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Effective Use of Cinnamon Essential Oil Encapsulated in β-cyclodextrin Citrate as an Antimicrobial for Food Packaging

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ABSTRACT

Essential oils are natural plant extracts that contain bioactive compounds with antioxidant and antimicrobial properties. This study examined the antibacterial activity of different essential oils; Marjoram (*Origanum majorana*), Nigella sativa, Olive (*Olea europaea*), Moringa (*Moringa oleifera*) and cinnamon (*Cinnamomum verum*) against a number of *Salmonella* strains isolated from different food items (vegetables and meats). *Salmonella* strains were resistant to at least three antibiotics, which indicates that they are multidrug-resistant. Results revealed that cinnamon essential oil treatment exhibited a greater effect on the pathogenic bacteria than other essential oils used in the study giving inhibition zone (32±0.20 - 38±0.04 mm). To reduce the volatility and hydrophobicity of cinnamon oil, it was encapsulated into β-cyclodextrin citrate and tested in the encapsulated form and the free form. The encapsulated form provided a controlled release of essential oil while maintaining this oil's antimicrobial effectiveness for a period lasted for one month. Thus, the encapsulated form is recommended to be used as a preservative or to incorporate into food packaging as an antimicrobial agent.

INTRODUCTION

Various food items as meat, vegetables, fish, fruits, and bread are exposed to contamination with pathogenic organisms, which leads to serious damage to human health (Berjia *et al.*, 2014; Ölmez, 2016). Examples of pathogenic microorganisms are *Salmonella spp.*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Campylobacter jejuni* (EFSA and ECDC, 2017).

The genus *Salmonella*, belonging to the family Enterobacteriaceae, is one of the most dangerous organisms that cause food contamination and lead to serious infections for organisms that threaten their life. In addition, *Salmonella* is also common and infects more than one host (Vivek *et al.*, 2012).

To ensure food safety, the growth of pathogenic microorganisms must be prevented through a set of chemicals, physical and physiological processes that ensure the validity of these foods during the preservation process (Nerin *et al.*, 2017).

Essential oils (Eos) are classified as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration because they are natural plant sources and have antimicrobial and antioxidant properties (Manso *et al.,* 2011; Wrona *et al.,* 2015). Despite the multiple benefits of essential oils, several obstacles are facing their use as food preservatives as they (1) volatile (2) having hydrophobic nature make them react with fats in foods (4), (3) they are often added in high concentrations to have an antimicrobial effect, and this leads to negative sensory changes (Emiroglu *et al.,* 2010).

To overcome these problems, essential oils can be encapsulated to protect their biological, functional properties, control their release (Vergis *et al.,* 2015), and prolong shelf life during storage (Vemmer and Patel, 2013; Martins *et al.,* 2014).

There are several factors through which the encapsulating material is selected, as it must be non-toxic, bioactive, and inexpensive (Dalmoro *et al.,* 2012).

Cinnamon oil is one of the important essential oils that is preferred to be used because of its strong effect against a group of pathogenic organisms during the preservation process (Manso *et al.,* 2014). Several studies include cinnamon oil packaging such as cyclodextrins that are non-toxic and some of them are used as novel food or food additives (Munhuweyi *et al.,* 2018). *Bacillus macerans* transform starch into cyclic oligosaccharides, which are known as cyclodextrins. CDs α-, β- and γ- are commercially generated from CDs (Chen & Liu, 2016).

The homogenous, crystalline, and non-hygroscopic nature of these CDs distinguishes them. The common types (α-, β- and γ-CDs) are composed of six to eight (α-1,4)-linked -D-glucopyranose units. The ability of CDs to form inclusion complexes with a variety of small molecules via molecular complexation is their most prominent property. CDs can form such complexes in solutions (Cusola *et al.,* 2013). Because guest molecules are briefly housed within the host cavity, inclusion in CDs has a significant impact on their physicochemical properties (Zhou *et al.,* 2009).

β-cyclodextrin, with 7 sugar units, has been the most commercially appealing (more than 95 percent consumed) due to its simple synthesis, availability, and price (Ji *et al.,* 2010). It contains a polar cavity that allows it to house the most crucial molecules and allows for easy crystallization recovery (Wang *et al.,* 2011), its production is economically viable (Szente and Szejtli 2004), non-toxic because it is not absorbed by the gastrointestinal system or lipophilic biological membranes (Liang *et al.,* 2012). Other characters distinguish β-CD, as its cavity of it is hydrophobic while the external section is hydrophilic. Like other polysaccharides, β-CD is stable in alkali solutions and is sensitive to acid hydrolysis (Voncina & Vivo, 2013).

Taking all these into consideration, this work is aimed at testing several essential oils as antimicrobial agents against a number of multidrug-resistant *Salmonella* sp. Then the most promising essential oil was chosen for being encapsulated into β- cyclodextrin citrate for developing an antimicrobial active food packaging.

MATERIALS AND METHODS

Essential Oils (EOs):

Five essential oils were used in this experiment: Marjoram (*Origanum majorana*), Nigella sativa, Olive (*Olea europaea*), Moringa (*Moringa oleifera*) and cinnamon (*Cinnamomum verum*). All EOs were purchased by the producer (National Research Center, Dokki, Egypt), and maintained at 4˚C in dark glass vials until their employment.

Collection of Microbial Pathogens:

Different *Salmonella* sp. were isolated from different food items (vegetables and fruits). Three strains of *S. typhinurius* and one strain of *S. enteritidis* were isolated from vegetables. In addition, two strains of *S. typhimurium*, one strain of *S. Kentucky*, and another strain of *S. Anatum* were isolated from meat products. These strains were obtained from Animal Health Research

Institute, Dept. of Serology, Dokki branch and Dept. of Microbiology, Zagazig branch, Egypt.

Antibiotic Susceptibility Profiling:

Disc diffusion assay was applied for investigating the susceptibility of *Salmonella* strains to different broad-spectrum antibiotics (Hudzicki, 2009). Single colonies from the strains under study were inoculated into the nutrient broth and incubated for 24 h at 37 ˚C. After that, the cultures were spread on the surface of nutrient agar using sterile swabs. Then antibiotic discs were distributed onto the surface. Nine broad-spectrum antibiotics (Amoxicillin (AMC 20 µg), Ceftazidime (CAZ 30 µg), Ceftriaxone (CRO 30 µg), Ciprofloxacin (CIP 5 µg), Amikacin (AK 30 µg), Cefotaxime (CTX 30 µg), Ampicillin (AMP 10 µg), Gentamycin (CN 30 µg) and Nitrofurantoin (F 300 µg) were applied. Inhibition zones (mm) for different antibiotics were measured after incubating plates at 37˚C for 24 h. The results we obtained were interpreted according to the Clinical & Laboratory Standards Institute (CLSI, 2011) guidelines.

Agar Well Diffusion Test:

Antibacterial Activity of Essential Oils:

The antibacterial activity of the five different essential oils was tested against eight *Salmonella* sp. that were isolated from different food items using the agar-diffusion approach. Bacteria were inoculated in a nutrient broth medium at 37˚C for 24 h. Then bacteria (1.5 $X10^8$ cfu) were swabbed on the nutrient agar plates. 100µL of 5% dimethylsulphoxide (DMSO) was added to (100µL) of each oil extract to solubilize it. EOs were deposited in wells (diameter 7 mm) formed in the agar plates, and those plates were cultured at 37˚C for 24 h. Then, the ranges of the inhibition zones were measured. All samples were measured in triplicates.

Fabrication of β-cyclodextrin Citrate:

β-cyclodextrin citrate was prepared by sedimentary reaction between β-cyclodextrin (Acros Organic, USA) and citric acid as previously was reported by Abeer *et al.* (2019). In brief, 2.00 g β-CD was added to 2.03 g of citric acid dissolved in 12 mL deionized water. The reaction mixture was refluxed at 100°C for 4 h after which excess of isopropanol was added to precipitate β-cyclodextrin citrate.

Antibacterial Activity of Cinnamon Oil Encapsulated In β -Cyclodextrin Citrate:

0.5 g of β-cyclodextrin citrate was mixed with 10 ml cinnamon oil. Cinnamon oil was tested alone and in the encapsulated form against tested *Salmonella* sp. using the agar diffusion approach as mentioned before. This experiment lasted for one month.

RESULTS AND DISCUSSION

***Salmonella* Strains Showed Multidrug Resistance:**

Data shown in Table 1 distinguish the efficacy of different antibiotics against *Salmonella* strains. All *Salmonella* sp. were resistant to different antibiotics as Amoxicillin and Ampicillin (100%), Nitrofurantoin and Ciprofloxacin (75%), Cefuroxime, and Gentamicin (62.5%), Ceftriaxone and Ceftazidime (37.5%), Amikacin (12.5%). On the other hand, *Salmonella* strains were sensitive to Amikacin (87.5%), Cefuroxime (37.5%), Gentamicin (37.5%), Ciprofloxacin (25%), Nitrofurantoin (25%), and Ceftriaxone (12.5%). Many studies have reported similar results as Zhao et al. (2008) who mentioned that all *Salmonella* isolates were susceptible to ceftriaxone and ciprofloxacin and exhibited resistance to streptomycin (37.8%), sulfamethoxazole-trimethoprim (27.7%), and gentamicin (25.7%). Also, Al-Sultan *et al.*, (2012) found that susceptibility of *Salmonella* isolates to gentamicin, ciprofloxacin, and chloramphenicol was 95%, 90%, and 80% respectively and a high level of resistance was observed against amoxicillin-clavulanic acid (100%) and erythromycin (80%).

Table 1. Susceptibility differences of *Salmonella* sp. isolates against different antibiotics.

Antibiotic	Conc. (µg/ disc)	Resistant (R)		Intermediate (I)		Susceptible (S)	
		No. *	%#	No. *	%#	No. *	%#
Amoxicillin	20	8	100	0	0	0	0
Ceftriaxone	30	3	37.5	4	50	1	12.5
Ceftazidime	30	3	37.5	5	62.5	0	0
Nitrofurantoin	300	6	75	0	0	2	25
Ciprofloxacin	5	6	75	0	0	2	25
Cefuroxime	30	5	62.5	0	0	3	37.5
Amikacin	30	1	12.5	0	0	7	87.5
Ampicillin	10	8	100	0	0	0	0
Gentamicin	10	5	62.5	0	0	3	37.5

Expressed as percent in reference to all *Salmonella* sp. isolates per each antibiotic studied.
* Denotes for number of *Salmonella* sp. isolates.

Essential Oils Had Antibacterial Activity against MDR bacteria:

Data in Table (2) demonstrates the activity of different essential oils against tested *Salmonella* sp. Cinnamon oil showed the highest antibacterial activity against all tested strains (32±0.20 - 38±0.04 mm). That is because cinnamon oil contains cinnamaldehyde as bioactive material with antibacterial effect (Abdelwahab *et al*., 2014), this material penetrates bacterial membranes causing their lysis (Vani and Lakshmi, 2014). Also, Marjoram and Nigella sativa essential oils gave remarkable activity. As Marjoram oil gave (21±0.0.08- 28±0.23 mm), while Nigella sativa oil gave (20±0.04- 23±0 mm). On the other hand, no antibacterial effect was detected when adding olive or moringa oils except for olive oil that showed an inhibition zone (18±0.12 mm) against *S. enteritidis* M6. This negative effect of essential oils can be due to the outer membrane that acts as an insulator against these oils in Gram-negative bacteria from Gram-positive bacteria (Ismail *e*t *al.*, 2017).

Table 2. The growth inhibition zones (expressed in mm) obtained testing the selected *Salmonella* strains against the assayed EOs.

Samples	Essential oils				
	Marjoram	Nigella sativa	Olive	Moringa	Cinnamon
	M SD	M SD	M SD	M SD	M SD
S. typhinurius M1	28±0.23	0.0±0	0.0±0	0.0±0	38±0.04
S. typhinurius M5	23±0.18	23±0	0.0±0	0.0±0	33±0.16
S. typhinurius M7	22±0.20	20±0.09	0.0±0	0.0±0	38±0.16
S. enteritidis M6	24±0.18	20±0	18±0.12	0.0±0	33±0.21
S. typhimurium S1	21±0.08	0.0±0	0.0±0	0.0±0	35±0.32
S. typhimurium S2	26±0.16	20±0.08	0.0±0	0.0±0	34±0.20
S. Kentucky S3	23±0.08	20±0.04	0.0±0	0.0±0	33±0.18
S. Anatum S4	0.0±0	22±0.20	0.0±0	0.0±0	32±0.20

Legend—M: mean expressed in mm; SD: standard deviation.

EOs exert antimicrobial effects by degrading cell walls, penetrating cell membranes and damaging cells, damaging membrane proteins, leakage of cell contents, cytoplasmic coagulation as shown in Fig. 1B. (Burt, S., 2004).

Several studies have investigated the antimicrobial activity for different essential oils. In this regard, (Teixeira *et al.*, 2013) found that at least four pathogenic organisms among the seven studied organisms were affected by using 17 types of essential oils. Also, (Pesavento *et al.*, 2015) revealed that

essential oils of thymol, rosemary, and oregano inhibited the growth of *Listeria monocytogenes* and *Staphylococcus aureus.*

Characterization of Fabricated β-cyclodextrin Citrate:

The poor solubility of β-cyclodextrin in water at room temperature can be overcome via an esterification process with citric acid, in presence of sodium hypophosphite as a catalyst. The primary hydroxyl groups of β-cyclodextrin are more accessible for esterification than a secondary one. The fabricated β-cyclodextrin citrate exhibited better solubility. Figure 2A. denotes the probable esterification reaction products of β-cyclodextrin with citric acid. ESEM in Figure 2C. reveals the surface morphology structure of fabricated β-cyclodextrin citrate.

The FT-IR spectra for β-cyclodextrin and β-cyclodextrin citrate are shown in figure 2B. Similar absorption bands characteristic of polysaccharides are observed in the two spectra. Though, a new peak had appeared at 1731cm^{-1} in β-cyclodextrin citrate spectrum due to C=O vibration consistent to ester group formed between the primary hydroxyl groups of β-cyclodextrin and carboxyl groups from citric acid. Moreover, the band at 890 cm^{-1} was the distinctive band of α-(1,4) glucopyranose in β-cyclodextrin citrate (Yuan *et al.,* 2013).

The very poor solubility of β-CD in water, which does not allow the direct dissolution for film casting procedure, can be improved through chemical modification of the external hydrophilic groups at 2, 3, and 6 positions (Kang, *et al.,* 2015).

Antibacterial Activity of Cinnamon Oil Encapsulated in β-cyclodextrin Citrate:

A quantitative evaluation of antimicrobial activity has been carried out on cinnamon oil free form and cinnamon oil/β-CD citrate against different *Salmonella* sp. as mentioned in Table 3 & Fig. 3. Cinnamon oil encapsulated in β -CD citrate had a promising antibacterial effect that lasted for a longer time with a slower rate of release. As the inhibition zone in the case of the free oil form was fixed and, in some cases, decreased. On the other hand, the encapsulated form caused an increased inhibition zone of all treatments. This can be attributed to the long path where the essential oil has to pass through, and the intense contact between the oil and the hydrophobic cavity of β-CD (Chen & Liu, 2016). In this regard, (Fathi et al., 2012) found that diffusion is the mechanism used in a core release, in which the active compound is released slowly by permeating the coating's wall without compromising its physical integrity, or through a release trigger, which involves a change in pH, mechanical stress, temperature, enzymatic activity, time, or osmotic force, among other triggers, that promotes capsule breakdown and releases the active compound instantly.

Our results are in accordance with, Babaoglu *et al.* (2017) who encapsulated clove essential oil in hydroxypropyl-beta-cyclodextrin using the kneading method. He found that the inclusion complex had greater stability and the antioxidant properties increased due to the encapsulation process. Also, (Hill *et al.,* 2013) encapsulated different oils like cinnamon bark extract, eugenol, clove bud extract inβ-cyclodextrin. They found effective antimicrobial activity against *Salmonella enterica* serovar Typhimurium LT2 and *Listeria innocua*.

Table 3. Effect of different forms of cinnamon oil (free and encapsulated) on inhibition zones of *Salmonella* strains**.**

Salmonella Sp.	Inhibition zone diameter (mm)			
	After 2 days		After 28 days	
	Cinnamon oil extract	Cinnamon oil extract encapsulated into β-cyclodextrin citrate	Cinnamon oil extract	Cinnamon oil extract encapsulated into β-cyclodextrin citrate
***S. typhinurius* M1**	30	24	30	30
***S. typhinurius* M5**	32	28	32	31
***S. typhinurius* M7**	28	24	28	27
***S. enteritidis* M6**	28	24	28	29
S. typhimurium S1	30	26	29	28
S. typhimurium S2	29	28	29	29
***S. Kentucky* S3**	32	30	30	32
***S. Anatum* S4**	31	29	32	32

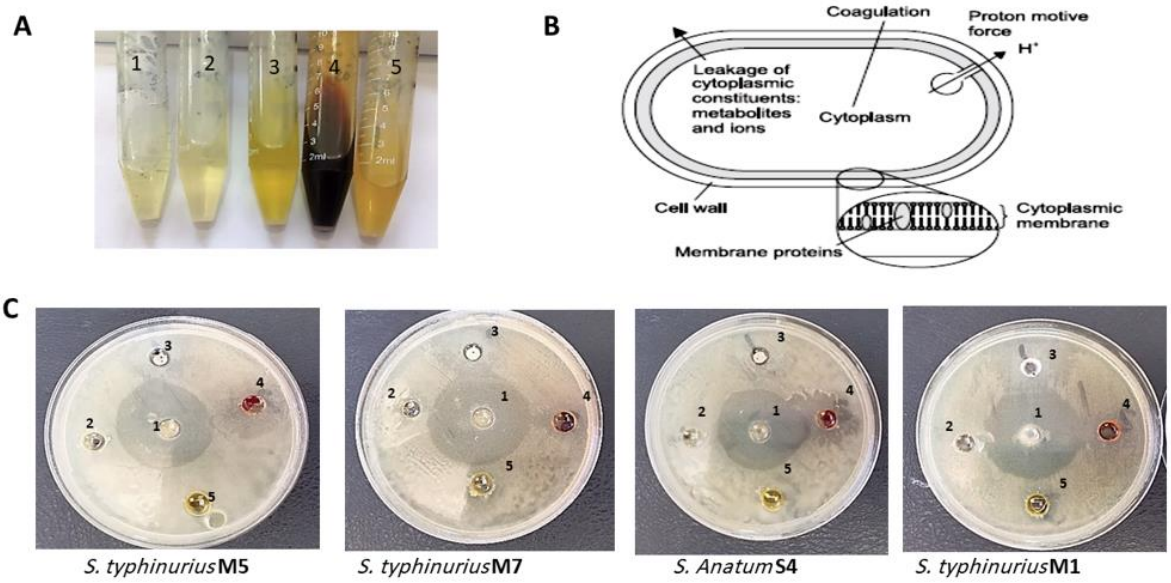

Fig. 1. Antibacterial activity of *Salmonella* strains against the assayed EOs.

A- Different essential oils used in the study.
B- Antibacterial activity mechanisms in the bacterial cell.
C- Effect of different essential oils.

1 – Cinnamon essential oil 2- Moringa oil 3- Marjoram oil 4- Nigella sativa oil 5- Olive oil

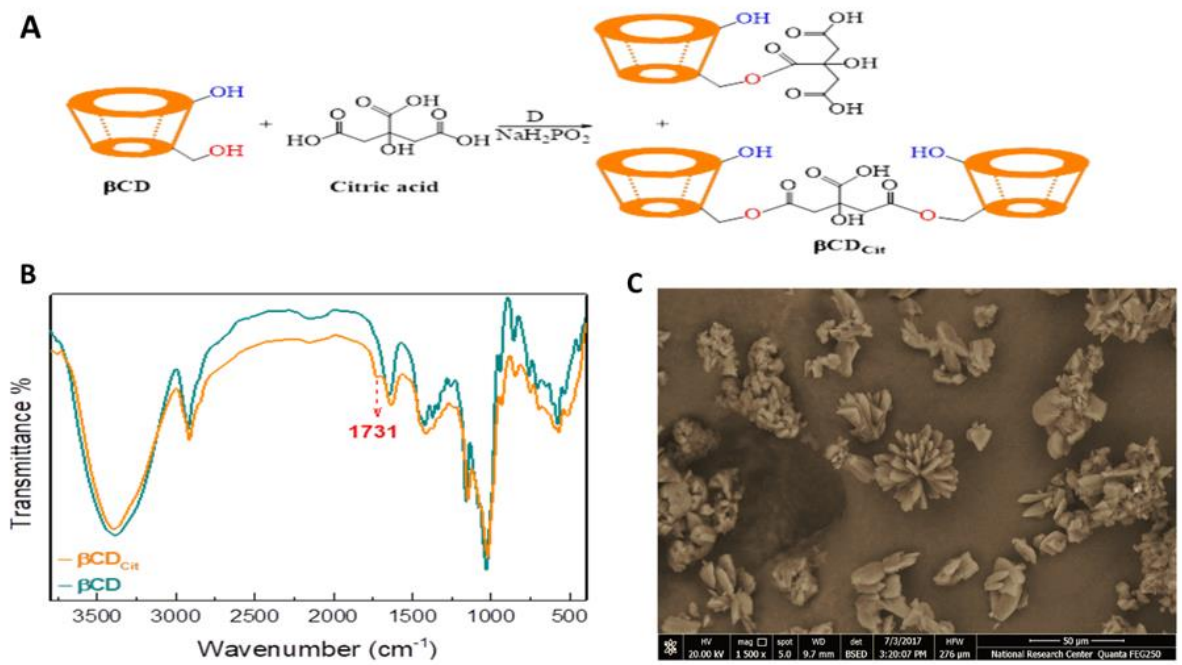

Fig. 2. Different characteristics of Fabricated β-cyclodextrin citrate

A- Schematic representation of esterification reaction of β-CD with citric acid
B- FTIR spectra of βCD and βCD_{Cit}
C- SEM of βCD_{Cit}

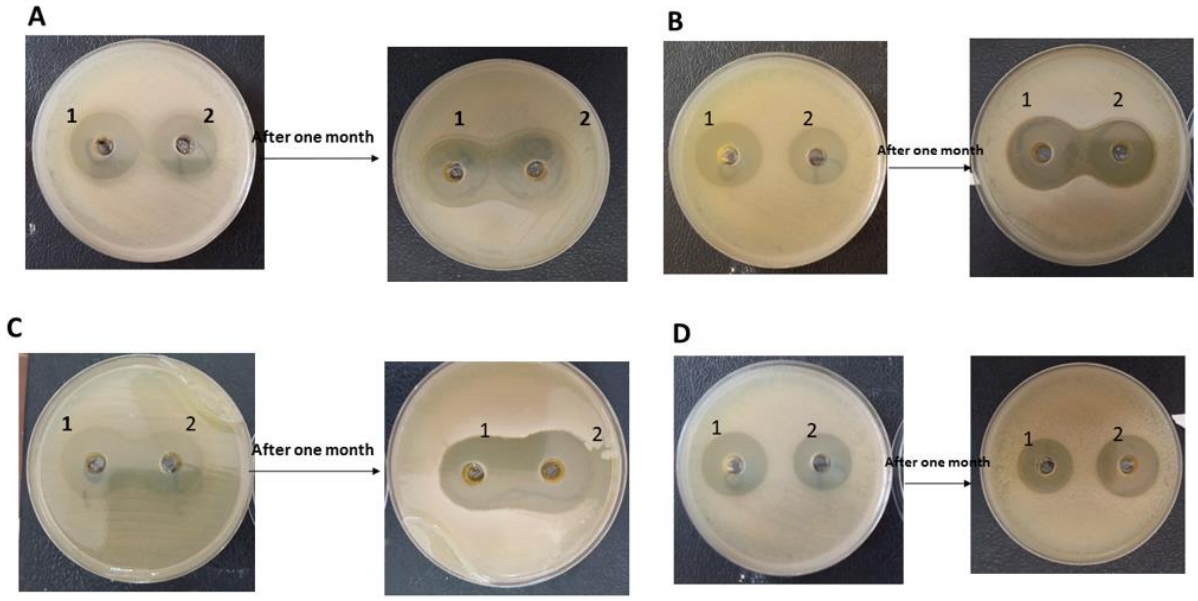

Fig. 3. Agar diffusion test of 1- Cinnamon oil free form & 2- cinnamon oil encapsulated into β-cyclodextrin citrate against several *Salmonella* strains.

A- *S. typhinurius* M5 B- *S. typhimurium* S2
B- C- *S. enteritidis* M6 D- *S. typhimurium* S1

Conclusion:

Essential oils proved to have antimicrobial activity against a number of multi-drug resistant *Salmonella* sp. Cinnamon oil was the most promising of these oils for affecting multidrug-resistant strains. For circumventing the different limitations in essential oils as lipophilic, immiscible with water, sensitive towards the chemical modification under the effect of some external factors such as temperature, light, presence of oxygen. β-cyclodextrin was evaluated as an

encapsulation strategy to promote the controlled release of cinnamon oil while also maintaining its antimicrobial activity. Besides cyclodextrins are virtually non-toxic and some of them are already approved as food additives or as novel foods.

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