Proceedings of the Indian National Science Academy (2024) 90:1095–1108
https://doi.org/10.1007/s43538-024-00300-2

RESEARCH PAPER

Thymus vulgaris essential oil enclosed in β-cyclodextrin as drug delivery system: a promising alternative for antibiotic usage in combating *Salmonella*

Fadia Benchallal[1] · Hayet Belkacemi[1] · Zahra Bellil[2] · Farid Chebrouk[3] · Abdelaziz Touati[2]

Received: 15 December 2023 / Accepted: 8 April 2024 / Published online: 13 April 2024

Abstract
The essential oil of *Thymus vulgaris* (TVEO) has been used in traditional medicine for centuries. However, its low solubility and high volatility have limited its usage. In this study, TVEO was extracted, and its chemical composition was identified. The TVEO was extracted using a Clevenger-type apparatus and identified using Gas Chromatography/Mass Spectrometry (GC/MS). An inclusion complex was then formulated with β-cyclodextrin (βCD) to enhance its solubility, and the antibacterial activity was tested against 14 multidrug-resistant bacteria. When treated with the complex, the inactivation kinetics of *Salmonella* typhimurium were evaluated under simulated intestinal conditions. Thymol and carvacrol were identified as the significant components of TVEO, comprising 47.53% and 19.27% of the oil, respectively. The solubility diagram of enclosed Thymol exhibited a Bs-type profile, with an association constant (Ks) of 202.35 M^{-1} and a complexation efficiency of 55.07% for the formulated complex TVEO-βCD. The antibacterial activity demonstrated significant inhibition against all tested strains except for three. The study demonstrates the effectiveness of the TVEO-β-CD complex against *S.* typhimurium in intestinal conditions, a novel application. Unlike prior research, our work focuses on this complex's efficacy, specifically against *Salmonella*, showing prolonged inhibitory effects. This reveals the enhanced antibacterial activity of β-CD in solubilising TVEO, offering potential as an antimicrobial agent.

Keywords *Thymus vulgaris* essential oil · β-cyclodextrin · Inclusion complex · Antibacterial effect · Simulated intestinal conditions

Introduction

In recent years, herbal products have increased in popularity due to increased awareness of the benefits of natural healthcare and alternative therapy methods. One medicinal plant that has gained widespread recognition is *Thymus vulgaris*, also known as common thyme.

✉ Fadia Benchallal
fadia.benchallal@univ-bejaia.dz

[1] Université de Bejaia, Faculty of Technology Laboratory of Materials Technology and Process Engineering (LTMGP), 06000 Bejaia, Algeria

[2] Laboratoire d'Ecologie Microbienne, FSNV, Université de Bejaia, Bejaia, Algeria

[3] Centre de recherche Scientifique et Technologie en Analyse Physico-Chimique, BP 384, Zone industrielle Bou-ismail RP, 42004 Bou-Ismail, Tipaza, Algeria

Multiple studies have been conducted on *T. vulgaris* essential oil to assess its chemical composition and various properties, including antibacterial (Nadia and Rachid 2013), antioxidant (Ismaili et al. 2017), anti-inflammatory (Abdelli et al. 2017), antiseptic, and analgesic effects (Parsaei et al. 2016; Rašković et al. 2021). The chemical profile of the oil often reveals the presence of monoterpene alcohols such as carvacrol and Thymol. However, this composition can vary based on factors such as climate, soil composition, extraction methods, origin, and harvest time of the plant (Lemos et al. 2017; Nezhadali et al. 2014). Nonetheless, most studies support the efficacy of *T. vulgaris* for these properties. Despite its potential, the low solubility, high volatility, and rapid oxidation of its essential oil in the presence of oxygen have limited its effectiveness and application (Capelezzo et al. 2018), as well as its bioavailability in vivo (Nieddu et al. 2014).

Some studies have attempted to form an inclusion complex of *T. vulgaris* essential oil (TVEO) or one of its major

compounds, such as Thymol, using amphipathic molecules like β-cyclodextrin (β-CD) (Dou et al. 2018; Lin et al. 2018) and γ-cyclodextrin (Tuță-Sas 2019). β-Cyclodextrin, a type of native cyclodextrin (CD), comprises seven glucose units linked by glycosidic bonds. This structure creates a cone-shaped molecule with a hydrophobic cavity inside and a hydrophilic exterior. CDs can increase the solubility of hydrophobic molecules in water by allowing the guest molecules to enter and bond with the lipophilic part of the CD's cavity (Tiwari et al. 2010).

The solubility of a substance is commonly used to assess the stability and composition of an inclusion complex. Higuchi and Connors have categorised solubility diagrams into two types: Profile A and Profile B. Profile A is characterised by a linear increase in guest molecule solubility with increasing CD concentrations, indicating a 1:1 stoichiometry between CD and the guest molecule. Positive deviations from linearity in Profile A, or Profile Ap, suggest the formation of multiple stoichiometries at different CD concentrations. Negative deviations from linearity, called Profile AN, indicate reduced efficacy of the CD at high concentrations. Profile B usually indicates the formation of a complex with limited solubility. Profile Bs initially follow the linear pattern of Profile A but lead to complex precipitation with further CD additions. Profile BI is similar, but the complex is so insoluble that the initial increase in solubility cannot be detected (Higuchi and Connors 1965). Bs and Bi types of phase-solubility diagrams are commonly observed when natural cyclodextrins (CDs) such as αCD, βCD, and γCD are utilised as complexation media for drugs. Bs-type diagrams depict a plateau representing the total solubility of the drug, including its intrinsic solubility and the solubility of the drug in the form of CD-complexes, with the ascending part resembling an A-type diagram, indicating the saturation of available drug molecules in the complexation medium. In contrast, Bi-type profiles also signify limited solubility of drug-CD complexes in the complexation media, but the complexes formed are insoluble in the medium, leading to a plateau in the diagram representing the total drug solubility. In both cases, the decline in total drug solubility at higher CD concentrations is due to the saturation of available drug molecules in the complexation media, highlighting the importance of understanding the complexation behaviour for optimising CD-based pharmaceutical formulations.

Several methods have been used to synthesise the inclusion complex between Thymol and β-CD, such as co-evaporation, kneading, and co-precipitation (Cid-Samamed et al. 2022; Tao et al. 2014). Among these methods, co-precipitation has been widely utilised and demonstrated to be highly effective in forming the inclusion complex. The inclusion complex of Thymol and β-CD has shown enhanced antimicrobial activity compared to free Thymol. This enhancement is attributed to Thymol's increased stability and solubility in the inclusion complex (Tao et al. 2014). Furthermore, the inclusion complex has a longer shelf life than free Thymol (Nieddu et al. 2014).

Antibiotic resistance has become a pressing global health issue, with overuse and misuse of antibiotics being significant contributing factors (Chokshi et al. 2019). The poultry industry, a significant consumer of antibiotics in agriculture, plays a critical role in developing antibiotic-resistant bacteria (Agyare et al. 2018). Antibiotics are frequently used in poultry production to combat and prevent bacterial infections, particularly those caused by *Salmonella*, which poses a significant threat to public health (Dar et al. 2017). However, the widespread emergence of antibiotic-resistant bacteria has diminished the effectiveness of antibiotics, making it more challenging to treat bacterial infections in poultry (Hedman et al. 2020).

Therefore, alternative strategies, such as natural antimicrobials, must be explored and implemented to reduce antibiotic dependence in poultry production. Essential oils from plants like *T. vulgaris* have been shown to possess antimicrobial properties (Ahmadzadeh et al. 2022; Hosseini and Meimandipour 2018; Khan et al. 2012). Co-administration of essential oils with antibiotics has been found to reduce the required antibiotic dosage, thus lowering the risk of antibiotic resistance (Amouei et al. 2021). Additionally, using essential oils as an alternative to antibiotics in poultry production offers advantages such as reducing the development of antibiotic-resistant bacteria, cost savings, and improved economic viability. Furthermore, essential oils provide a more sustainable and environmentally friendly alternative to traditional antibiotics, benefiting both the poultry industry and the environment (Abd El-Hack et al. 2022).

This study aimed to identify the chemical composition of the essential oil extracted from *T. vulgaris* grown in a specific region of Algeria. The study will also investigate the oil's antibacterial properties against 14 different strains of bacteria. To enhance the oil's solubility, it will be encapsulated in β-CD and evaluated for its ability to inhibit the growth of *Salmonella* typhimurium, a bacterium that causes intestinal diseases in poultry.

This study presents a novel investigation into the antibacterial activity of the complex formed between *T. vulgaris* essential oil and β-cyclodextrin in simulated intestinal conditions, specifically targeting *Salmonella*. Our research addresses a critical gap in the existing literature by evaluating the effectiveness of this complex in combating *Salmonella* within an environment that closely mimics the complex conditions of the poultry intestinal tract. By focusing on this specific aspect, our study offers valuable insights into the potential application of this complex as a natural antibacterial agent against *Salmonella* infections. These findings open up new avenues for developing targeted interventions

to promote gastrointestinal health in poultry, thereby contributing to advancements in the field.

Materials and methods

Materials

All chemicals and reagents used were of analytical grade and were sourced from Sigma-Aldrich.

Extraction of *Thymus vulgaris* essential oils

The plant was harvested in the Amizour region (coordinates: 36° 36′ 22.5864″ North, 4° 52′ 42.3912″ East, altitude: 226 m) in July 2022 at the El Hamma Garden of Algiers. A voucher specimen of *T. vulgaris* (N° HBGH1898) was deposited at the departmental herbarium of this garden. The aerial parts of the harvested plant were washed with distilled water and then air-dried for one week. The TVEO was extracted using a Clevenger-type apparatus for 6 h with appropriate water. The obtained TVEO was then stored in a shaded bottle between 4–5 °C for characterisation.

Methods

Determination of TVEO composition by gas chromatography–mass spectrometry and quantification by gas chromatography-flame ionization detector

The chemical composition of TVEO was analysed using GC–MS on a Hewlett-Packard computerised system. The system consisted of a 6890 gas chromatograph coupled to a 5973A mass spectrometer with an HP-5MS column (30 m × 0.25 mm ID, 0.25 µm film thickness). Helium was used as the carrier gas with a flow rate of 0.5 mL/min, and the injection volume was 0.2 µL (split 1:20) at a temperature of 250 °C. The oven temperature program started at 60 °C and was held for 8 min, then increased at a rate of 2 °C/min to 250 °C and held for 10 min. The mass range and scan time were set to 50–550 m/z and 283 s, respectively. Electronic impact at 70 eV was used as the ionisation mode.

GC analyses were conducted on a Hewlett-Packard 6890 gas chromatograph using the same HP-5MS column. The following conditions were used: carrier gas nitrogen with a flow rate of 0.6 mL/min, injection volume of 0.2 µL with a 1:20 split ratio, and an injection temperature of 250 °C. The temperature program was the same as that used for GC–MS. Detection was performed by flame ionisation detection (FID) at 300 °C.

Identification and quantification of TVEO

Identification of volatile constituents was based on comparing their Kovats indices (KI) with literature values, including a homologous series of C8–C28 n-alkanes and those stored in the NIST 20 and Wiley 07 MS libraries. The KI indices were determined using the following formula:

$$RI(x) = 100 * z + 100 * \frac{RT(x) - RT(z)}{RT(z+1) - RT(z)}$$

where (x) is the unknown compound, (z) is the number of carbon atoms of the n-alkane eluted before the unknown compound x, (z + 1) is the number of carbon atoms of the n-alkane eluted after the unknown compound x, RI(x) is the retention index of the unknown compound x, RT(x) is the retention time of the unknown compound x, RT(z) is the retention time of the preceding n-alkane and RT(z + 1) is the retention time of the succeeding n-alkane.

Mass spectra literature data were also used to confirm identification (Adams 2017). The relative concentrations of components were represented as a percentage (% content) and calculated by integrating their corresponding GC-FID chromatographic peak areas using ChemStation software.

Enhancing solubility of TVEO

Determination of solubility diagram and association constant of an inclusion complex

The solubility diagram provides an overview of the stoichiometry of the guest and host molecules. The slope of the linear portion of the solubility diagrams allows for the calculation of the association constant (stability constant) Ks using Eq. (1) (Hill et al. 2013):

$$\mathrm{Ks} = \frac{\alpha}{\mathrm{So} * (1 - \alpha)} \quad (1)$$

where α is the The slope of the linear part of the curve, So is the aqueous solubility in mol/L of the guest molecule without CD and Ks is the stability constant in M^{-1}.

Preparation of TVEO-βCD inclusion complex

The preparation process of the inclusion complex between βCD and TVEO involves several steps, following the method described by Al-Nasiri et al. (Al-Nasiri et al. 2018) with some modifications. Firstly, 3.8 g of βCD were mixed with a solution containing equal parts ethanol and water at a temperature of 55 °C. Then, 1 g of TVEO, previously dissolved in 10 millilitres of ethanol, was added to the mixture. The solution was stirred for 4 h at room temperature and left to

rest overnight at 7 °C. Subsequently, the resulting powder was collected through vacuum filtration and allowed to air dry for one week.

Solubility diagram of thymol in TVEO entrapped in βCD

An excess of TVEO was added to seven flasks, and an aqueous solution containing various concentrations of βCD was added. The flasks were then continuously agitated for seven days, after which the solutions were filtered using microfilters (ISOLAB 094.05.003) with a PV DF 0.45 µm pore size. The solubilised Thymol was quantified using Ultra High-Performance Liquid Chromatography (UHPLC) with a mobile phase consisting of a 50:50 (v/v) mixture of acetonitrile/water and a detection wavelength of 274 nm on a Thermoscientific Chromeleon (c) Dionex Version 7.2.0.3765 system. A calibration curve of Thymol was generated under the same conditions, following the method validated by Hajimehdipoor et al. (2010).

Determination of the inclusion efficiency of TVEO-βCD complex

A 40-mg sample of the TVEO-βCD inclusion complex was dissolved in 25 millilitres of ethanol and subjected to stirring. The solution was then filtered using a 0.45 µm microfilter (Al-Nasiri et al. 2018) and analysed using UHPLC with a mobile phase consisting of a 50:50 (v/v) mixture of acetonitrile and water (Hajimehdipoor et al. 2010) The inclusion efficiency is calculated using the following Eqs. (2) (Wen et al. 2010):

$$IE\,(\%) = \frac{CE}{CT} * 100 \tag{2}$$

where IE (%) is the The inclusion efficiency, CE is the Concentration of TVEO entrapped and CT is the Total concentration of TVEO.

Characterisation of TVEO-βCD with scanning electron microscopy (SEM)

The morphologies of βCD and the TVEO-βCD inclusion complex were examined using a Thermo Fisher Prisma E Scanning Electron Microscope. The instrument was operated at a voltage of 10 kV and a magnification of 5000X. The working pressure was set at 30 Pa, and the working distance was 10.3 mm.

Antibacterial activity of TVEO and thymol

The antibacterial activity of TVEO was evaluated against 14 pathogenic bacterial strains, including Gram-negative and Gram-positive bacteria, which were isolated from various sources. Notably, many of these strains are considered multidrug-resistant (Table 1).

The antibacterial activity of TVEO and Thymol solutions, prepared in DMSO (20 mg in 10 mL of DMSO), was evaluated using the agar-well diffusion method on

Table 1 Bacterial strains used in the study

Strains	Source
1. *1-Klebsiella pneumoniae* producing CTX-M-15	Clinical
2. *Acinetobacter baumannii* producing OXA-23	Clinical
3. *Pseudomonas aeruginosa producing VIM-1*	Clinical
4. *Escherichia coli* producing CTX-M-15- and OXA-48-	Clinical
5. *E. coli* ATCC25922	Laboratory
6. *Acinetobacter baumannii* producing OXA-58	Clinical
7. *Serratia marcescens*	Clinical
8. *Enterobacter cloacae* overexpressing AmpC	Clinical
9. *Salmonella* typhimurium Resistant to ciprofloxacin	Poultry
10. *Providencia rettgeri*	Clinical
11. *Staphylococcus epidermidis* Methicillin Resistant	Clinical
11. *Staphylococcus aureus* Methicillin Resistant	Clinical
12. *Staphylococcus saprophyticus*	Clinical
13. *Enterococcus faecalis* Vancomycin Resistant	Fecal carriage

1. Strain that produces the CTX-M-15 extended-spectrum beta-lactamase (ESBL) enzyme, which confers resistance to a range of β-lactams including penicillins, cephalosporins, and monobactams

2. Strain that that produces the OXA-23 carbapenemase enzyme, which can break down and inactivate carbapenem antibiotics and confers resistance to most of β-lactams

3. Strain that produces the VIM-4 metallo-beta-lactamase enzyme, which can also break down carbapenem antibiotics and confers resistance all of β-lactams except aztreonam

4. Strain that produces both of the CTX-M-15 ESBL and the OXA-48 carbapenemase, making it resistance to most of β-lactams

5. A laboratory strain of *E. coli* commonly used as a quality control reference

6. Strain that produces the OXA-58 beta-lactamase enzyme, which can also break down carbapenem antibiotics and confers resistance to most of β-lactams

7. Has a naturally occurring chromosomal AmpC beta-lactamase enzyme that can confer resistance to a range of antibiotics, including narrow-spectrum penicillins and cephalosporins, cefuroxime, cephamycins, and aztreonam

8. Strain that overexpresses the AmpC beta-lactamase enzyme, which can confer resistance to most of β-lactams

9. Resistant to ciprofloxacin due to mutations in the quinolone resistance-determining region (QRDR) of the DNA gyrase

10. Strains that is naturally resistant to tetracyclines, some penicillins, and older cephalosporins

11. Strain that is resistant to most of β-lactams

12. Strain that is susceptible

13. Strain that is resistant to the antibiotic vancomycin, and can cause a range of infections including bloodstream infections and urinary tract infections

Mueller–Hinton agar (Fluka, St Louis, USA) (Ashraf et al. 2017). Mueller–Hinton agar medium was poured into sterile Petri dishes and allowed to solidify. Wells were made in the agar medium using a sterile pipette with spacing between them.

To prepare a standardised inoculum, a 0.5 McFarland inoculum (10^8 CFU/mL) was used (Fani and Kohanteb 2017). This standardised inoculum was then swabbed onto the Mueller–Hinton surface for each bacterial strain. Subsequently, 50 µL of TVEO and 50 µL of Thymol solution were poured into the wells. The Petri dishes were kept at 4 °C for 2 h for the essential oil diffusion and then incubated at 37 °C for 18 h.

Determination of the minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) values of TVEO and its formulated complex, TVEO-βCD, against *S.* typhimurium, were determined using the macrodilution method. A series of dilutions of TVEO and the complex in 1 millilitre DMSO were added to 4 millilitres of Mueller Hinton Broth (Fluka, St Louis, USA). The bacterial culture was introduced into the broth dilution at a final concentration of 10^4 CFU/ml. Additionally, a positive control (Mueller Hinton broth inoculated with bacteria without TVEO-βCD) and a negative control (sterile Mueller Hinton broth) were included in the experiment. All cultures were incubated at 37 °C for 18 h. The MIC is defined as the lowest concentration that can inhibit the visible growth of microorganisms.

Effect of TVEO-βCD on the inactivation kinetics of *S.* typhimurium

The effect of the TVEO-βCD complex on the growth of *S.* typhimurium bacteria was investigated in a laboratory environment simulating the intestinal tract conditions. One gram of the TVEO-βCD complex was added to 200 mL of peptone water (Fluka, St Louis, USA), maintained at a pH of 6.8 to simulate duodenal pH and a temperature of 37 °C with shaking. To assess the growth of the bacteria in the presence and absence of the complex, one millilitre of the culture was taken every 15 min from both the experimental and control media and then plated after serial dilutions on TSA agar (Fluka, St Louis, USA). The plates were incubated for 24 h at 37 °C, and after incubation, a count of CFUs was conducted.

Experimental evaluation of the reversible inhibition of *Salmonella* growth by TVEO-βCD complex

In this study, the biological activity of the TVEO-βCD complex against *Salmonella* was evaluated using a series of experimental procedures. Initially, *Salmonella* cultures were treated with TVEO-βCD and incubated for 24 h to assess its inhibitory effects on bacterial growth in the same conditions as the inactivation kinetics. Following treatment, the cultures were subjected to filtration to remove the TVEO-βCD complex, and the growth of *Salmonella* was monitored over an additional 24h period in a fresh growth medium. Control experiments were conducted using untreated *Salmonella* cultures to establish baseline growth conditions. The growth of *Salmonella* was quantified using colony counting.

Results

GC/MS analysis shows Thymol and Carvacrol as the main components of TVEO

A total of 22 compounds were identified in TVEO through GC/MS analysis, as presented in Table 2. The primary compound was thymol Fig. 1a, accounting for 47.53% of TVEO, followed by Carvacrol Fig. 1b at 19.27% and γ-Terpinene at 14.94%. Three other compounds, P-cymene (3.96%), α-Terpinene (2.82%), and Z-Caryophyllene (1.72%), had percentages more significant than 1%.

Determination of solubility diagram of TVEO enclosed in βCD

The solubility diagram of Thymol in TVEO enclosed in βCD, shown in Fig. 2, resembled profile Bs as per the classification by Higuchi and Connors in Fig. 3. The stoichiometry of the complex was 1:1 when the concentration of βCD was between 0 and 4 mM. However, when the concentration of βCD exceeded four mM, the solubility of Thymol in TVEO remained constant, and the formation of βCD precipitate occurred.

The association constant, Ks, of the complex formed was determined using the linear portion of the plot shown in Fig. 4. Based on Higuchi's equation, the Ks value was estimated at 202.35 M^{-1}.

Quantification of TVEO entrapment efficiency in β-cyclodextrin complex using GC/MS and UHPLC analysis

The concentration of Thymol in the formed complex was calculated based on the ratio of βCD to Thymol in TVEO used to prepare the complex and the GC/MS analysis results. The theoretical concentration of Thymol in TVEO was found to be 0.138 g/L. To determine the concentration of Thymol entrapped in the βCD, the chromatogram area in

Table 2 Composition of Thymus vulgaris EO

No	Retention time (min)	KI (Obs)	KI (Lit)	Compounds	EO %
1	9.22	924	924	α-Thujene	0.56
2	9.58	930	932	α- pinène	0.33
3	12.047	979	979	Oct-1-en-3-ol	0.22
4	12.89	986	983	3-Octanone	0.21
5	13.11	990	988	β-Myrcene	0.99
6	13.87	1002	1004	α-Phellandren	0.15
7	14.74	1015	1017	α-Terpinène	2.82
8	15.38	1024	1024	P-Cymene	3.96
9	17.94	1061	1059	γ-Terpinene	14.94
10	19.7	1086	1088	Terpinolene	0.09
11	20.75	1101	1101	Cis-Sabinene hydrate	0.39
12	26.09	1175	1177	Terpinène-4-ol	0.25
13	27.44	1194	1196	p-Cymen-8-ol	0.27
14	30.17	1234	1235	Thymol methylether	0.2
15	30.79	1243	1244	Carvacrol methylether	0.24
16	34.99	1301	1289	Thymol	47.53
17	35.57	1310	1298	Carvacrol	19.27
18	42.16	1408	1418	Z-Caryophyllene	1.72
19	47.77	1455	1509	β-Bisabolene	0.96
20	48.6	1561	1523	δ-Cadinene	0.23
21	49.8	1571	1531	Cis- α-bisabolen	1.05
22	51.89	1589	1573	Caryophyllene oxide	1.10
	Grouped compounds Monoterpene hydrocarbons (No. 1,2,5–12,18–21) Oxygenated monoterpenes (No. 3,4,13–15,22) Sesquiterpene hydrocarbons (No. 18,19,20) Oxygenated sesquiterpenes (No. 22)				97.48%

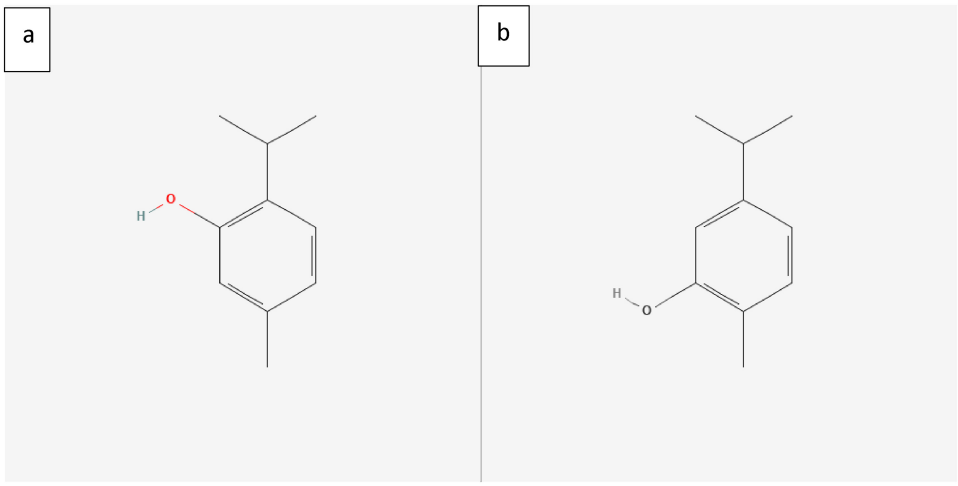

Fig. 1 Chemical structure of Thymol (**a**) and Carvacrol (**b**)

Fig. 5 was calculated using UHPLC and a calibration curve of Thymol Fig. 6. The inclusion efficiency of Thymol into β-cyclodextrin was estimated to be 55.07% using Eq. (2).

Morphological analysis of β-cyclodextrin and TVEO-βCD complex

The SEM images presented in Fig. 7 showed the morphologies of βCD and the TVEO-βCD complex. As observed in image (A) with circles surrounding the particles, βCD

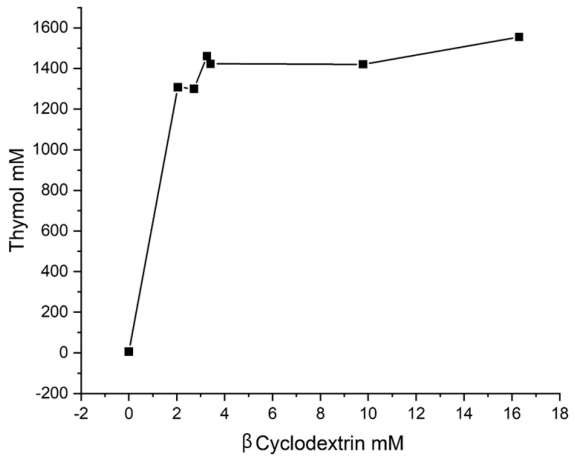

Fig. 2 Solubility diagram of Thymol in TVEO into βCD

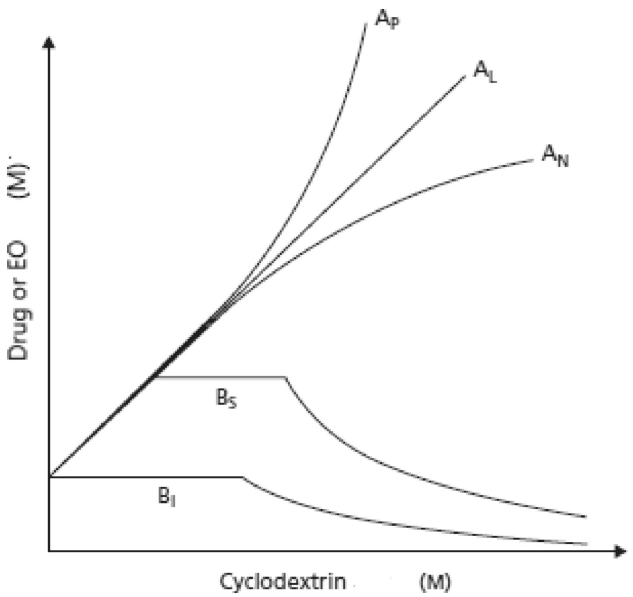

Fig. 3 Phase-solubility diagrams according to Higuchi and Connors

took a block form with different sizes ranging from 8 to 34 µm, whereas the dimensions of the complex in image (B) appeared as small, well-organised particles with homogeneous crystalline parallelogram structure with a uniform size of approximately 0.85 µm.

The reduction in the size of β-Cyclodextrin after loading with Thyme essential oil (TVEO) can be attributed to the formation of an inclusion complex between β-CD and the hydrophobic components of TVEO. When TVEO is loaded into the β-CD cavity, it forms a stable complex through hydrophobic interactions, resulting in a more compact structure. This inclusion process causes the β-CD molecule to encapsulate the TVEO molecules, leading to a decrease in the overall size of the TVEO-βCD complex compared to the uncomplexed β-CD molecule. Additionally, the encapsulation of TVEO within the β-CD cavity may induce conformational changes in the β-CD structure, further contributing to the reduction in size. This phenomenon is commonly observed in the formation of inclusion complexes and indicates successful encapsulation of TVEO within the β-CD cavity.

Antimicrobial activity and MIC of thyme essential oil and thymol against various bacterial strains

All tested strains were susceptible to TVEO and Thymol, with inhibition halos ranging between 20 and 55 mm, despite their multidrug and natural resistances, as shown in Table 3. However, the *Pseudomonas aeruginosa* strain showed a weak inhibition zone with an inhibition halo of 8 mm for TVEO and 6 mm for the Thymol solution.

Among the tested strains, *Serratia marcescens*, which was resistant to amoxicillin, amoxicillin + clavulanic acid, cephalothin, cefamandole, cefuroxime, colistin, and polymyxin B, showed the highest susceptibility to TVEO with an inhibition halo of 52 mm.

TVEO exhibited higher inhibition against Gram-negative strains compared to Gram-positive ones, where resistance was shown *by Staphylococcus epidermidis* and *Enterococcus faecalis*. Both *Escherichia coli* ATCC, susceptible to all antibiotics, and *E. coli* producing extended-spectrum β-lactamase (ESBL) CTX-M-15 showed a similar inhibition halo of 32.5 mm. Great susceptibility was shown by both *Acinetobacter baumannii* strains producing carbapenemase OXA-58 and OXA-23, with diameters of 40 mm and 33 mm, respectively. The *S.* typhimurium strain was also susceptible to TVEO and Thymol, with inhibition halos of 23 mm and 25 mm, respectively (Table 3).

The MIC of *S.* typhimurium against TVEO was 3.35 mg/ml. However, no inhibition was observed for TVEO-βCD.

Inactivation kinetics of *S.* typhimurium treated with TVEO-βCD complex

The kinetics shown in Fig. 8 indicated that in the intestinal environment, the growth of *S.* typhimurium increased exponentially during the first 120 min of the experiment, from 4.14×10^7 CFU/ml until it reached 1.88×10^{11} CFU/ml. However, when the bacteria were treated with the inclusion complex, the CFU/ml decreased from 4.34×10^9 to 1.30×10^7 within 60 min under the same intestinal conditions. The bacterial concentration remained between 1.3×10^7 and 1.37×10^8 CFU/ml from t = 60 to t = 120 min.

The observation of bacterial growth restarting and increasing after 30 min of contact between TVEO and Thymol suggests a temporal limitation in the antibacterial

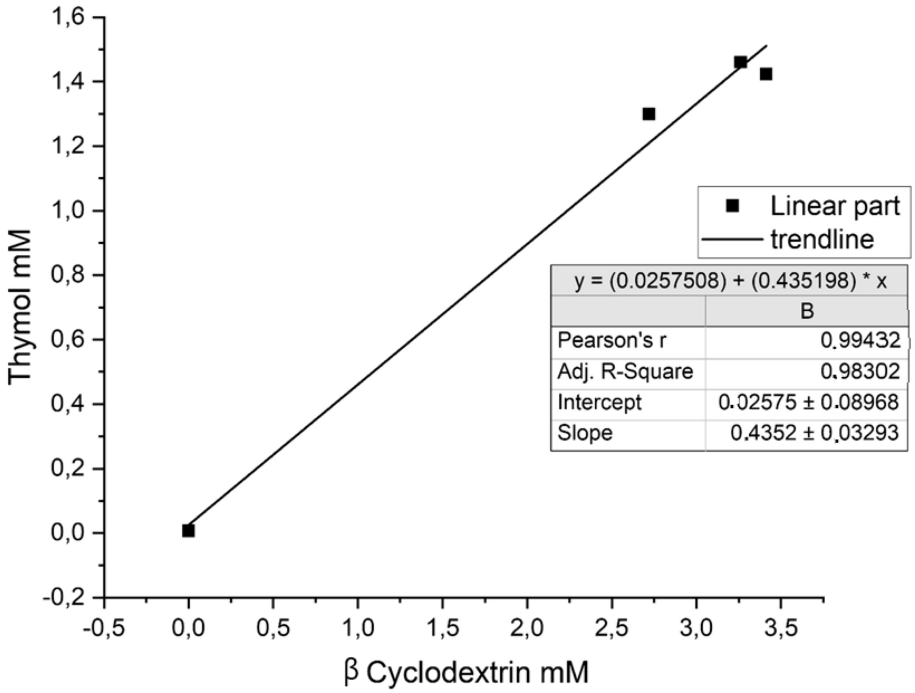

Fig.4 The linear part of the solubility diagram of thymol into β-Cyclodextrin

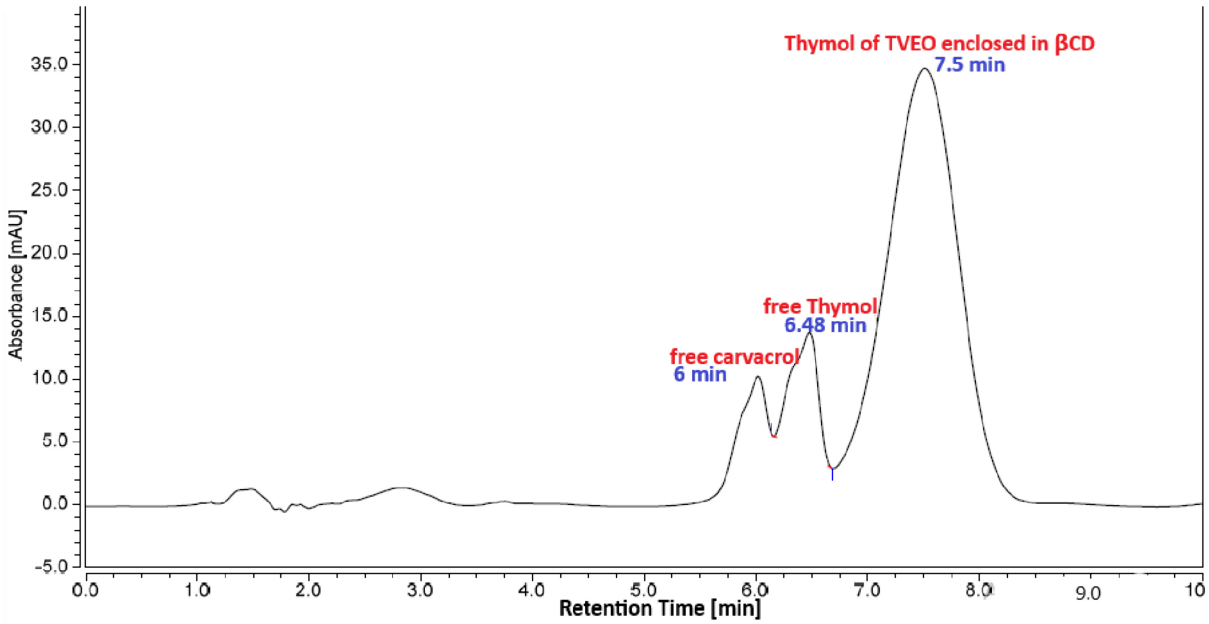

Fig. 5 The UHPLC chromatogram of TVEO-βCD dissolved in Ethanol

activities of these compounds. This phenomenon indicates that the efficacy of TVEO and Thymol does not persist over time, allowing a fraction of bacteria that were not initially killed to resume growth. However, for the TVEO- βCD complex, the resurgence of bacterial growth is only observed after 60 min of contact. This delayed onset of bacterial growth resumption suggests that the complex may exhibit prolonged or sustained antibacterial activity compared to TVEO and Thymol alone. Such observations underscore the importance of considering the immediate antibacterial effects and the activity duration when evaluating antimicrobial agents' efficacy, highlighting the potential advantages of complexation strategies in enhancing the temporal stability of biological activities.

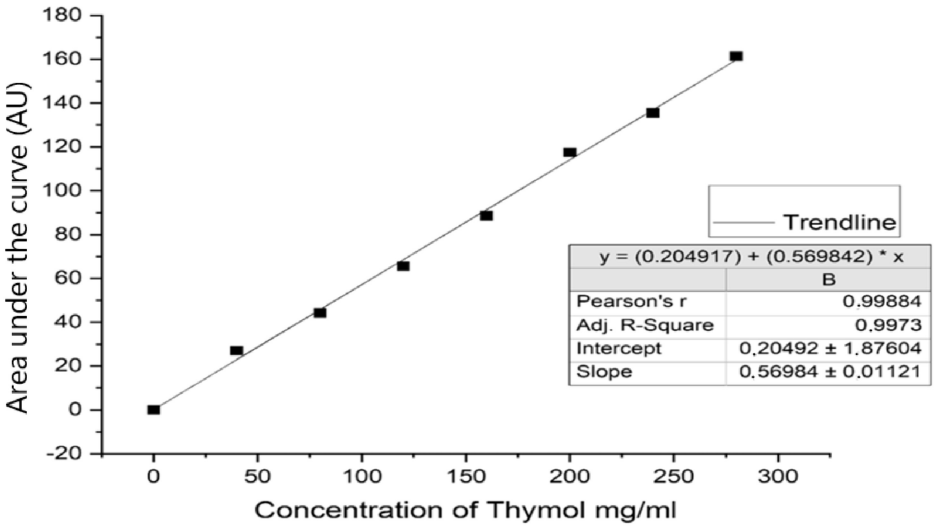

Fig. 6 Calibration curve of thymol in ethanol Quantified by UHPLC

Experimental evaluation of the irreversible inhibition of *Salmonella* growth by TVEO-βCD complex

In our study of the biological activity of the TVEO-βCD complex against *Salmonella*, the irreversible action experiment revealed substantial growth suppression upon initial treatment. After 24 h, colony counts in treated cultures decreased significantly to 9.65×10^7 CFU/ml despite separation of complex by filtration, in contrast to controls not treated with complex, which exhibited exponential growth of *Salmonella*. the action of the TVEO-βCD complex against *Salmonella* appears to be irreversible. This is indicated by the significant suppression of bacterial growth observed after treatment.

Discussion

This study aimed to characterise the chemical composition of TVEO, evaluate its antibacterial activity, and develop an inclusion complex of Thymol in TVEO using βCD to enhance its solubility. Furthermore, we aimed to assess the ability of the inclusion complex to inactivate the growth kinetics of *S.* typhimurium under simulated intestinal conditions.

The chemical analysis of TVEO revealed that Thymol Fig. 1a and Carvacrol Fig. 1b were the major components, accounting for 47.53% and 19.27%, respectively. These values were higher than those reported in previously published studies (Chbel et al. 2022; Fadil et al. 2018; Giordani et al. 2008). Therefore, our study demonstrated that monoterpenes were the predominant class in TVEO, which is consistent with the chemical composition of the Lamiaceae family.

The inclusion efficiency of TVEO-βCD was estimated to be 55.07%, which was lower than the values reported by Al-Nasiri et al. and Dou et al. (Al-Nasiri et al. 2018; Dou et al. 2018) but higher than the inclusion efficiency reported by Abraca et al. (Abarca et al. 2016) The solubility diagram profile of the complex is similar to that of the B_s type, which has also been reported by Tao et al. (Tao et al. 2014). The stability constant of the complex was found to be 202.35 M^{-1}, indicating an interaction between Thymol in TVEO and β-cyclodextrin. The equilibrium shift towards forming a complex indicated that a limited amount of the ligand would be released.

While TVEO has been studied for its potential applications, its use in vivo is limited due to cytotoxicity, low solubility, instability, unpleasant taste, and uncontrolled release (Alsakhawy et al. 2022). To overcome these challenges, researchers have turned to cyclodextrins, which are biodegradable and non-toxic and can encapsulate hydrophobic essential oils. βCD is a preferred option due to its better solubility in water, optimal cavity size for the inclusion of most essential oils, greater stability, wider availability, and cost-effectiveness, making it a practical choice for industrial applications.

Zoonotic diseases pose a significant public health concern, accounting for 60% of human infectious diseases and causing severe illnesses (Kulkarni et al. 2015). Poultry farming can be a vector for zoonotic diseases, as livestock carries bacteria such as *Salmonella* and *Campylobacter* that can cause human infections (Rahman et al. 2020). These bacteria are frequently found in the intestines of birds and can be transmitted to humans through undercooked chicken meat or exposure to bird faeces. However, the uncontrolled use of antibiotics in the poultry industry has contributed to the emergence of antibiotic-resistant bacteria, including *Salmonella spp.,* that produce extended-spectrum beta-lactamase

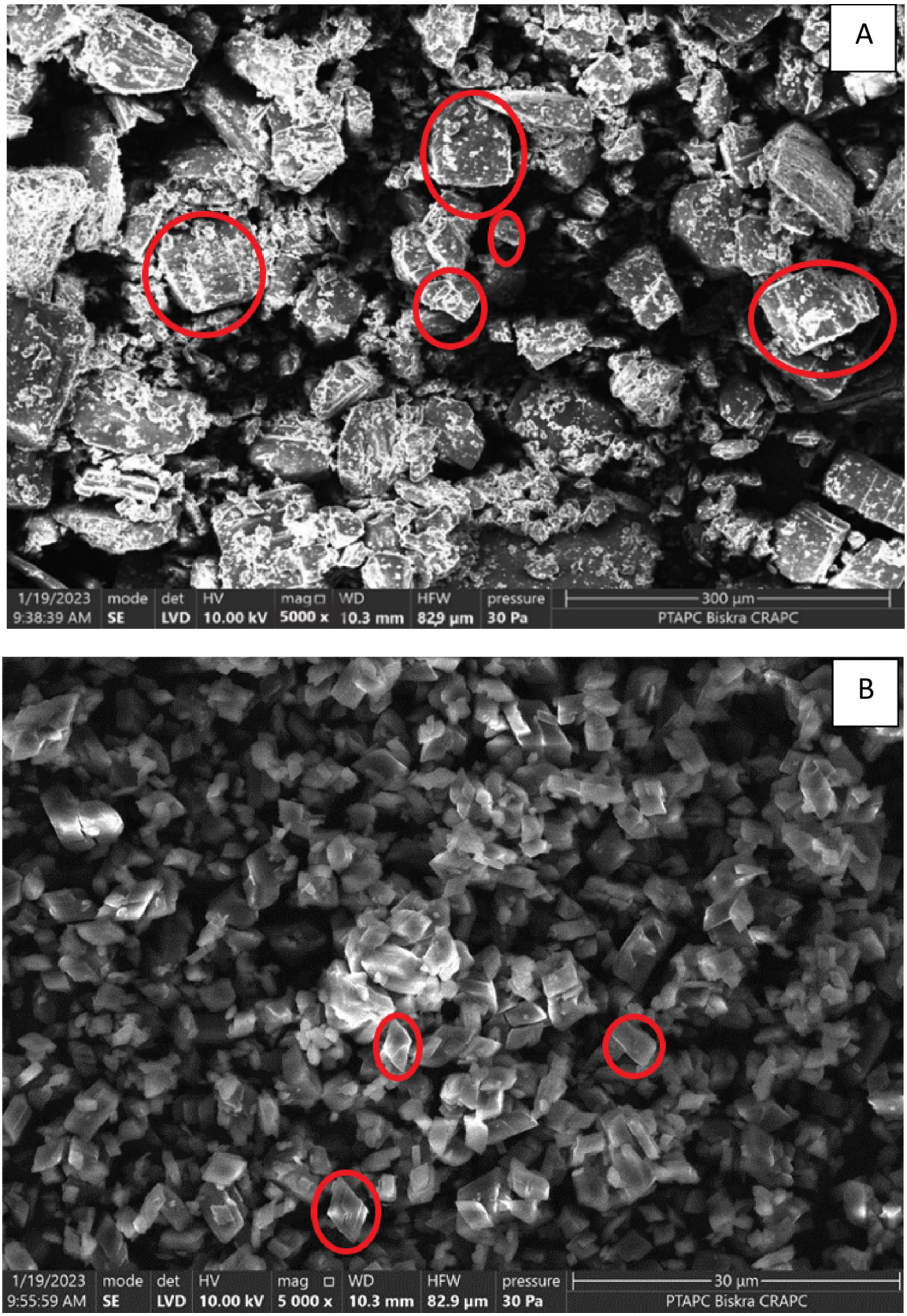

Fig. 7 SEM images of β-Cyclodextrin (**A**), Complex TVEO-β-Cyclodextrin (**B**)

Table 3 Inhibition halos of TVEO and Thymol against 14 bacterial strains

Species	Inhibition halos of essential oil (mm)	Inhibition halos of Thymol (2 mg/ml)
Klebsiella pneumonia producing CTX-M-15	44 ± 2.8	44 ± 0
Acinetobacter baumannii producing OXA-23	33 ± 1.4	49 ± 1.4
VIM-1 producing *Pseudomonas aeruginosa*	8 ± 2.8	5 ± 0.5
Escherichia coli producing CTX-M-15- and OXA-48-	32.5 ± 3.5	24 ± 1.2
E. coli ATCC25922	32.5 ± 3.5	29.5 ± 0.7
Acinetobacter baumannii producing OXA-58	40 ± 0	55 ± 0.5
Serratia marcescens	52 ± 0.7	32 ± 1.4
Enterobacter cloacae overexpressing AmpC	30.5 ± 2.8	19 ± 1.4
Salmonella typhimurium Resistant to ciprofloxacin	23 ± 2.8	25 ± 0
Providencia rettgeri	45 ± 1.4	34 ± 1.4
Staphylococcus epidermidis Methicillin Resistant	No zone	No zone
Staphylococcus aureus Methicillin Resistant	37.5 ± 2.1	44 ± 2.1
Staphylococcus saprophyticus	35 ± 2.1	45 ± 0.2
Enterococcus faecalis Vancomycin Resistant	No zone	No zone

Results represented by: mean ± standard deviation

(ESBL), plasmid-mediated quinolone resistance (PMQR), and colistin resistance (Ziech et al. 2016).

Infectious diseases can significantly impact the poultry industry, causing economic losses for chicken producers. Furthermore, residues of antibiotics have been found in poultry muscles and eggs, indicating that the unregulated use of antibiotics in the industry poses a significant risk to both public health and the poultry industry (Yamaguchi et al. 2015). The emergence of multidrug-resistant (MDR) bacteria and the ban on some antibiotics has made it necessary to find alternative treatments that are effective against MDR bacteria without posing any risks. In our study, TVEO showed excellent antibacterial activity against the MDR strains tested. TVEO has shown promising results when used alone or with antibiotics against some MDR bacteria (Alibi et al. 2020; Benameur et al. 2019; Rosato et al. 2020; Sienkiewicz et al. 2012). Listorti et al. demonstrated that TVEO was effective against 25 MDR *Salmonella* strains (Listorti et al. 2020). Moreover, *T. vulgaris* is a well-known plant that is safe for human consumption, even when used to treat chicken meat, since it is already commonly used as a spice in food.

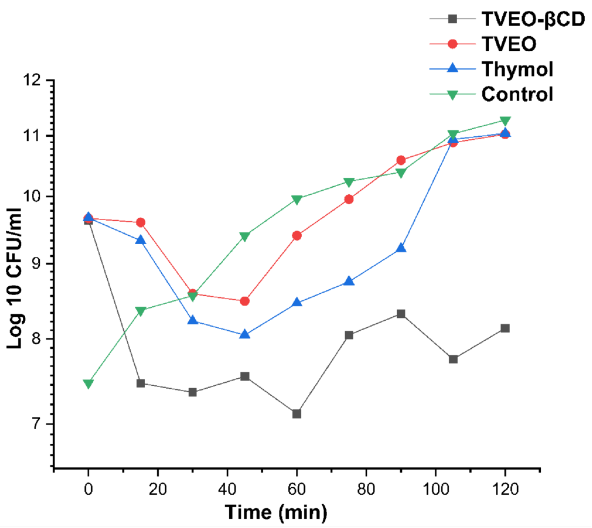

Fig. 8 Survival of *S.* typhimurium in the intestinal environment alone and treated with TVEO,Thymol and TVEO-βCD complex

The antibacterial activity of TVEO-βCD against *S.* typhimurium and other strains was studied by Ahmed et al. The authors found low antibacterial activity, which was attributed to the incomplete dissolution of the complexes (Ahmed et al. 2022). It is well-known that the release of the guest molecule from cyclodextrins occurs gradually and can be promoted by several conditions, such as agitation and temperature. Furthermore, it is essential to note that the release and performance of the complex in vivo may differ from that in vitro, as it will encounter the dynamic and complex conditions of the gastric and intestinal environments. Therefore, to test the effectiveness of TVEO-βCD in vivo and improve its bioavailability at the target site, it is crucial to study the behaviour of the inclusion complex in a simulated environment that closely mimics the organism. This will help to understand the complex's behaviour better and optimise its release and performance in vivo.

In our study, we aimed to evaluate the effectiveness of the TVEO-βCD complex as a drug delivery system in inhibiting the growth of *S.* typhimurium under intestinal conditions to develop an alternative to the usage of antibiotics for broiler chickens. The kinetics study revealed that the complex had a significant lethal effect on the growth of *S.* typhimurium. Within the first 15 min of exposure, the strain experienced

a 50% inhibition, and there was a reduction from 4.34×10^9 to 1.30×10^7 CFU/ml during the first hour. Throughout the experiment, the growth rate of the treated strain remained consistently lower than that of the untreated strain. Furthermore, the complex was found to have an irreversible effect and exerted a persistent inhibition.

Additionally, the complex improved the bioavailability and absorption of Thymol, the active compound in TVEO, and controlled its release. Over the 120-min experiment, each increase in bacterial growth was followed by inhibition due to the release of Thymol fractions that inhibited bacterial growth. This controlled release prevented high concentrations of Thymol, which could induce toxicity while extending the antibacterial effect. By combining the antibacterial potential of TVEO, which is safe, widely available, and cultivated globally, with the solubilisation and controlled-release properties of βCD, we have developed a complex that could be used as an alternative to conventional antibiotics in broiler production. This approach aims to promote antibiotic-free livestock production, safeguard the economy, and protect humans from life-threatening zoonotic infections without exposing them to antibiotic residues in broiler meat.

Conclusion

In conclusion, our study successfully demonstrated the novel application of the complex TVEO-β-CD in combating *Salmonella* typhimurium under conditions simulating the intestinal environment. While previous research has investigated the complexation of TVEO with β-CD, the novelty of our work lies in the utilisation of this complex specifically against *Salmonella* in conditions mimicking the intestinal tract. Our findings reveal that this complex effectively reduces *Salmonella's* growth, maintaining an irreversible and prolonged inhibitory effect, unlike free TVEO. This indicates the enhanced efficacy of β-CD in solubilising the essential oil and prolonging its antibacterial activity. Notably, the enhanced solubility of TVEO resulting from its complexation with β-CD has not been previously reported in combating *Salmonella* under intestinal conditions. Therefore, our study contributes valuable insights into the potential use of the TVEO-βCD complex as a promising antimicrobial agent for combating *Salmonella* infections in the gastrointestinal tract.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43538-024-00300-2.

Acknowledgements The Algerian Ministry of Higher Education and Scientific Research (MERS), the General Directorate of Scientific Research and Technological Development (DGRSDT), and the University of Bejaia supported the research. We want to thank Mr. Abdenour BOUMECHHOUR and Mr. Mustapha ABOU MUSLIM from the Research Center of Physical and Chemical Analysis (CRAPC), Tipaza, for their assistance with the GC-MS and GC-FID analysis.

Author contributions FB was responsible for data generation, analysis, interpretation, and manuscript writing. ZB participated in the antibacterial activity analysis. FC was involved in the extraction and characterisation of the essential oil. HB supervised the research work. Finally, AT reviewed and corrected the article.

Funding This paper was not funded.

Declarations

Competing interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Abarca, R.L., Rodríguez, F.J., Guarda, A., Galotto, M.J., Bruna, J.E.: Characterisation of beta-cyclodextrin inclusion complexes containing an essential oil component. Food Chem. **196**, 968–975 (2016). https://doi.org/10.1016/j.foodchem.2015.10.023

Abd El-Hack, M.E., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Soliman, M.M., Youssef, G.B., Taha, A.E., Soliman, S.M., Ahmed, A.E., El-Kott, A.F.: Alternatives to antibiotics for organic poultry production : types, modes of action and impacts on bird's health and production. Poult. Sci. **101**, 101696 (2022)

Abdelli, W., Bahri, F., Romane, A., Höferl, M., Wanner, J., Schmidt, E., Jirovetz, L.: Chemical composition and anti-inflammatory activity of algerian thymus vulgaris essential oil. Nat. Prod. Commun. **12**(4), 611–614 (2017)

Adams, R.P.: Adams. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured, Carol Stream (2017)

Agyare, C., Boamah, V.E., Zumbi, C.N., Osei, F.B.: Antibiotic use in poultry production and its effects on bacterial resistance. In: Antimicrobial Resistance—A Global Threat. IntechOpen (2018). https://doi.org/10.5772/intechopen.79371.

Ahmadzadeh, A., Nobakht, A., Mehmannavaz, Y.: Supplementary prebiotics, probiotics, and thyme (thymus vulgaris) essential oil for broilers : performance, intestinal morphology, and fecal nutrient composition. Probiotics Antimicrob. Proteins (2022). https://doi.org/10.1007/s12602-022-09927-3

Ahmed, J., Mulla, M.Z., Al-Attar, H., AlZuwayed, S., Ejaz, M., Al-Jassar, S., Jacob, H., Thomas, L., Al-Ruwaih, N., Joseph, A.: Thermal, structural, antimicrobial, and physicochemical characterisation of thyme essential oil encapsulated in β- and γ-cyclodextrin. J. Microencapsul. **39**(4), 364–379 (2022). https://doi.org/10.1080/02652048.2022.2090626

Alibi, S., Selma, W.B., Ramos-Vivas, J., Smach, M.A., Touati, R., Boukadida, J., Navas, J., Mansour, H.B.: Antioxidant, antibacterial, anti-biofilm, and anti-quorum sensing activities of four essential oils against multidrug-resistant bacterial clinical isolates. Curr. Res. Transl. Med. **68**(2), 59–66 (2020)

Al-Nasiri, G., Cran, M.J., Smallridge, A.J., Bigger, S.W.: Optimisation of β-cyclodextrin inclusion complexes with natural antimicrobial agents: thymol, carvacrol and linalool. J. Microencapsul. **35**(1), 26–35 (2018)

Alsakhawy, S.A., Baghdadi, H.H., El-Shenawy, M.A., Sabra, S.A., El-Hosseiny, L.S.: Encapsulation of thymus vulgaris essential oil in caseinate/gelatin nanocomposite hydrogel: in vitro antibacterial activity and in vivo wound healing potential. Int. J. Pharm. **628**, 122280 (2022). https://doi.org/10.1016/j.ijpharm.2022.122280

Amouei, H., Ferronato, G., Qotbi, A.A.A., Bouyeh, M., Dunne, P.G., Prandini, A., Seidavi, A.: Effect of essential oil of thyme (*Thymus vulgaris* L.) or increasing levels of a commercial prebiotic (TechnoMOS®) on growth performance and carcass characteristics of male broilers. Anim.: Open Access J. MDPI **11**(11), 3330 (2021). https://doi.org/10.3390/ani11113330

Ashraf, S.A., Al-Shammari, E., Hussain, T., Tajuddin, S., Panda, B.P.: In-vitro antimicrobial activity and identification of bioactive components using GC–MS of commercially available essential oils in Saudi Arabia. J. Food Sci. Technol. **54**, 3948–3958 (2017)

Benameur, Q., Gervasi, T., Pellizzeri, V., Pl'uchtová, M., Tali-Maama, H., Assaous, F., Guettou, B., Rahal, K., Grul'ová, D., Dugo, G.: Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with cefotaxime against bla ESBL producing multidrug resistant Enterobacteriaceae isolates. Nat. Prod. Res. **33**(18), 2647–2654 (2019)

Capelezzo, A.P., Mohr, L.C., Dalcanton, F., de Mello, J.M.M., Fiori, M.A.: β-Cyclodextrins as encapsulating agents of essential oils. In: Cyclodextrin: A Versatile Ingredient, pp. 169–200 (2018)

Chbel, A., Elmakssoudi, A., Rey-Méndez, M., Barja, J.L., Filali, O.A., Soukri, A., Khalfi, B.E.: Comparative study of essential oil composition, antibacterial and antioxidant activities of the aerial parts of *Thymus vulgaris* grown in Morocco and France. J. Essent. Oil Bear. Plants, pp. 1–13 (2022)

Chokshi, A., Sifri, Z., Cennimo, D., Horng, H.: Global contributors to antibiotic resistance. J. Glob. Infect. Dis. **11**(1), 36 (2019)

Cid-Samamed, A., Rakmai, J., Mejuto, J.C., Simal-Gandara, J., Astray, G.: Cyclodextrins inclusion complex: preparation methods, analytical techniques and food industry applications. Food Chem. **384**, 132467 (2022). https://doi.org/10.1016/j.foodchem.2022.132467

Dar, M.A., Ahmad, S.M., Bhat, S.A., Ahmed, R., Urwat, U., Mumtaz, P.T., Bhat, S.A., Dar, T.A., Shah, R.A., Ganai, N.A.: *Salmonella typhimurium* in poultry: a review. Worlds Poult. Sci. J. **73**(2), 345–354 (2017). https://doi.org/10.1017/S0043933917000204

Dou, S., Ouyang, Q., You, K., Qian, J., Tao, N.: An inclusion complex of Thymol into β-cyclodextrin and its antifungal activity against Geotrichum citri-aurantii. Postharvest Biol. Technol. **138**, 31–36 (2018)

Fadil, M., Fikri-Benbrahim, K., Rachiq, S., Ihssane, B., Lebrazi, S., Chraibi, M., Haloui, T., Farah, A.: Combined treatment of *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Myrtus communis* L. essential oils against *Salmonella typhimurium*: optimisation of antibacterial activity by mixture design methodology. Eur. J. Pharm. Biopharm. **126**, 211–220 (2018). https://doi.org/10.1016/j.ejpb.2017.06.002

Fani, M., Kohanteb, J.: In vitro antimicrobial activity of *Thymus vulgaris* essential oil against major oral pathogens. J. Evid.-Based Complement. Altern. Med. **22**(4), 660–666 (2017)

Giordani, R., Hadef, Y., Kaloustian, J.: Compositions and antifungal activities of essential oils of some Algerian aromatic plants. Fitoterapia **79**(3), 199–203 (2008)

Hajimehdipoor, H., Shekarchi, M., Khanavi, M., Adib, N., Amri, M.: A validated high performance liquid chromatography method for the analysis of Thymol and carvacrol in *Thymus vulgaris* L. volatile oil. Pharmacogn. Mag. **6**(23), 154 (2010)

Hedman, H.D., Vasco, K.A., Zhang, L.: A review of antimicrobial resistance in poultry farming within low-resource settings. Animals **10**(8), 1264 (2020)

Higuchi, T., Connors, K.A.: Advances in analytical chemistry and instrumentation. Phase Solubility Stud., 117–212 (1965)

Hill, L.E., Gomes, C., Taylor, T.M.: Characterisation of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT Food Sci. Technol. **51**(1), 86–93 (2013). https://doi.org/10.1016/j.lwt.2012.11.011

Hosseini, S.A., Meimandipour, A.: Feeding broilers with thyme essential oil loaded in chitosan nanoparticles: an efficient strategy for successful delivery. Br. Poult. Sci. **59**(6), 669–678 (2018). https://doi.org/10.1080/00071668.2018.1521511

Ismaili, R., Houbairi, S., Sanâa, L., Khadija, M., Abdeslam, L.: Etude de l'activité antioxydante des huiles essentielles de plantes aromatiques et médicinales marocaines. Eur. Sci. J. **13**(12), 323 (2017)

Khan, R.U., Naz, S., Nikousefat, Z., Tufarelli, V., Laudadio, V.: *Thymus vulgaris*: alternative to antibiotics in poultry feed. Worlds Poult. Sci. J. **68**(3), 401–408 (2012)

Kulkarni, M.A., Berrang-Ford, L., Buck, P.A., Drebot, M.A., Lindsay, L.R., Ogden, N.H.: Major emerging vector-borne zoonotic diseases of public health importance in Canada. Emerg. Microbes Infect. **4**(1), 1–7 (2015). https://doi.org/10.1038/emi.2015.33

Lemos, M.F., Lemos, M.F., Pacheco, H.P., Guimarães, A.C., Fronza, M., Endringer, D.C., Scherer, R.: Seasonal variation affects the composition and antibacterial and antioxidant activities of *Thymus vulgaris*. Ind. Crops Prod. **95**, 543–548 (2017)

Lin, L., Zhu, Y., Thangaraj, B., Abdel-Samie, M.A.S., Cui, H.: Improving the stability of thyme essential oil solid liposome by using β-cyclodextrin as a cryoprotectant. Carbohydr. Polym. **188**, 243–251 (2018). https://doi.org/10.1016/j.carbpol.2018.02.010

Listorti, V., Battistini, R., Ercolini, C., Tramuta, C., Razzuoli, E., Vencia, W., Decastelli, L., Gallina, S., Masotti, C., Serracca, L.: In vitro susceptibility of multidrug resistant strains of Salmonella to essential oils. Nat. Prod. Commun. **15**(1), 1934578X19878904 (2020)

Nadia, Z., Rachid, M.: Antioxidant and antibacterial activities of *Thymus vulgaris* L. Med. Aromat. Plant Res. J. **1**(1), 5–11 (2013)

Nezhadali, A., Nabavi, M., Rajabian, M., Akbarpour, M., Pourali, P., Amini, F.: Chemical variation of leaf essential oil at different stages of plant growth and in vitro antibacterial activity of *Thymus vulgaris* Lamiaceae, from Iran. Beni-Suef Univ. J. Basic Appl. Sci. **3**(2), 87–92 (2014)

Nieddu, M., Rassu, G., Boatto, G., Bosi, P., Trevisi, P., Giunchedi, P., Carta, A., Gavini, E.: Improvement of thymol properties by complexation with cyclodextrins: in vitro and in vivo studies. Carbohyd. Polym. **102**, 393–399 (2014)

Parsaei, P., Bahmani, M., Karimi, M., Naghdi, N., Asadi-Samani, M., Rafieian-Kopaei, M.: A review of analgesic medicinal plants in Iran. Der Pharm. Lett. **8**(2), 43–51 (2016)

Rahman, M., Sobur, M., Islam, M., Ievy, S., Hossain, M., El Zowalaty, M.E., Rahman, A.M.M., Ashour, H.M.: Zoonotic diseases: etiology, impact, and control. Microorganisms **8**(9), 1405 (2020)

Rašković, A.L., Kvrgić, M.P., Tomas, A.D., Stilinović, N.P., Čabarkapa, V.S., Stojšić-Milosavljević, A.Đ., Kusturica, M.N.P., Rakić, D.B.: Antinociceptive activity of Thyme (*Thymus vulgaris* L.) and interactions with neurotropics and analgesics. Braz. J. Pharm. Sci. (2021). https://doi.org/10.1590/s2175-97902020000318819

Rosato, A., Sblano, S., Salvagno, L., Carocci, A., Clodoveo, M.L., Corbo, F., Fracchiolla, G.: Anti-biofilm inhibitory synergistic effects of combinations of essential oils and antibiotics. Antibiotics **9**(10), 637 (2020)

Sienkiewicz, M., \Lysakowska, M., Denys, P., Kowalczyk, E.: The antimicrobial activity of thyme essential oil against multidrug resistant clinical bacterial strains. Microb. Drug Resist. **18**(2), 137–148 (2012)

Tao, F., Hill, L.E., Peng, Y., Gomes, C.L.: Synthesis and characterisation of β-cyclodextrin inclusion complexes of thymol and thyme oil for antimicrobial delivery applications. LWT Food Sci. Technol. **59**(1), 247–255 (2014). https://doi.org/10.1016/j.lwt.2014.05.037

Tiwari, G., Tiwari, R., Rai, A.K.: Cyclodextrins in delivery systems: applications. J. Pharm. Bioallied Sci. **2**(2), 72 (2010). https://doi.org/10.4103/0975-7406.67003

Tuță-Sas, I.: *Thymus vulgaris* extract formulated as cyclodextrin complexes: synthesis, characterisation, antioxidant activity and in vitro cytotoxicity assessment. Farmacia **67**(3), 442–451 (2019). https://doi.org/10.31925/farmacia.2019.3.10

Wen, Z., Liu, B., Zheng, Z., You, X., Pu, Y., Li, Q.: Preparation of liposomes entrapping essential oil from *Atractylodes macrocephala* Koidz by modified RESS technique. Chem. Eng. Res. Des. **88**(8), 1102–1107 (2010)

Yamaguchi, T., Okihashi, M., Harada, K., Konishi, Y., Uchida, K., Do, M.H.N., Bui, H.D.T., Nguyen, T.D., Nguyen, P.D., Chau, V.V.: Antibiotic residue monitoring results for pork, chicken, and beef samples in Vietnam in 2012–2013. J. Agric. Food Chem. **63**(21), 5141–5145 (2015)

Ziech, R.E., Lampugnani, C., Perin, A.P., Sereno, M.J., Sfaciotte, R.A.P., Viana, C., Soares, V.M., Pinto, J.P.A.N., Bersot, L.S.: Multidrug resistance and ESBL-producing *Salmonella* spp. Isolated from broiler processing plants. Braz. J. Microbiol. **47**, 191–195 (2016). https://doi.org/10.1016/j.bjm.2015.11.021