Evaluation of Natural Plant Powders with Potential Use in Antimicrobial Packaging Applications

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ABSTRACT

This study investigates the antimicrobial effects of vanillin, turmeric and curcumin in the forms of Dimethyl Sulfoxide (DMSO) solution as well as polymer packaging. Five types of common pathogens and food spoilage bacteria were used in this study: Staphylococcus aureus and Listeria monocytogenes representing gram-positive bacteria; Shigella sonnei, Salmonella typhimurium as well as E.coli O157:H7 representing gram- negative bacteria. Turmeric is chosen as the antimicrobial agent to be incorporated into packaging materials. In order to study the antimicrobial effect as packaging application, two types of the polymer based turmeric products were produced. They are LDPE based turmeric pellets produced by a single screw extruder, and turmeric coated BOPP films. Finally, a comparison on antimicrobial effect was conducted. between two solvents regent alcohol and DMSO.

KEYWORDS: *vanillin, turmeric, curcumin, antimicrobial and films*

1.0 INTRODUCTION

Natural plants, which have antimicrobial agents, have been widely used in ethno-medicine around the world dating back to a thousand years ago. In the first century A.D., Dioscorides wrote De Materia Medica, which offers descriptions of approximately 30 healing plants. However, since the development of antibiotics in the 1950s, the production of antimicrobial agents derived from plants has been rarely needed[1]. Currently, the use of natural plants with antimicrobial activity have come back into our lives: essential oils such as Cinnamomum zeylanicum, Thymus vulgaris and Origanum vulgaris were studied as antimicrobial solutions in paper packaging[2]. Antimicrobial compounds from plants can be divided into several major groups, which include Phenolics and Polyphenols, Terpenoids and Essential Oils, Alkaloids, Lectins and Polypeptides, Mixtures and Other Compounds[1]. Our study focuses on three plant extracts namely vanillin, turmeric, curcumin.

The natural product of the extracts of vanilla, vanillin is a phenolic aldehyde that has received considerable attention as a possible antimicrobial agent [3-6]. The antimicrobial property of vanillin is due to its phenolic compound in chemical structure. Vanillin has been selected as an antimicrobial agent for coating paperboard intended for packaging bakery products. Rakchoy *et al.* [2] evaluated the inhibitory effects of vanillin solutions and investigated MIC of the solution against selected bacteria including *E.coli* (DMST 4212), *Staphylococcus aureus* (DMST 8840) and *Bacillus cereus* (DMST 5040) by agar well diffusion method. Three types of vanillin coating solutions were prepared and evaluated, including vanillin/ DMSO (10,5,2.5,1.25%(w/w)), vanillin/ ethyl alcohol (10,5,2.5,1.25%(w/w)) and vanillin/chitosan (10,5,2.5,1.25%(w/w)).

Turmeric, a bright yellow-orange substance, obtained from the root of the plant Curcuma longa, has long been used as a traditional spice in both China and India. Turmeric is mostly used in flavored milk drinks, cultured milk and desserts to obtain lemon and banana colors in dairy products. In previous research, turmeric has been shown to have anti-HIV activity [1], wound-healing [7] as well as antimicrobial activity [8]. The main components of turmeric are: curcumin (60%), desmethoxycurcumin, monodemethoxycurcumin, bisdemethoxycurcumin, dihydrocurcumin and cyclocurcumin [9]. Bhavani Shankar and Sreenivasa Murthy [10] evaluated the activity of turmeric fractions on the growth of some bacteria in vitro. In their work, both curcumin and the oil fraction were tested against several bacteria like *Staphylococcus* and *Lactobacillus*. However, curcumin only inhibited *Staphylococcus aureus* in the range of 2.5mg/ml to 50mg/ml [11]. Negi *et al.*[12]extracted turmeric oil from the mother liquor using hexane at 60 °C, and then studied the antimicrobial activity by pour plate method. The results of the study show that turmeric oil possessed antibacterial activity against tested bacteria including *Staphylococcus aureus* and *E.coli*. Another antimicrobial study conducted by Seher et al. [8] on methanolic extract of turmeric powder showed that turmeric powder was most effective on *Staphylococcus aurues* (COWAN 1) and had a slight effect on Listeria monocytogennes (SCOOT A). However, it was not effective on *E. Coli* (ATCC 25921).

Curcumin is the most biologically active constitute of turmeric. The characteristic yellow color of turmeric was isolated in the 19th century and was named curcumin. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial and antitumor activities [1,7,11] . The chemical structure of curcumin was determined by Roughley and Whiting in 1973 [11]. Antimicrobial activity of indium curcumin against *S. aureus* (ATCC 25923) and *S.epidermidis* (ATCC 14990) as well as minimum inhibitory concentration (MIC) has been reported [14]. It was reported that curcumin inhibits the growth of varieties of microbes such as viruses, bacteria and some pathogenic fungi [13]. Other studies on indium curcumin and microcapsule curcumin evaluated the antimicrobial effects for particular bacteria [14-15].

In 2011,Cheng et al.[5] investigated the antimicrobial effects of the aforementioned three anti microbial agents using regent alcohol as solution. The study showed that vanillin, turmeric and curcumin are promising natural antimicrobial agent. But, the insoluble particles were found in both curcumin and turmeric solutions at high concentrations.

Little literature can be found how the three antimicrobial agents are incorporated into a polymer for inhibiting microbial growth. Sangsuwan *et al.* [6] incorporated vanillin directly into chitonsan-methyl cellulose based films with polyethylene glycol (PEG) 400 as a plasticizer. Furthermore, they analyzed the impact of different vanillin concentration on mechanical properties, water vapor and oxygen permeability, opacity and thermal properties of the coating films. The results indicated that both vanillin and PEG concentration affected all the tested properties of chitosan-methyl cellulose based film.

The study is aimed at studying the antimicrobial effect of three natural plant powders, namely vanillin, turmeric and curcumin in agar diffusion method, in the forms with DMSO solution as well as incorporated in packaging materials. Such applications could be effectively used for foods products, not only in the form of films but also as containers.

Specifically, the study

- Investigate inhibitory effects of all solutions on Mueller Hinton II agar plates against five types of common bacteria: E.coli O157:H7, Staphylococcus aureus, Shigella sonnei, Salmonella typhimurium, and Listeria monocytogenes.
- Compare the results and find minimum inhibitory concentration (MIC) for each solution.
- Apply natural plant powders on LDPE resin and BOPP film to investigate their antimicrobial effect.
- Compare the DMSO based results to regent alcohol based results by Cheng et al. in 2011[5].

2.0 EXPERIMENTAL

2.1 Materials

The materials involved in this study are natural plant powders, solvent, microorganism as well polymers.

Antimirobial agents used in this study are Vanillin,Tumeric powders and Curcumin. Turmeric powders were purchased from nuts.com. Vanillin (94752, Fluka) and Curcumin (C1386, Sigma) were obtained from Sigma-Aldrich Co. LLC. U.S.

The solvent used in the experiment was Dimethyl sulfoxide (DMSO, 276855 anhydrous, ≥99.9%, purchased from Sigma-Aldrich Co. LLC. U.S.). In this experiment, three types of solutions were prepared based on DMSO, using a series of half-fold dilutions. The coating solutions involving these three antimicrobial agents are vanillin/DMSO (10, 5, 2.5, 1.25, 0.625 and 0.3125% (w/w)), turmeric/DMSO (10, 5, 2.5, 1.25, 0.625 and 0.3125% (w/w)), and curcumin/DMSO (10, 5, 2.5, 1.25, 0.625 and 0.3125% (w/w)).

Five types of common pathogenic and food spoilage bacteria as microorganisms were selected. They are (a) gram-positive bacteria: *Staphylococcus aureus* and *Listeria monocytogenes;* (b) gram-negative bacteria: *Shigella sonnei, Salmonella typhimurium, E. coli* O157:H7. The Department of Biotechnology and Molecular Bioscience, Rochester institute of technology, NY, U.S, provided all bacterial strains.

The antimicrobial activities of vanillin, turmeric and curcumin solutions were analyzed on Mueller Hinton II agar (VWR) by agar diffusion method. The polymers used for testing were low-density polyethylene resin (LDPE, NA 214-000, Lyondell Company for making the pellets, and biaxially oriented polypropylene (BOPP) film (Bicor™ 100 SLP, ExxonMobil) as base film for the antimicrobial agents.

2.2 Methods

The antimicrobial activities of vanillin, turmeric and curcumin solutions were determined by agar diffusion method. Each type of bacteria was streaked onto Mueller Hinton II agar plates. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates uniformly. Using blank antibiotic assay discs of 6mm in diameter impregnated with 40µl of vanillin, turmeric and curcumin solutions and was placed on the surface of the agar respectively. The solutions diffused out from the antibiotic assay disc into the agar. Then, the plates containing the testing samples were incubated for 24 hours at 35.5°C. Control samples were performed triplicate on each bacteria with 40µl solvent. Vanillin, turmeric and curcumin solutions were performed triplicate for different concentrations. In order to obtain comparable results, all prepared solutions were applied under the same conditions and the same incubation environment.

The antimicrobial activities of all solutions were detected as clear zones around the antibiotic assay discs, and the diameters of the clear zones measured by a ruler in millimeters (mm). If the solution is effective against bacteria at a certain concentration, no colonies will grow wherever the concentration in the agar is greater than or equal to that effective concentration. This region is called the "zone of inhibition." Thus, the size of the zone of inhibition is a measure of the compound's effectiveness. The larger the clear zone around the antibiotic assay disc, the more effective the solution is. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of solutions that completely inhibit the growth of each bacterial strain being tested [15].

The antimicrobial agents are incorporated into polymers in two ways: pellets produced by single extruder and the adhesive coating for the film.

The natural plant powders are blended with polymer resin LDPE into a master batch in concentration of 2.5% (w/w). 600 grams of resin was added to the mixer with 7.5 grams of turmeric powders. The blended material was fed into a single screw extruder, with the working temperature at 305°F. Material was first cooling immediately by water after exited the die, then ground in a knife mill to produce pellets (5mm×3mm×2mm). The pellets were collected separately in ten containers every three munities. In order to obtain comparable results, all pellets used in antimicrobial testing was melted and reshaped into discs of approximately 6mm in diameter and 1mm in thickness.

Adhesive was prepared by using Ethyl Acetate, 555 (Dow Chemical Company) and 536B (Dow Chemical Company) mixture in 33.46g, 38.46g and 5g respectively. The turmeric powders were blended with prepared adhesive (Ethyl Acetate, 555,536B) made a mixture concentration of 5%(w/w). 3.85g turmeric powders were added to the prepared adhesive, forming a total of 80.77g coating solution. BOPP film (22mm×40mm) coated by draw down machine (EZ Coater EC-100, Fairfield. Ohio, U.S.A.), with 3.5ml of the coating solution covering the entire film and was dried at 120°C-130°C in oven for 24 hours. Then the film was cut into test samples (13mm×13mm).

The antimicrobial activities of natural plant powder containing LDPE discs and turmeric coated BOPP films were also determined by agar diffusion method.

3.0 RESULTS AND DISCUSSION

The antimicrobial activity of natural plant powders in DMSO with different concentration are summarized in figures 1 and tables 1-3 (pages 34-36). Antimicrobial effectiveness of the blends of LDPE and the coating on BOPP film with natural plants agent are also discussed in figure 2. A comparison table (table 4) between DMSO solution and Regent alcohol is stated in table 4.

3.1 Antimicrobial Effects of Vanillin, Turmeric and Curcumin in DMSO Solution

The average diameter and standard deviation of clear zone of inhibition on control sample, vanillin/DMSO solution, turmeric/DMSO solution and curcumin/ DMSO solution are illustrated in Table 1. The Standard deviation observed in the study stated in table 1 is small, all tested powders were dissolved uniformly in DMSO. In table 1, if there was no distinct clear zone of inhibition observed in the test, the diameter marked as "-". Standard deviation marked as "-" illustrated that there were no distinct clear zone of inhibition observed in three replicates.

Different bacteria species showed variable sensitivity. Of the different agents tested for their antibacterial effect, vanillin/DMSO solution was the most susceptible to inhibited the growth of *Listeria monocytogenes* with the MIC at 1.25%(w/w). Vanillin/DMSO solution also inhibited the growth of *Staphylococcus aureus* and *Shigella sonnei* to a moderate extent with the MIC at 5%(w/w). In addition, the same solution has slight less antimicrobial activity on *Salmonella typhimurium* and *E.coli* O157:H7, with the MIC at 0.3125%(w/w) and 5%(w/w) respectively. The results contradicts previous study on the antimicrobial effect of vanillin/ DMSO solution in inhibiting *E.coli* O157:H7 by Rakchoy *et al.* (2009) who reported that vanillin/DMSO solution was more effective over *E.coli* O157:H7 with the MIC at 2.5%(w/w). However, the results are in good agreement with pervious research from [3] who reported vanillin/DMSO solution was more effective over *Staphylococcus aureus.*

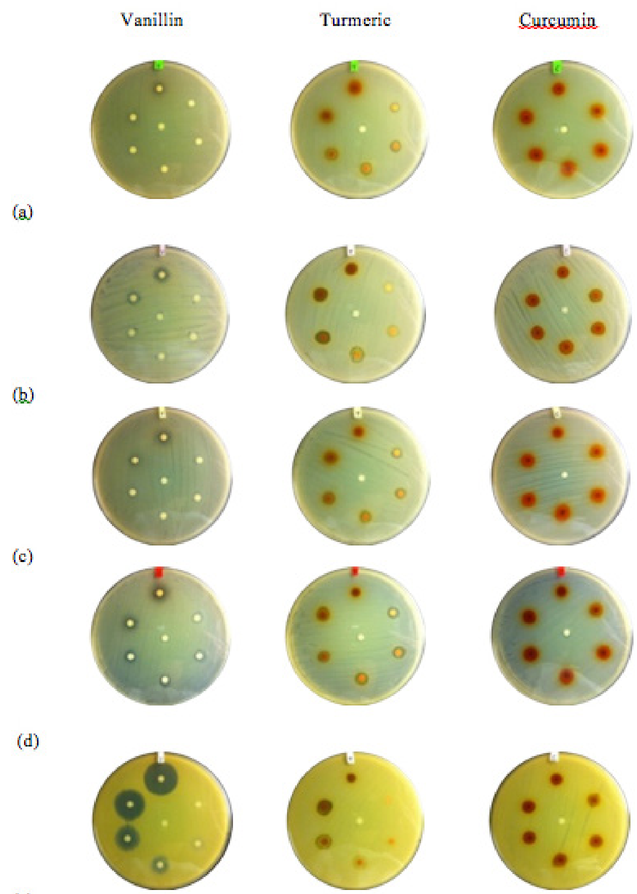

Figure 1. The illustration above shows zones of inhibition around antibiotic assay discs saturated with 40 µl of vanillin, turmeric and curcumin solutions respectively. (a) E. Coli O157:H7, (b) Staphylococcus aureus, (c) Shigella sonnei, (d) Salmonella typhimurium, (e) Listeria monocytogenes.

Table 1 Antimicrobial Effects of Vanillin, Turmeric and Curcumin in DMSO Solution. The influence of concentration on antimicrobial activity over each bacterial.

Bacteria	Control sample		Concentration (% w/w)	Vanillin		Turmeric		Curcumin	
	Average diameter (mm)	STDEV		Average diameter (mm)	STDEV	Average diameter (mm)	STDEV	Average diameter (mm)	STDEV
Listeria mono-cytogenes	0	0	10	29.67	0.58	4.33	1.53	4	0
			5	25	1	8.67	1.15	6.67	0.58
			2.5	19	0	7.67	0.58	6	0
			1.25	10.33	0.58	8	0	6.67	0.58
			0.625	-	-	-	-	7	0
			0.3125	-	-	-	-	7.67	0.58
Staphylococcus aureus	0	0	10	8.67	0.58	5.67	1.53	4	1
			5	5.33	0.58	7	1	6.33	0.58
			2.5	-	-	8.33	0.58	6.33	0.58
			1.25	-	-	8	0	8.68	0.58
			0.625	-	-	-	-	7.33	0.58
			0.3125	-	-	-	-	8	1
Salmonella typhimurium	2.33	0.58	10	5.33	1.53	2.67	0.58	3.33	0.58
			5	4	0	5.33	1.15	5.67	0.58
			2.5	5	0	5.66	0.58	6	0
			1.25	5	0	7	0	8	1
			0.625	5.33	0.58	7.33	0.58	7	0
			0.3125	5	0	5.33	0.58	7	0
E.coli O157:H7	2.00	1.73	10	4.33	0.58	7	1	4	0
			5	4	0	7.67	0.58	6.33	0.58
			2.5	-	-	6.33	0.58	6.67	0.58
			1.25	-	-	7	0	7.33	0.58
			0.625	-	-	6	0	6.33	0.58
			0.3125	-	-	4	0	6.33	0.58

According to the results, turmeric/DMSO solution has a significant antimicrobial activity, with the MIC at 1.25%(w/w), towards Gram-positive bacteria: *Listeria monocytogenes* and *Staphylococcus aureus.* On the other hand, Gram-negative bacteria including *Shigella sonnei, Salmonella typhimurium* and *E.coli* O157:H7 were sensitive to turmeric/DMSO solution and showed moderate sensitivity. The MIC of turmeric/DMSO solution is 0.3125%(w/w) on all tested Gram-negative bacteria.

Curcumin/DMSO solution possessed antibacterial activity against all tested bacteria. Amongst those tested bacteria, *Listeria monocytogenes, Staphylococcus aureus* and *Shigella sonnei* had a higher sensitivity on curcumin/DMSO solution than *Salmonella typhimurium* and *E.coli* O157:H7. Moreover, MIC was observed at 0.1325%(w/w) for all tested bacteria, which means such solution still possessed antimicrobial effect on all tested bacteria even though the concentration of solution decreased to the minimum in the experiment.

The measured clear zone of inhibition diameter after subtracted control sample effects for each tested DMSO solution are illustrated in Table 1. Compared with vanillin/DMSO solution, turmeric/DMSO solution and curcumin/DMSO solution showed a greater inhibitory effect at lower concentrations on all tested bacteria. However, when the solution's concentration was above 1.25%(w/w), vanillin/DMSO solution had a stronger inhibition effect on *Listeria monocytogenes* than turmeric/DMSO solution and curcumin/DMSO solution did.

Based on the results, vanillin/DMSO solution showed increasing antimicrobial activity over selected bacteria strains as the concentration increased. However, data from turmeric/DMSO solution and curcumin/DMSO solution demonstrated a decreasing concentration of solution resulted in a first increased followed by decreased antimicrobial activity on certain concentration. The insoluble particles in the certain solution at higher concentration might prevent the active agents diffusing in the agar plate.

If we subtracted the additional effect from control sample, the largest inhibition zone diameters for the selected bacteria should be showed in Table 2.

Table 2 The most effective agent solution for the selected bacteria

Type of bacteria	Bacteria	Combination	Concentration (%w/w)	Inhibition zone diameters (mm)
Gram positive	Listeria monocytogenes	Vanillin/DMSO	10	29.67
	Staphylococcus aureus	Turmeric/DMSO	2.5	8.33
		Curcumin/DMSO	1.25	8.68
Gram negative	Shigella sonnei	Turmeric/DMSO	5.00	6.67
		Curcumin/DMSO	0.625	7.33
	Salmonella typhimurium	Turmeric/DMSO	0.625	5.00
		Curcumin/DMSO	1.25	5.67
	E.coli O157:H7	Turmeric/DMSO	5.00	5.67
		Curcumin/DMSO	1.25	5.33

Table 3: The Minimum Inhibitory Concentration of selected agents solutions

Bacteria	Vanillin (%w/w)	Turmeric (%w/w)	Curcumin (%w/w)
	DMSO	DMSO	DMSO
Listeria monocytogenes	1.25	1.25	0.3125
Staphylococcus aureus	5	1.25	0.3125
Shigella sonnei	5	0.3125	0.3125
Salmonella typhimurium	0.3125	0.3125	0.3125
E.coli O157:H7	5	0.3125	0.3125

Table 4 The MIC of selected agents in Reagent alcohol [5]and DMSO solutions

Bacteria	Vanillin (%w/w)		Turmeric (%w/w)		Curcumin (%w/w)	
	Reagent alcohol	DMSO	Reagent alcohol	DMSO	Reagent alcohol	DMSO
Listeria monocytogenes	2.5	1.25	10	1.25	1.25	0.3125
Staphylococcus aureus	10	5	10	1.25	2.5	0.3125
Shigella sonnei	2.5	5	-	0.3125	1.25	0.3125
Salmonella typhimurium	10	0.3125	-	0.3125	-	0.3125
E.coli O157:H7	2.5	5	-	0.3125	-	0.3125

Selected bacteria can be effectively suppressed by using the particular solution with the largest inhibition zone diameter. However, agent's cost as well as antimicrobial activity should take into account when it comes to commercial use and industrial application. In general, optimum option must have lower cost with effective function for particular application. Thus, the Minimum Inhibitory Concentration (MIC) is an important index in this study. As the antimicrobial activity against certain bacteria is related to the type of bacteria as well as the type of powder's solution and its concentration, choose the combination of powder is crucial (Table 2-page 34).

3.2 The Minimum Inhibitory Concentration (MIC)

The MIC of selected agents solution on five bacteria is illustrated in Table 3. Although vanillin in DMSO solution showed greater antimicrobial activity on selected bacteria, turmeric and curcumin were both have lower MIC, which indicates turmeric and curcumin can inhibit selected bacteria when the concentration goes down. Turmeric/DMSO solution has MIC of 1.25%(w/w) on both *Listeria monocytogenes* and *Staphylococcus aureus*, and 0.3125%(w/w) on *Shigella sonnei, Salmonella typhimurium* and *E.coli* O157:H7. On the other hand, curcumin/DMSO solution showed 0.3125%(w/w) on all tested bacteria.

3.3 Effect of solvent

Compared with Reagent alcohol [5], the results in DMSO reflects the actual effect from selected powders. Overall, DMSO has a better solubility than Reagent alcohol, especially when it is used with curcumin and turmeric powders. Solvent may influence the effectiveness of selected powders in two aspects. First, solvents may possess antimicrobial activity, which causes an additional effect on tested bacteria. In table 4, the MIC of all selected agents in Regent alcohol is larger than in DMSO. Second, if the solubility of a solvent is poor for some agents such as Tumeric and Curcumin, those agents may migrate slowly through the solvent to the agar and weaken the antimicrobial activity from agents. Thus, the selection of an adequate solvent to dissolve an antimicrobial agent is important for this study.

3.4 Antimicrobial effect of turmeric incorporated polymer

The selection of an antimicrobial agent is often limited by the incompatibility of that agent with packaging material or by its heat instability during the extrusion process. Turmeric is selected as incorporated antimicrobial agent to be applied to blend and coat with the polymer packaging materials due to the following reasons: (1) In all the three powders, turmeric powder has the acceptable melting point that can be used in single screw extruder. (2) It has antimicrobial activity. (3) Turmeric powder is the lowest cost among three powders.

After 48 hours and 72 hours in the incubation chamber at 36°C, there is no inhibition zone observed for the LDPE pellets on the agar plate.

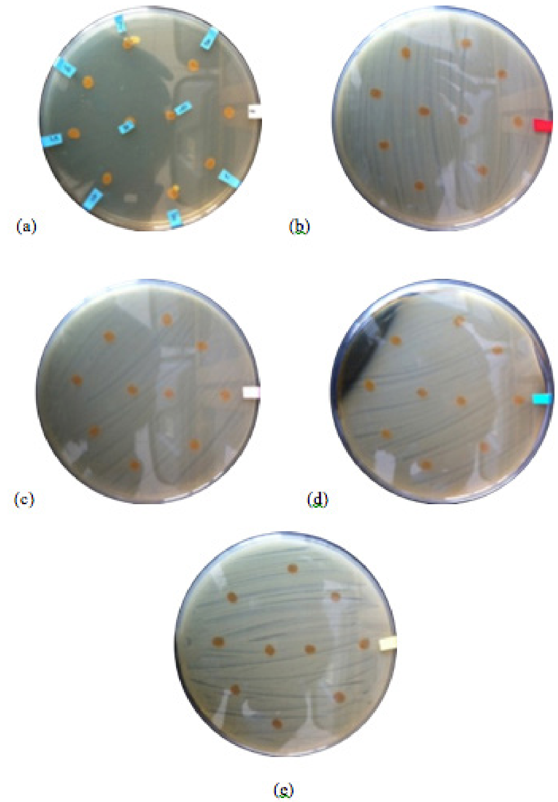

Figure 2 Turmeric containing LDPE resin after 72 hours: (a) Listeria monocytogenes, (b) E. Coli O157:H7, (c) Salmonella typhimurium, (d) Shigella sonnei, (e) Staphylococcus aureus.

Similarly, there is no inhibition zone observed for the Turmeric powder incorporated coated BOPP film on the agar plates after 72 hours in the incubation chamber at 36°C. This result might be due to several factors including the chemical and physical structure of the polymer, temperature during processing and moisture of the environment. The configuration of the polymers' matrix, like the low presence of a porous medium, can influence the diffusion phenomenon of turmeric powders. The chemical structure such as hydrogen bond and electrostatic interactions also play a role in diffusion. On the other hand, the antimicrobial activity of turmeric powder may be influenced by high temperature during the single screw extruder processing. Moreover, insufficient moisture in the testing environment for such products prevent the migration of turmeric powders out of polymer matrix.

CONCLUSIONS

According to this study, natural powders including vanillin, curcumin and turmeric are inhibitory to all tested bacteria to varying degrees. Vanillin/DMSO solution inhibits the growth of bacteria, with *Listeria monocytogenes* being the most susceptible with MIC at 1.25%(w/w). The same solution possesses a moderate to lower effect on *Staphylococcus aureus, Shigella sonnei, Salmonella typhimurium* and *E.coli* O157:H7. On the other hand, curcumin and turmeric powders in DMSO solution has antimicrobial effects. Gram-positive bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* are generally more sensitive to curcumin and turmeric than Gram-negative bacteria such as *Shigella sonnei, Salmonella typhimurium* and *E.coli* O157:H7. The study also found that the antimicrobial activity against certain bacteria is related to the type of bacteria as well as the type of the powder's solution and its concentration. Therefore choosing the proper combination of powder and solvent is very important.

The study reported that it appears not effective to incorporate turmeric directly into the plastic film and coating, despite that the turmeric has an antimicrobial effect in the Agar diffusion method. Future studies on packaging materials with a high presence of a porous medium may be conducted to understand the feasibility of polymer incorporated antimicrobial material. Particle size, solubility, and adaptability to industry processing of natural antimicrobial spices should be also considered.

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