease preventives (Wolfender *et al.*, 2011; Georgiev, 2014).

Plant derived food and spices are safe food supplements and are in use for ages in culinary. The ancient Egyptians, Arabs and the Romans made extensive uses of spices. They were not using it only for taste development in food and beverages, but also as a medicine, such as, stimulants, incenses, disinfectants and aphrodisiac agents. In other parts of the world it has been also used for food products (Nisar *et al.*, 2015). It is commonly used for taste development, flavoring and Seasoning and sometime particularly as an herbal medicine. Beside many other functions of spices, it can be used for the preservation and shelf life extension of different foods (Nadeem & Riaz, 2012).

Turmeric locally called Haldi and Cumin locally known as Zeera are common food spices with known functional and medicinal values. It has healing potential against different diseases, such as anthelmintic, anticancer, antiparasitic, antiseptic, antimicrobial and antifungal potentials. Turmeric (*Curcuma longa* L.)¹ is also best known for its antitumour, antiphlegmatic, antiviral, antioxidant and blood purifier, clear skin color, remove

wound maggots, antimicrobial properties against foodborne pathogens (Nisar *et al.*, 2015).

Cumin is belongs to *Ranunculaceae* family, widely found in the Mediterranean countries, Middle East, Eastern Europe and Western Asia. It has been used as a spice in several foods due to its hot-peppery taste and considered as a valuable functional food (Zehra *et al.*, 2018; Lutterodt, 2010) and used extensively in indigenous medicines in Pakistan, India, China, Saudi Arabia. It is used for asthma, cough, bronchitis, headache, rheumatism, fever, kidney and liver disorders, influenza, eczema, and as a diuretic, lactagogue, carminative and vermifuge. It reported as anticarcinogens, antiulcer, antibacterial and antifungal, antihypertensive, hepatoprotective, anti-inflammatory, antipyretic, antioxidant. It has been also used as a food additive for preservation and shelf life extension of different perishable foods (Toma *et al.*, 2015). Different phytochemical potential of Cumin and Turmeric has been explored by researchers and a verity of phytochemicals such as fatty and essential oils, proteins, alkaloids, flavonoids, saponins, phenols and flavonoids has been reported (Sajfrtova *et al.*, 2014; Farag 2014; Farasata 2014; Fico 2001).

Herbs have been used in principle medicinal therapy from ancient ages before the arrival on new age medicines (Shinwari *et al.*, 2015). Antioxidant potential of these herbs prevent the abnormal actions of reactive oxygen species and free radicals are usually produced in body during normal metabolisms (Yan and Asmah 2010). In this study, we have explored the functional and phytochemical potential of locally produced Cumin and

Turmeric seeds. Antibacterial and antifungal activities of the extracts were determined against a wide range of pathogenic bacteria. The extracts were also used against foodborne pathogens for its preservation potential against foodborne pathogens.

Materials Methods

Sample preparation and extraction: The dried samples (200 g) of Turmeric (*Curcuma longa*) and Cumin (*Cuminum cyminum*) seeds were crushed to uniform small pieces with the help of clean electronic grinder. An amount equal to 50 g of the crashed samples were mixed in 500 mL of methanol and left for 2 days with manual shaking in dark place. The methanol mixture was filtered with Whatman filter No. 1. The extracts were dried with the help of Rotary evaporator (IKA Germany). The dried extracts were used for further analysis.

Phytochemical analysis: Different Phytochemical analysis of the methanol extracts of Cumin and Turmeric was conducted using standard procedures and following (Akter *et al.*, 2016; Sofowara 1993; Trease & Evans, 1983; Harborne, 1973).

Test for phlobatannins: Each extract mixed with 2% aqueous HCl was heated to boil. The results showing red precipitate deposition in the test tube was considered as positive for the presence of phlobatannins.

Test for flavonoids: Dilute ammonia solution (5 mL) were mixed with a portion of the extracts and concentrated H₂SO₄ was added to the mixture. The pearance of yellow coloration was indicating the presence of flavonoids. The yellow coloration disappeared after some time.

Test for alkaloids: An amount 2 mL Wagner's reagent was added to the extracts and mixed well. The reddish brown precipitate was considered as the presence of alkaloids.

Test for quinones: A small amount of dilute NaOH was mixed with the crude extract (1mL). The appearance of blue green or red coloration was taken as positive results for the presence of quinones.

Test for coumarin: To the extract 10 % of NaOH was added and chloroform was further added for observation of yellow color, which showed the presence of Coumarin.

Test for terpenoids (Salkowski test): An amount 5ml of crude extracts was mixed with chloroform (2 mL) and concentrated H₂SO₄ (3 mL) was carefully added drop wise in a manner that it formed a thin layer. The reddish brown color showed the presence of terpenoids.

Antioxidant activity: The antioxidant activities of extracts were evaluated by DPPH radical scavenging assay. An amount 1 mL of 0.1 mM DPPH (For making DPPH 0.1 mM, add 0.0039432 g of DPPH in absolute methanol 100 mL) solution was mixed with 0.5 mL of sample extracts. The solution was mixed well by shaking

properly and then incubated for 30 min in the dark area under room temperature. The measurement of DPPH free radical reduction was analyzed under the OD 517 nm using spectrophotometer. The DPPH solution without any extract and methanol was used as control. The experiment was repeated. Percentage of inhibition of DPPH free radical was calculated by the following formula. Methanol absolute was taken as blank (Hsu 2007).

$$\% \text{ Scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

where A₀ = Control

A₁ = Test sample

Total phenolic compounds: The total polyphenolic contents of each extracts were analyzed with the help of Folin-Ciocalteu reagent following Akbar *et al.*, (2014a). Extracts (1 mL) were transferred to 25 mL volumetric flask containing 9 mL of ultrapure water and 1 mL of Folin-Ciocalteu reagent (1 mL) was added and mixed properly. The solution was incubated for 5 min at room temperature, 10 mL of sodium carbonate (7 %) was added and the volume was completed with ultrapure water. After incubation in dark (90 min) at room temperature, the optical density was analyzed at 750 nm with the help of spectrophotometer. The results were calculated comparing with a pre-prepared tannic acid calibration curve (0–100 mg/L). Same procedure was applied for the preparation of blank using 1 mL of ultrapure water replacing extract.

FTIR analysis: The crude extracts of Turmeric and Cumin were analyzed for its FTIR. The spectra range from 500 – 4000 cm⁻¹ were adjusted for sample analysis using FTIR spectrometer (Nicolet, Avatar 360).

Antimicrobial activity determination of crude extracts

Antibacterial activity: Antimicrobial activities of crude extracts were determined against different types of pathogenic bacteria namely *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Dry crude extracts were dissolved in dimethyl sulphoxide (DMSO) and sterilized with the help of membrane filter with 0.45 µm pore size diameter (Minisart Ltd., Germany). Fresh culture of target bacteria adjusted to 1×10⁸ CFU /mL with 0.5 McFarland standards were inoculated over the surface of sterilized dry plates of Mueller Hinton Agar (Oxoid UK) using sterilized cotton swab. Wells (6 mm) were bored in the media plates and of extracts (100 µL) were aseptically poured into each well. The Patri dishes were placed incubator for 16-24 h at 37°C. The diameter of the inhibition zone was recorded in millimeter (mm). The DMSO were used as negative control and antimicrobial drug Doxycycline (DO 30 µg) were employed as positive control in this study.

Antifungal activity: Antifungal activity was investigated by agar mix method. An amount of 5 mL crude extracts was mixed with 20 mL of molten Potato Dextrose Agar. Petri dishes were left for solidification. An inoculum of 4 mm in diameter pieces of seven days old fungal cultures (*Mucor mucedo*, *Aspergillus flavus*, *Aspergillus niger*) were

used for the inoculation of test and control. For yeast growth, an agar surface streaking method was used. The PDA supplemented with simple DMSO and antifungal drugs were used as negative and positive control. All the plates were incubated for one week at $28 \pm 2^\circ\text{C}$. The growth inhibition of the target fungal species was observed daily, and results were recorded after one week incubation. Growth in the compound amended media was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control.

Sabouraud dextrose agar were poured in sterile petri dishes and lawn of Yeast spp. (*Saccharomyces boulardii*) were made over it using sterile swab. Wells (6 mm) were bored in the agar and 100 μL of each extract was poured in the well. The plates were incubated for 24 h at 37°C . Zone of inhibition were measured by calculating the diameter in millimeter (mm).

Extract incorporated film preparation and antibacterial activity determination: Sodium alginate (1 g) (Viv Interchem Co. Ltd. Thailand) were mixed with 50 mL of by de-ionized water and kept on stirring for 1 h at room temperature. The 1 mL solution (5% w/v) of calcium chloride (Ajax Finechem, Australia) were poured drop wise into the alginate solution following Akbar & Anal (2014b). An amount of 2 mg extracts was mixed to the solution. The solution was then poured into clean sterile petri dishes (150 mm) and kept at 50°C for 6 h in hot air oven. The dried film was cut in 10 mm circular pieces and used against pathogenic bacteria (*E. coli* and *S. aureus*) for its antibacterial activity evaluation. Film prepared using the same materials except extracts were used as a control in this study.

Application of cumin activated film for pathogen reduction in meat: Ready-to-eat poultry meat sausages were purchased from the local market and refrigerated for 15 days before its use in the study. Fresh target bacterial culture was adjusted to cell count 10^7 - 10^8 CFU/mL in normal saline (0.9 % NaCl) using McFarland standards. The meat pieces (10 g) free from target bacteria were contaminated with 1 mL of target bacterial cell adjusted and kept for 30 minutes under aseptic condition. The meat pieces were then wrapped in extract incorporated film kept at low temperature (5 ± 1) at refrigerator. Unwrapped meat pieces with equal target bacterial cells kept at same condition were used as a control. Target bacterial number were counted from day 1 to 5.

Results and Discussion

Phytochemical and functional properties analysis of Cumin and Turmeric extracts confirmed the presence of different phytochemicals in both spices, at different level. In Cumin, the phytocomponents such as, phlobatannins, flavonoids, alkaloids and quinone were absent, whereas coumarin and terpenoids were present. On the other hand, Turmeric was rich in phlobatannins, flavonoids, alkaloids, terpenoids and quinone except Coumarin, which were not present in Turmeric extracts in the current study (Table 1). The presence of phytochemicals in spices and plant materials enhances its functional properties including its antimicrobial potential (Shiney & Ganesh 2012). Methanolic extraction were preferred due to its higher capability for phytochemical

release compared to counterparts (Altemimi *et al.*, 2017; Dhawan & Gupta, 2017).

The Phytochemical groups usually responsible for biological activity are polyphenolic compounds, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Lai & Roy, 2004). These biologically active compounds have potential anti-inflammatory, antibacterial, antifungal, anti-parasitic, antioxidant, and anticarcinogenic properties (Akteer *et al.*, 2016).

In this study the total phenolic contents of Cumin and Turmeric were estimated 51.2 mg/g and 20 mg/g of dry weight equivalent to gallic acid. Maizura *et al.*, (2011) reported the phenolic contents of Turmeric 67.89 mg GAE/100 g extract. Yan & Asmah (2010) reported 348.75 mg GAE/100 g polyphenolic contents in fresh turmeric leaf. Kim *et al.*, (2011) reported 825.58 mg and 582.8 mg GAE/100 g of phenolic contents in Turmeric powder. Asimi *et al.*, (2013) reported the total phenolic contents of turmeric ranging from 1.416 ± 0.38 to 147.333 ± 3.98 and cumin ranging from 3.666 ± 0.38 to 58.000 ± 1.32 in different concentration solutions.

The DPPH radical scavenging activity of Cumin and Turmeric extracts were recorded 44% and 46 % respectively. Maizura *et al.*, (2011) reported $64.6 \pm 2.4\%$ antioxidant activity of Turmeric extracts. Yan & Asmah (2010) reported 24.93% of antioxidant activity of fresh turmeric leaf. Nisar *et al.*, (2015) reported the DPPH radical scavenging activities of different Turmeric extracts ranging from 31.33 to 52.19%. Asimi *et al.*, (2013) reported the antioxidant activity of Turmeric ranging from 9.355 ± 0.46 to 89.263 ± 0.15 Cumin ranging from 9.430 ± 0.45 to 87.440 ± 0.27 mg/mL in different concentration solutions. Cumin exhibited high antioxidant activity due to the presence of linalool, carvacrol, anethole and estragole, flavonoids and other polyphenolic compounds (Johri, 2011).

Antioxidant potential of plant extracts is its capability to capture the free radicals (Stoilova *et al.*, 2007). The DPPH assay is considered an easy and valid method for the estimation of antioxidant potential of any compound (Suhaj 2006). The presence of phenolics in spices directly contribute to their antioxidant activities (Wong *et al.*, 2006). The antioxidant activities are associated with the presence flavonoids and phenolic compounds present in the extracts (Akteer *et al.*, 2016).

The FTIR analysis established the presence of different functional groups preliminary conformed by chemical analysis. The FTIR spectra band range at 3400 cm^{-1} showed the O-H and N-H bond, whereas the band on 2800 - 2900 cm^{-1} are related to C-H vibration. The band on 1600 cm^{-1} is assigned to C=O and C=C, showing the presence of alkaline. The band between 1000 - 1100 cm^{-1} is possibly corresponding to the presence of terpenoids in the extracts (Nithyadevi & Sivakumar 2015). Different peaks of functional groups with some impurities were found during the FTIR analysis of extracts (Fig. 1a, b).

Cumin and Turmeric extracts showed tremendous antibacterial potential against a variety of gram positive and gram-negative pathogens. The Cumin and Turmeric extracts were found equally active against all target pathogens with a minimum zone of inhibition 25 ± 3 mm (Cumin) and 20 ± 2 mm (Turmeric). The turmeric extracts showed relatively small zone of inhibition against gram positive bacteria (*S. aureus*). Activity of both extracts against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and

Escherichia coli are mentioned in (Table 2). Enhanced antibacterial activity of cumin was noted compared to turmeric extracts (Fig. 2a). All the bacterial strains showed sensitivity against antibiotic doxycycline used a positive control in the study. The Cumin seed oil and alcoholic has been reported with good antimicrobial activities against the clinical isolates of *Klebsiella pneumoniae* (Johri 2011). EL-Farmawi *et al.*, (2014) reported comparatively less or no activity of Turmeric extracts against different pathogens including *Klebsiella pneumoniae* and *S. aureus*. These results were not in agreement to our study as in our study the turmeric was found active against verity of pathogens. Although its activity was narrow as compared to Cumin extracts against all pathogens. Asimi *et al.*, (2013) reported antibacterial activity of Cumin extracts against *S. aureus* and *M. luteus*. In a similar study Akgul & Kivanc (1989) reported the inhibitory effect of Cumin against *S. aureus* and *K. pneumoniae* and *P. aeruginosa*. Sunilson *et al.*, (2009) and Dhiman *et al.*, (2016) reported the antimicrobial activities of Turmeric against different foodborne pathogens. Our study is in agreement with the results of their studies.

Table 1. Phytochemical constituents of the Cumin and Turmeric extracts.

S. No	Phytochemical test	Cumin	Turmeric
1.	Phlobatannins	-	+
2.	Flavonoids	-	+
3.	Alkaloids	-	+
4.	Coumarin	+	-
5.	Terpenoids	+	+
6.	Quinon.	-	+

Note: - = Not present, + = Present

Table 2. Antibacterial and yeast activity of Cumin and Turmeric extracts.

Target microbes	Cumin	Turmeric
<i>Salmonella typhi</i>	35 ± 2 mm	30 ± 1 mm
<i>Pseudomonas aeruginosa</i>	25 ± 3 mm	28 ± 2 mm
<i>Staphylococcus aureus</i>	36 ± 3 mm	20 ± 2 mm
<i>Escherichia coli</i>	31 ± 1 mm	32 ± 1 mm
<i>Saccharomyces boulardii</i>	18 ± 2 mm	0 mm

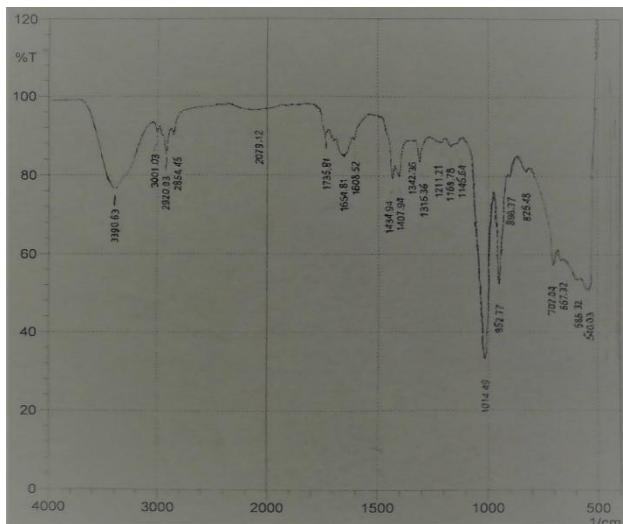


Fig. (1a). The fourier transform infrared spectroscopy pattern of Cumin extract.

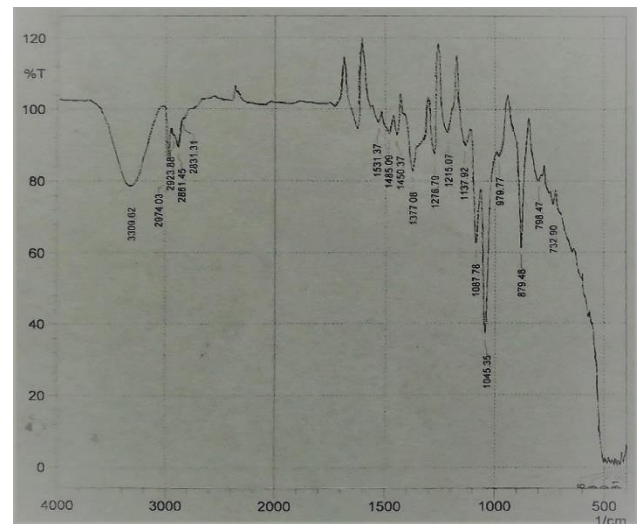


Fig. (1b). The fourier transform infrared spectroscopy pattern of Turmeric extract.

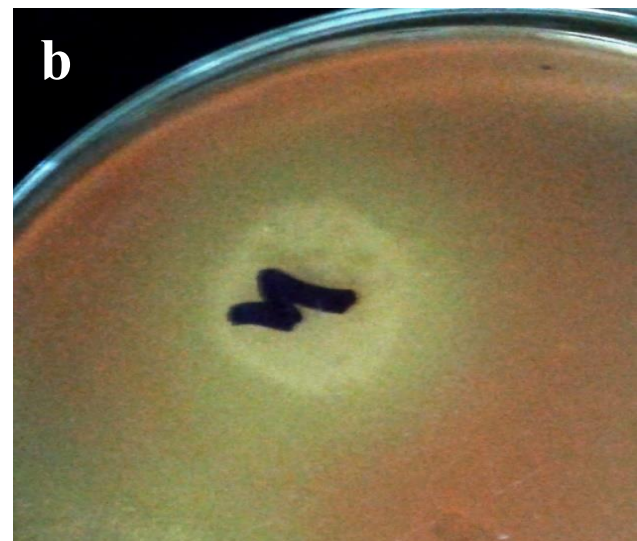
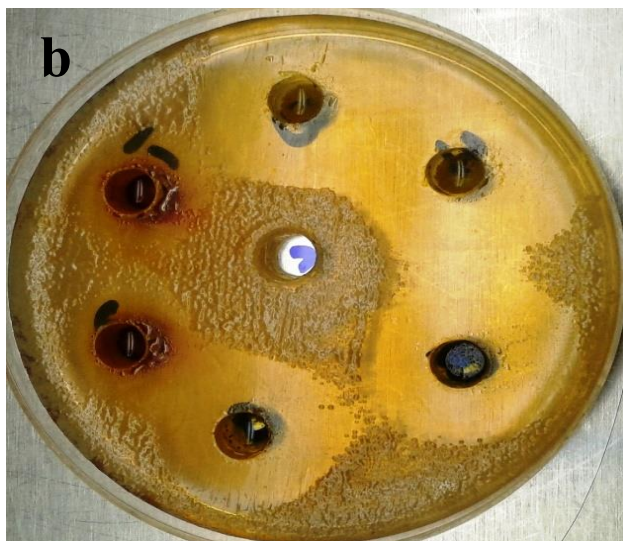


Fig. (2a). Zone of inhibition of Cumin and Turmeric extracts against *Salmonella typhi* (2b). Antibacterial zone of cumin extracts incorporated film.

Antifungal potential of both extracts was evaluated against three filamentous fungi (*Mucor mucedo*, *Aspergillus flavus*, *Aspergillus niger*) and one yeast spp. (*Saccharomyces boulardii*). Cumin extracts were found active against all four fungi with zero percent growth on Cumin extracts supplemented PDA and SBA. The turmeric extracts were found active against filamentous fungi but showed no effect on the growth of probiotic yeast spp. (*Saccharomyces boulardii*). *Cuminum cyminum* was found active in a similar study against different fungi with higher activities against dermatophytes, particularly *Trichophyton rubrum* (Romagnoli *et al.*, 2010). Mohammadpour *et al.*, (2012) reported the activity of Cumin essential oil against *Aspergillus parasiticus* NRRL-2999, *Aspergillus niger*, *Aspergillus flavus* PICC-AF24, and *Aspergillus flavus* PICC-AF39. In a similar study, Martins *et al.*, (2009) reported the positive activities of curcumin against different species of *Aspergillus*, *Candida* and *Paracoccidioides* and found curcumin with higher effect on *Paracoccidioides brasiliensis* MG05 compared to fluconazole a commonly used antifungal drug. Agaoglu & Alemdar, (2007) reported the extended antimicrobial activities of Cumin, cloves and Cinnamon against *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis*, *Escherichia coli*, *Enterococcus faecalis*, and *Candida albicans* in a study used the disc diffusion assay. The secondary metabolites such as tannins, flavonoids, coumarins, thiosulfinates, glucosinolates, and saponins are responsible for the antimicrobial and medicinal properties of plants (Dhiman *et al.*, 2015).

The research interests in plant and food-based preservatives and antimicrobial are getting appraisal from industries and consumer due its consumer and environmental friendly natures (Tajkarimi, 2010). The use of spices and their essential oil has increased as a natural bio-preservatives since nineties. These natural antimicrobials can increase the shelf life of food and reduce pathogens (Simitzis *et al.*, 2008). Alginate film incorporated with Cumin and Turmeric extracts were found active against target bacterial pathogens, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Fig. 2b). The extracts incorporated film were used against *E. coli*. It was selected for this study because of its importance as a common foodborne pathogen. The target bacterial count was found reducing with the passage of shelf life time under low temperature and was reduced 3 log decimals during 5 days of incubation. The initial count 1×10^7 CFU/ g was reduced to 2×10^4 CFU/ g in case of Cumin activated film and 9×10^4 CFU/ g with turmeric extracts incorporated film. Whereas the bacterial growth in control samples were remained almost constant with no significant reduction in bacterial cell numbers after 5 days of incubation under same condition (Fig. 3).

Food industries have shown great interest in edible film with antimicrobial potentials. As such films and coating provides barriers against pathogens as well as

moisture and gaseous exchanges, results in the improvement of food quality (Jang *et al.*, 2011). Such type of packaging and films can reduce the environmental pollution replacing the existing plastic films (Akbar & Anal 2014b). In a similar study, Ayala-Zavala *et al.*, (2013) reported the use of antimicrobial and edible film with cinnamon leaf oil for the preservation of fresh-cut peaches. Raybaudi-Massilia *et al.*, (2008) successfully used active film against on fresh cut Fuji apples *E. coli* O157:H7 and reported four logs reduction in target pathogens number. Ravishankar *et al.*, (2009) has effectively used apple-based edible films containing plant antimicrobials against *S. enterica* and *E. coli* O157:H7 on poultry, and against *L. monocytogenes* on ham.

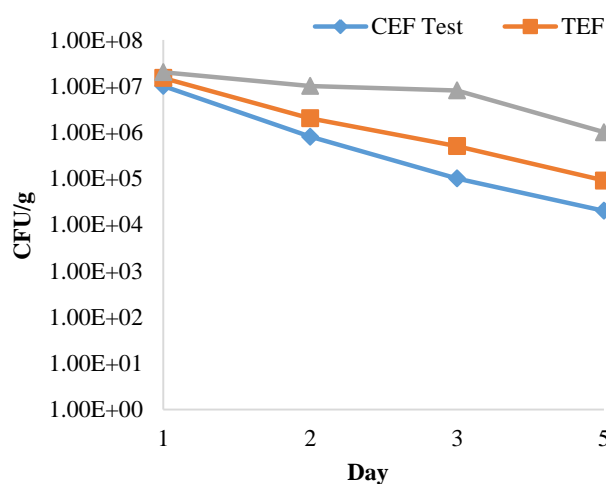


Fig. 3. Growth reduction of *Escherichia coli* in the presence of extracts incorporated film, Cumin extracts incorporated film (◆) Turmeric extracts incorporated film (■), control (▲).

Conclusion

It was concluded from this study, that both common spices are rich in phytochemical with antioxidant potential. The cumin and turmeric extracts were found active against different bacterial pathogens and fungal species important in food industries. The cumin extracts was with higher spectrum of activities against both fungal and bacterial species. The Cumin and turmeric extracts activated edible films were found active against foodborne pathogenic *E. coli* and the number of *E. coli* were reduced by 3 log folds during the challenge study. These common kitchen spices can be useful for the control of foodborne pathogens in daily life and in edible packing for food industries.

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