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Comparison of Antioxidant and Antibacterial Activities of Free and Encapsulated Garlic Oil with Beta-cyclodextrin

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Abstract

Background and Objectives: Application of garlic oil in food industry can be improved by encapsulation. There is no study about the formation of inclusion complex of garlic oil by beta-cyclodextrin. The aim of the present study is comparison of the antioxidant and antibacterial activities of free and encapsulated garlic oil with beta-cyclodextrin.

Materials and Methods: Antioxidant activity was determined by 1, 1- diphenyl-2-picryl-hydrazyl assay, and antibacterial properties by agar well diffusion, minimum inhibitory concentration, minimum bactericidal concentration and bacterial growth assay. Statistical analysis was performed by Minitab statistical software.

Results and conclusion: Garlic oil had poor antioxidant activity (EC_{50}, 5222 µg ml^{-1}) and EC_{50} because garlic oil/beta-cyclodextrin (containing 1495 µg ml^{-1} released garlic oil) was achieved after 5 h and 25 min. Agar well diffusion showed no inhibition zone on Muller Hinton Agar for garlic oil and garlic oil/beta-cyclodextrin (with initial release (shaking at 150 rpm for 24 h at 37ºC) and without initial release). *Staphylococcus aureus* was the most susceptible bacterium to garlic oil, and garlic oil/beta-cyclodextrin with and without initial release (minimum inhibitory concentration 10^{-5}, 10^{-4} and 10^{-3} % w v^{-1}, respectively); however, *Bacillus cereus* was the most resistant. The effect of initial release for garlic oil/beta-cyclodextrin on inhibiting the growth of all four bacteria was significant. There was no significant difference ($P>0.05$) between the inhibitory effect of garlic oil and garlic oil/beta-cyclodextrin with initial release on *Staphylococcus aureus* and *Bacillus cereus,* also *Salmonella entrica* and *Escherichia coli.* Garlic oil showed a weak antioxidant activity in 1, 1- diphenyl-2-picryl-hydrazyl assay. Garlic oil and its complex were not able to penetrate to the solid media; therefore, no inhibition zone and no antibacterial activity in the agar well diffusion assay were observed. Initial release of garlic oil/beta-cyclodextrin had significant impact on the inhibition of four bacterial growth, similar to free garlic oil. Since encapsulation of garlic oil can cover its drawbacks (low solubility in water, liquid form, and intense odor), garlic oil/beta-cyclodextrin could be considered as an nonsynthetic antibacterial agent.

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1. Introduction

Side effects of synthetic food additives and increasing resistance to using them have caused considerable consumer interest for natural ones, which can be found in plants, animals, bacteria, algae and fungi. Phenolic compounds or other hydrophobic components of essential oils can play a main role for their effectiveness as antioxidant and antimicrobial agents [1].

The cultivation and use of garlic as a condiment and medicine have a long history. Iran is the 16th country in the world in the ranking of garlic production, with 90913 tons production in 2013, and

about 50% production increase in the recent decade [2].

As soon as fresh garlic is crashed and injured, alliin converts to allicin by alliinase, which will immediately be inactivated at body temperature and pH below 3.5, the usual pH of gastric juice [3-5]. Garlic oil (GO) contains compounds which are produced from thiosulfates during the steam treatment; they are not found in intact plants due to changes during oil extraction and processing. Its therapeutic (anticancer, antioxidant, anti-inflammatory, hypoglycemic) properties and anti-microbial activity against a large number of gram-positive and gram-negative bacteria are known. Allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan have antimicrobial activity, by inactivation of microorganisms metabolic enzymes. As the antimicrobial activity of these allyl sulfur compounds increases with each additional S atom, GO's antimicrobial activity is more powerful than each of them individually [6,7].

GO is generally recognized as safe natural product by Food and Drug Administration, but some limitations such as volatility, low solubility in water, liquid form, intense odor of GO have restricted its application in food industry. These mentioned limitations can be improved by encapsulation. Hydrophobic active components of GO can be protected by hydrophobic cavity of beta-cycldextrin (beta-CD). Also hydrophilic outer surface of beta-CD possesses a hydrophilic property to GO and increases its aqueous solubility [7].

Cyclodextrins (CDs) are biodegradable and used as a food ingredient for protection of active ingredients against oxidation, light-induced reactions, heat-promoted decomposition, volatility, and sublimation. The other application of CDs can be elimination (or reduction) of undesired tastes/odors [8]. Among the three major types of CDs, beta-CD is the most widely used because of its relative suitable cavity volume, reasonable price, and being on the GRAS' list since 1998 [9,10].

Kumar and Berwal, by studying on the inhibitory activity of garlic (*Allium sativum*) against *Staphylococcus* (*S.*) *aureus, Salmonella* (*S.*) *typhi, Escherichia* (*E.*) *coli* and *Listeria* (*L.*) *monocytogenes,* showed that it has antibacterial potential [11]. The application of garlic as a preservative in tomato paste by the minimum inhibitory concentration (MIC) of fresh garlic and chloroformic extract of garlic against *Bacillus coagulance* (responsible for flat sour) indicated no significant effects on the chemical and physical characteristics of tomato paste [12]. Molana and Shahandeh evaluated the effect of raw, frozen and cooked garlic cloves and their extracts on *Pseudomonas* (*P.*) *aeruginosa* by disk-diffusion method. Although cooked cloves had the least effect on bacteria, bactericidal and bacteriostatic effects of garlic were shown [13]. Garlic choloroformic extract and allicin's effectiveness on *B. melitensis* and *B. abortus* were previously reported [14]. Study on the bacteriostatic effect of GO on *L. monocytogenes* in soft cheese and its antioxidant and antimicrobial activities in chicken sausages demonstrated effectiveness of garlic as a preservative [15,16]. Alipour Yeganeh et al. studied the inhibitory effect of garlic powder extract (GP) and garlic tablet (GT) extract on the growth of *S. typhimurium* and *Shigella* (*S.*) *dysenteriae* by determination of MIC and MBC. GP was much more effective than GT on *S. typhimurium* and Shigella [17]. Ayala-Zavala and Aguilar reported that free and encapsulated GO inhibited the growth of aerobic mesophilic microorganisms (bacteria, yeast and molds) in fresh-cut tomatoes [7]. Study of Ghanbari et al. on the antimicrobial effect of GO by uncommon antibiotics in veterinary medicine indicated that the inhibition zone diameter was less than antibiotics [18]. The antibacterial effects of garlic extract were proved in ready-to-cook chicken by MIC for *S. aureus* and *E. coli* [19]. Mozaffari-nejad et al. studied garlic aqueous extract's antibacterial effect on *S. aureus* in hamburger, and showed that it can be used not only as a flavor but also as a natural antibacterial agent [20]. The effectiveness of fresh garlic juice for preservation of fish was shown by reducing *Bacillus* (*B.*) *cereus, S. aureus, Enterococcus faecalis, E. coli and Proteus mirabilis* [21]. Li et al. studied the antimicrobial activities of fresh garlic extract on *S. aureus, P. aeruginosa,* and *C. albicans* and showed inhibition properties of it [22]. Fratianni et al., working on ultrapure water and acetone/ethanol/ ultrapure water extract of Italian garlic, confirmed their antioxidant and antimicrobial activity against *S. aureus, E. coli, P. aeruginosa* and *B. cereus* [23]. The antimicrobial, antioxidant activities and sensory properties of pressurized liquid extraction, fresh garlic, garlic powder, commercial GO in frankfurters during storage were studied by Horita et al. The results showed that pressurized liquid extraction had the highest allicin extract, and fresh garlic had potential antioxidant and antimicrobial effects during the shelf life [24].

To our knowledge, there is no research on comparison of the antioxidant and antibacterial properties of free and encapsulated GO by beta-CD; therefore, the objective of the present study was to evaluate the antioxidant and antibacterial activities of GO inclusion complex with beta-CD by determination of free radical scavenging capacity, MIC, MBC and antibiotic sensitivity.

2. Materials and Methods

2.1. Materials

GO containing diallyl disulphide (41.33%), diallyl trisulphide (28.82%), methyl allyl sulphide (7.83%), allyl sulphide (6.65%), diallyl tetrasulphide (3.93%), and 3-vinyl-1, 2-dithio-cyclohex-5-ene (3.73%), stored at 4ºC, was kindly offered by Magnolia Flavor & Fragrance Company (Kaveh Industrial City, Iran). Microorganisms, including

two gram-positive strains; *B. cereus* (PTCC 1015), *S. aureus* (PTCC 1431), and two gram-negative strains; *E. coli* (PTCC 1330), *S. entrica* (PTCC 1709) were obtained from Iranian Research Organization for Science and Technology (IROST). Antibiotic discs were purchased from Padtan Teb Company (Tehran, Iran), beta-CD and culture media were obtained from Sigma-Aldrich (Missouri, United States) and Merck (New Jersey, United States), respectively. All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Preparation of inclusion complex of GO with beta-CD

Inclusion complex of GO and beta-CD was prepared under optimized conditions (temperature; 35°C, GO/beta-CD; 8:100 and beta-CD/solvent (ethanol:distilled water, 1:2; 5.5:100), and the co-precipitation method and ultrasonic cleaner (optimized by response surface method, data not shown).

2.3. Antioxidant activity

DPPH (1, 1- diphenyl-2-picryl-hydrazyl) radical scavenging assay is based on electron donation of antioxidants for neutralizing DPPH radical. Bleaching of purple colored ethanolic solution of DPPH acts as an indicator of the antioxidant efficacy. GO (500 mg) was dissolved into absolute ethanol (50 ml), and then serial dilutions were prepared from this stock solution. The solution of GO/beta-CD was prepared at concentration of 50000 µg ml^{-1} in ethanol, by adding 1000 mg GO/beta-CD into 20 ml of absolute ethanol. The mixture was put in a shaking water-bath incubator reciprocating motion (150 rpm) for 24 h at 37ºC, and centrifuged (35329×g, 5 min, at 37ºC). Ascorbic acid solutions (positive control) were also prepared in distilled water at concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg ml^{-1}.

The DPPH assay was an adaptation of El-Ghorab et al.'s method [25]. Two ml of sample solutions was mixed with 2 ml of 4 mM DPPH in absolute ethanol. The mixture was shaken vigorously, maintained in dark, and monitored every 30 min (for ascorbic acid and GO) and 60 min (for GO/beta-CD) by a UV-Vis Spectrophotometer (Agilent Cary 60, CA, USA) using cell with 1 cm path length, at 517 nm. All samples were tested in triplicate. The scavenging activity or inhibition of DPPH activity was calculated by Eq.1:

$$\text{Scavenging activity } (\%)=(A_{control}-A_{sample}/A_{control})\times 100 \quad \text{Eq.1}$$

Finally, the results were reported as EC_{50} (effective concentration of the antioxidant necessary to decrease the initial DPPH concentration by 50%), the effective concentration of the antioxidant necessary to decrease the initial DPPH concen-tration by 50%, or scavenge 50% of the free radicals present. The antioxidant activity index (AAI) is used to standardize DPPH results, which is calculated as follows [26]:

$$AAI = \text{DPPH concentration in reaction mixture } (\mu g\ ml^{-1}) / EC_{50}\ (\mu g\ ml^{-1}) \quad \text{Eq. 2}$$

Based on AAI, samples can be assorted as having poor (AAI<0.5), moderate (0.5<AAI<1.0), strong (1.0<AAI<2.0), and very strong antioxidant activity (AAI>2.0).

2.4. Antibacterial activity

Antibacterial activities of GO and GO/beta-CD (with and without initial release) were studied by application of well diffusion (Kirby–Bauer antibiotic testing), MIC and MBC (Macro and Micro-dilution) methods on four above mentioned strains. GO and GO/beta-CD inhibition zones were compared with standard antibiotic discs [27].

Preparation of pure culture media: Pure cultures were prepared by inoculating freeze-dried micro-organisms into broth media, and incubated at 37ºC for 18-24 hours before microbial tests.

Standardization of inoculums: Standard pure cultures were prepared by suspending a loopful of each strain in 10 ml peptone water. After 18 h at 37°C, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard containing 1×10^6 CFU ml^{-1}. GO, GO/beta-CD-GO and GO/beta-CD (1% w v^{-1}) were prepared using DMSO (Dimethyl sulfoxide) and tween 80 (500 µg ml^{-1}), for with and without initial release, respectively. For initial release, GO/beta-CD suspension was incubated in 37°C for 24 h at 200 rpm in shaker incubator (J1SL50, Jal Tajhiz, Iran).

2. 5. Antibacterial tests

Agar well diffusion assay Kirby Bauer method was used. A loopful suspension of each pure bacterium was cultured in trypticase soy agar. After incubation at 37°C for 24 h, a pure colony of each bacterium was spread in Muller Hinton Agar (MHA) plates uniformly by means of a sterile swab (two plates for GO and GO/beta-CD). Three wells (5 mm in diameter) were punched in MHA, with sterilized pasteur pipette at a suitable distance. Approximately 100µl of GO or GO/beta-CD (1, 10, and 100% w v^{-1}) was dropped into each well, stabilized for 3 h, incubated at 37°C for 24 h. The diameter of inhibition zones was measured in mm, and the results were recorded [28].

2.6. Antibiotic sensitivity test

Inhibition zones of GO and GO/beta-CD were compared with standard antibiotics, including µg per disc: amikacin 30, nitro-furantoin 300, ciproflaxacin 5, sulfamethoxazol 3, gentamycin 10, cephalexin 30, and nalidixic acid 30. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile MHA plates. Then the antibiotic discs were asep-tically placed on the plates separately, and

incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured in mm [27].

Determination of MIC and MBC**:** Serial dilutions of 10^{-3} to 10^{-11} for GO or GO/beta-CD in 9 ml sterilized MHB were prepared. Then 1 ml of each standard pure culture (containing 1×10^{-6} CFU ml^{-1} active bacteria) was inoculated into all test tubes. Positive and negative controls were also prepared, with no GO or GO/beta-CD and bacteria, respectively. All tubes were incubated at 37°C for 24 h. The test tube with the lowest concentration of the GO or GO/beta-CD with no detectable growth (no turbidity) was considered as the MIC. The MIC dilutions were sub-cultured on MHA, and incubated at 37°C for 24 h. The lowest concentration with no growth on the MHA plate (99% bactericidal) was recorded as the MBC [29].

Bacterial growth curves**:** 300 µl from each of the mentioned macro dilution tubes was poured to the well of microplate (10×10-well Honeycomb with lid) put in Automated Microbiological Growth Analyser (Bioscreen C, FP- 1100 C, Finland) at 37°C for 24 h. Every 30 min, and before measurement of absorbance at 600 nm, the microplate was shaken for 10s (2 times). Four bacteria, media, DMSO, and Tween 80 were analyzed as controls. All samples were used in triplicate. The results were shown by two ways: a) the area under the curve, calculated by absorbance at 600 nm [30, 31], and b) growth inhibition, based on Casey et al.'s formula [32]:

The area under the curve (%) =A×100/ B Eq. 3

A=area under the curve of samples (strains containing GO or GO/beta-CD)

B= area under the curve of positive controls (selected strains)

Growth inhibition (%) = [(O-E)/O]×100 Eq. 4

O=absorbance of positive control (time of 24 h- time of 0 h, at 570 nm)

E =absorbance of samples (time of 24 h- time of 0 h, at 570 nm)

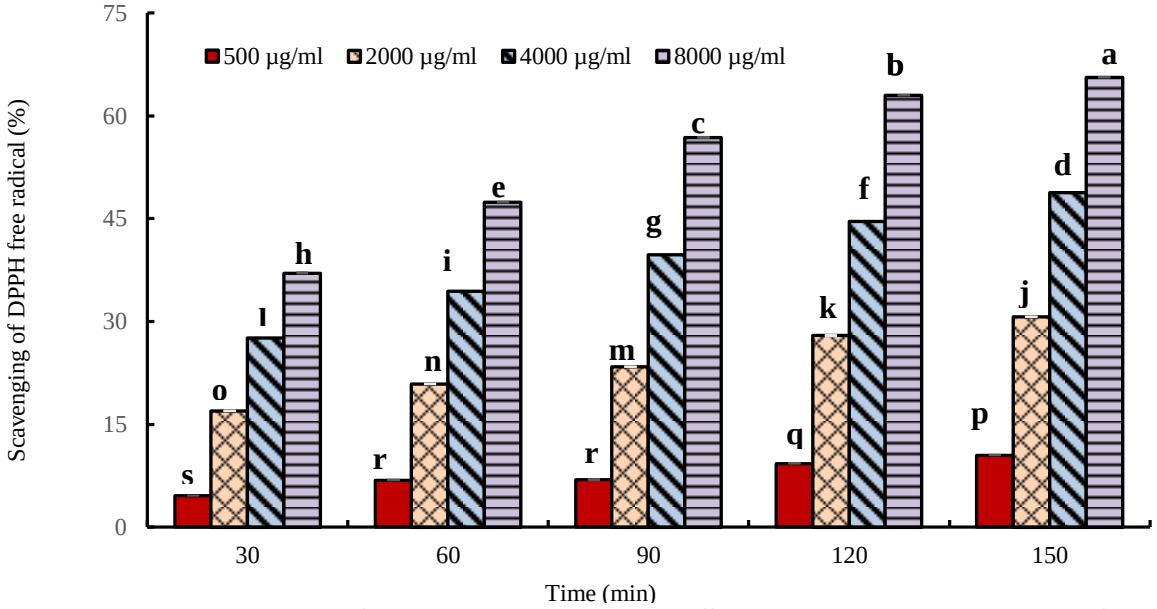


Figure 1. DPPH scavenging activity of garlic oil. Values denoted by different letters on each column are significantly different (P≤0.05).

2.7. Statistical analysis

The data were showed as the mean ± standard deviation of triplicate determinations and analyzed by an analysis of variance, ANOVA (P≤0.05), General Linear Model and Tukey's test. Statistical analysis was performed using Minitab (version 16) statistical software (Minitab Inc., State College, Pennsylvania, USA).

3. Results and Discussion

3.1. Measurement of antioxidant activity

Since odd electron of the nitrogen atom in the DPPH radical is reduced by receiving a hydrogen atom or electron from an antioxidant compound, the antioxidant activities of GO essential oil, GO/beta-CD, and ascorbic acid (positive control) were determined by DPPH method [33]. Scavenging activity of the ethanolic solutions of GO is shown in Fig. 1. By increasing the GO concentration, the scavenging ability of DPPH free radicals was increased. There is no significant difference between 60 and 90 min of GO in 500 µg ml^{-1}.

EC_{50} for GO was 5222 µg ml^{-1} after 150 min. EC_{50} of ascorbic acid was 32.81 µg ml^{-1} after 30 min, and EC_{50} for GO/beta-CD after 24 h stirring in water bath (37ºC) was 29.91% (1495 µg ml^{-1} GO). 50% of scavenging for GO/beta-CD inclusion complex was achieved after 5 h and 25 min. Considering the equations of 500 and 416 g GO concentration, time required for EC_{50} was calculated as 16 h and 18 min and 5 h and 16 min, respectively y=0.0477X+3.362, R^2=0.9544 and y=0.1148X+ 13.662, R^2=0.9932, respectively).

Thus, 325 min for 1495 µg ml^{-1} could be comparable with 316 min for 2000 µg ml^{-1}. AAI of ascorbic acid and GO was calculated 49.66 and 0.3, respectively. Based on Scherer and Godoy's classification, GO has poor antioxidant activity, whereas ascorbic acid is a strong antioxidant.

There is a few works on GO antioxidant activity, but a lot of work on the solvent extraction of GO and its drying. Of course, seemingly, no work has been done on GO/beta-CD antioxidant activity. It is to be mentioned that various methodologies have been used for DPPH assay and variation in concentrations of DPPH, sample volume has effect on the results. For instance, EC_{50} of ascorbic acid was reported from 11.85 to 629 µg ml^{-1} [34]. In the present study, GO had poor antioxidant activity (EC_{50} 5222 µg ml^{-1}, and AAI=0.31). Lawrence and Lawrence studied the antioxidant activity of garlic essential oil grown in north Indian plains, and claimed it has very good antioxidant properties. They reported EC_{50} value of 500 µg ml^{-1} for GO, which was 10 times more than that found in our study [35]. Teixeiraa et al. showed that garlic essential oil revealed almost no antioxidant activity (AAI < 0.5). It may be due to GO's volatile content, which may vary according to different varieties including agronomic and genetic varieties of the plant source [36]. This finding is similar to the present study.

Study of the antioxidant activity of the mangiferin (MGN) inclusion complex with beta-CD by Ferreira et al. showed that at a low concentration of MGN, MGN/beta-CD is more reactive than free MGN. It was attributed to the effect of beta-CD on the radical scavenging ability of DPPH. They showed that the complexation of cyclodextrin with polyphenols increased their antioxidant activities [37]. But, based on the theory of Lucas-Abbellán et al., since methanolic medium of DPPH may prevent complex formation by hydrophobic cavity of CDs, this method cannot be used to measure the antioxidant activity of complexes with CDs [38]. Tsae et al. studied the antioxidant activity of the inclusion complex of paeonol (PAE) with beta-CD by DPPH assay. There were some differences with the present work, such as initial preparation of PAE/beta-CD (incubation for 90 min at room temperature), DPPH concentration, and sample volume. With comparing the antioxidant activity of PAE and PAE/beta-CD at the same concentration, they showed that the complex of PAE with beta-CD improved the ability to eliminate the DPPH radical, by interaction of the hydroxyl group of beta-CD with PAE to form the intermolecular hydrogen bond [39]. This was also confirmed by comparing the time required for EC_{50} of GO solution containing 500 and 2000 µg ml^{-1} and GO/beta-CD containing 1495 µg ml^{-1} GO (978, 316, and 325 min, respectively).

3.2. Measurement of antimicrobial activity

3.2.1. Antibacterial tests

In Agar well diffusion assay, the results showed that GO, and GO/beta-CD (with and without initial release) had no inhibition zone on MHA in the presence of all four bacteria, and at all concentrations (1, 10, and 100% w v^{-1}). Both positive and negative gram bacteria were resistant to them. Neither GO nor GO/beta-CD had the ability to penetrate into the solid media (MHA); therefore, no inhibition zone and no antibacterial activity in agar well diffusion assay were observed.

Using standard antibiotic discs, bacterial responses were very different according to the inhibition zones (Table 1).

Table 1. Resistance pattern of selected microorganisms to garlic oil or garlic oil/beta-cyclodextrin and antibiotics.

Antimicrobial agent	Disc code	*B. cereus*	*E. coli*	*S. entrica*	*S. aureus*
Amikacin	AN-30	R	I	S	R
Cefalexin	CN-30	R	R	I	S
Ciprofloxacin	CIP-5	S	S	S	I
Gentamicin	GM-10	S	R	I	S
Nalidixic acid	NA-30	I	I	S	R
Nitrofurantoin	F/M-300	S	S	S	S
Garlic oil*		R	R	R	R
Garlic oil/beta-cyclodextrin*		R	R	R	R

R=Resistant; I=Intermediate; S=Sensitive. *In all three concentrations

Nitrofurantoin was the most effective, and the test strains had the most resistant to cefalexin and amikacin. *S. entrica* was the most sensitive strain on these antibiotics, and *E. coli* relative resistance, compared with the two other bacteria. While GO and GO/beta-CD had no sensitivity against the test strains.Both positive (*S. aureus* and *B. cereus*) and negative gram (*E. coli* and *S. entrica*) bacteria were resistant to GO, and GO/beta-CD (with and without initial release) at all concentrations (1, 10, and 100% w v^{-1}), and no inhibition zone on MHA was observed. Although there is no work on the antibacterial activities of GO and its inclusion complex, a few studies were done on the antibacterial activity of garlic aqueous extracts by agar well diffusion assay. Khusro et al. and Sah et al. showed their effectiveness on *B. licheniformis, Kelebciella pneumonia, E. coli, and S. aureus.* Garlic aqueous extracts at lower temperatures (26ºC versus 100°C) caused more inhibition zone [40,41]. In vitro antibacterial activity of garlic extract on *S. aureus* and *E. coli* by agar well diffusion revealed

maximum inhibition zone for *B. subtilis*, and the inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity [42]. Ghanbari et al. reported that diallyl disulfide, which was infused into sterile filter paper discs, had bactericidal effects much less than common antibiotics in veterinary medicine [18]. Fresh garlic extract displayed inhibition properties against *C. albicans* (larger sized inhibition zones compared with fluconazole and itraconazole), and weak inhibition properties against *P. aeruginosa* [22]. Cooked garlic cloves had the least bacteriostatic effects on *P. aeruginosa*, and garlic chloroform extract caused inhibition zone equivalent to the disk of gentamicin [13].

3.2.2. Determination of MIC and MBC

The results of MIC and MBC for GO and GO/beta-CD (with and without release) are shown in Table 2. All the tested strains were susceptible to GO and GO/beta-CD, but, the most sensitive bacteria was *S. aureus* to GO and GO/beta-CD (with and without initial release) at 10^{-5}, 10^{-4}, and 10^{-3}% w v^{-1} concentrations, respectively. *B. cereus* was the most resistance bacterium as since the MIC of this bacterium was (10^{-3}% w v^{-1}).

Table 2. MIC and MBC of garlic oil and garlic oil/beta-CD with selected bacteria

Microorganisms	MIC (w v^{-1})			MBC (w v^{-1})		
	Garlic oil	Garlic oil/beta-CD With initial release	Without initial release	Garlic oil	Garlic oil/beta-CD With initial release	Without initial release
S. aureus	10^{-5}	10^{-5}	10^{-3}	10^{-4}	10^{-4}	-
B. cereus	10^{-3}	10^{-3}	-	10^{-4}	10^{-3}	-
E. coli	10^{-4}	10^{-4}	-	10^{-3}	10^{-3}	-
S. entrica	10^{-4}	10^{-4}	-	10^{-3}	10^{-3}	-

The MIC of *E. coli* and *S. entrica* was the same (10^{-4}% w v^{-1}). GO/beta-CD without initial release had no inhibitory effects on the three other bacteria. The results of MBC for GO and GO/beta-CD (with and without initial release methods) showed their best bactericidal effect on *S. aureus*. In this way, *B. cereus* was the most resistant strain. In conclusion, the effect of initial release for GO/beta-CD on the growth inhibition of all four microorganisms was considerable; better results for GO/beta-CD with initial release comparing with GO/beta-CD without initial release. The same results of MIC and MBC for GO and GO/beta-CD were observed. Considering the GO loading of GO/beta-CD (11%), it may be due to evaporation of free GO during incubation.

Antimicrobial properties of garlic may be due to its organosulphur components, which inhibits acetyl CO-A enzyme, and therefore, biosynthesis of lipids, fatty acids, and finally, disturbance of cell existence. Diallyl disulphide (DDS) is the most effective garlic constituent, and the content of DDS in our GO was 41.33%. Alipour Yeganeh et al. studied on the MIC and MBC of garlic powder extract and garlic tablet on *S. typhimurium* and *S. dysenteriae*, and showed that the garlic powder extract was more effective than garlic tablet, either on Salmonella. or Shigella [17]. Durairaj et al. reported that aqueous fresh garlic extracts had less MIC for gram-positive than gram-negative organisms (more effective on *B. subtilis* and *S. aureus*) [42]. Fratianni et al. observed differences in the antimicrobial activities of Italian garlic extracts due to the different method of extraction used (solvent, with or without exposure to light, room or refrigerator temperature). Different Italian garlic extracts were the best against *S. aureus*, *E. coli*, and *P. aeruginosa;* in comparison with *B. cereus* [23]. Our results agreed with Fratianni et al.'s study in which *S. aureus* sensitivity to GO was more than that of *B. cereus*. There is no research on determination of MIC or MBC for GO/beta-CD [23]. Only Ayala-Zavala and Gonzalez Aguilar studied the antimicrobial pro-perties of packed GO/beta-CD in tree/paper cellulose fiber tea-bags (forming sachets) on fresh-cut tomato [7]. Microbial growth in fresh-cut tomatoes was signify-cantly affected by the treatment with free and encapsulated GO, respectively. The highest concentration of GO used, both free and encapsulated, caused the highest inhibition of microbial growth, with no acceptability sensory quality for panelists. In fact, the lowest free GO concentration showed the highest acceptability, with no achievement of a significant reduction of microorganisms [7]. The various results for antibacterial properties of garlic extracts can be due to differences in extraction methodology (aqueous and solvent extraction versus steam distillation), and components (fresh garlic extract versus garlic essential oil or GO).

3.2.3. Bacterial growth curves

All four microorganisms had normal growth phases (log and stationary phases) during 24 hours of incubation at bioscreen C (Fig 2.A.1 and 2.A.2).

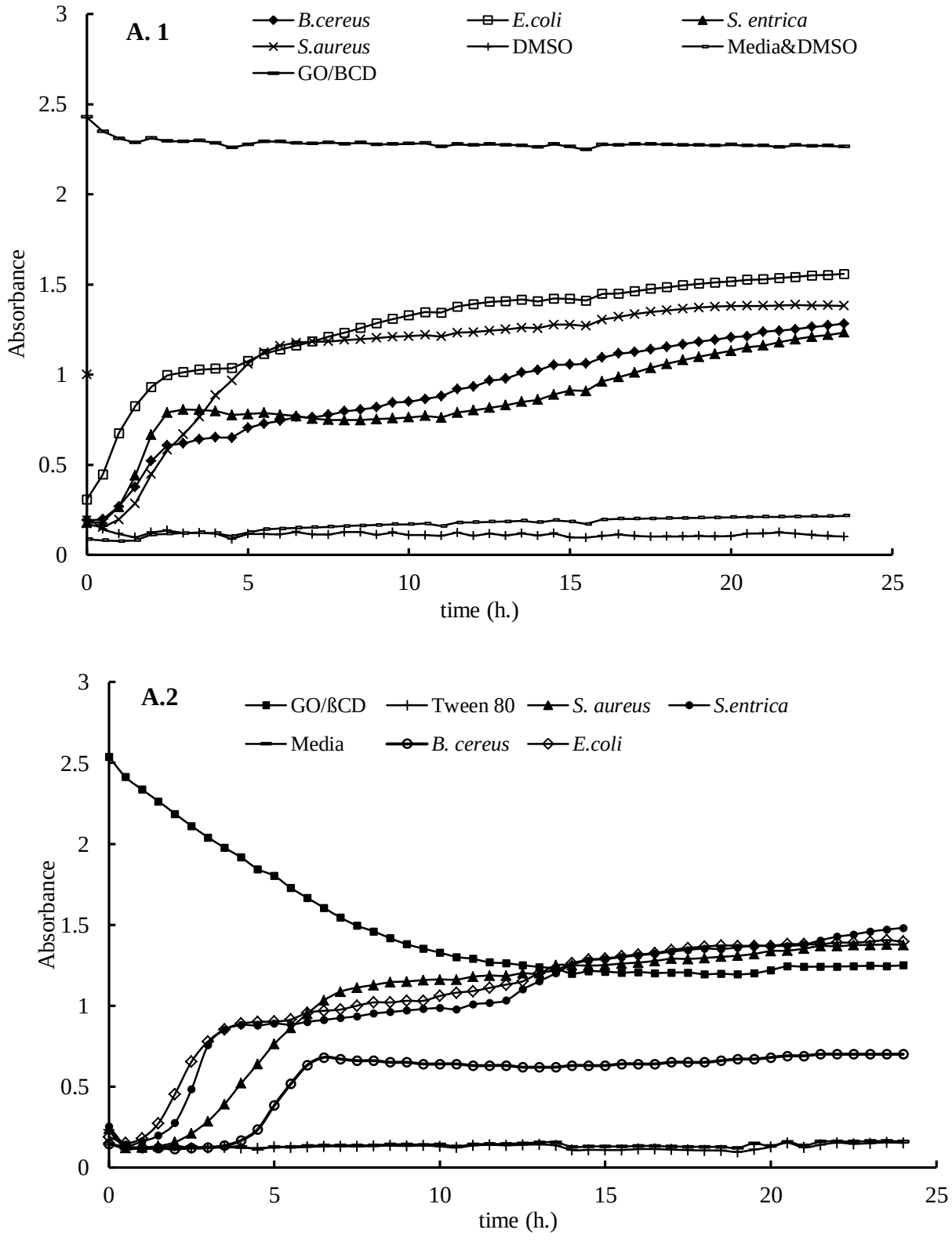
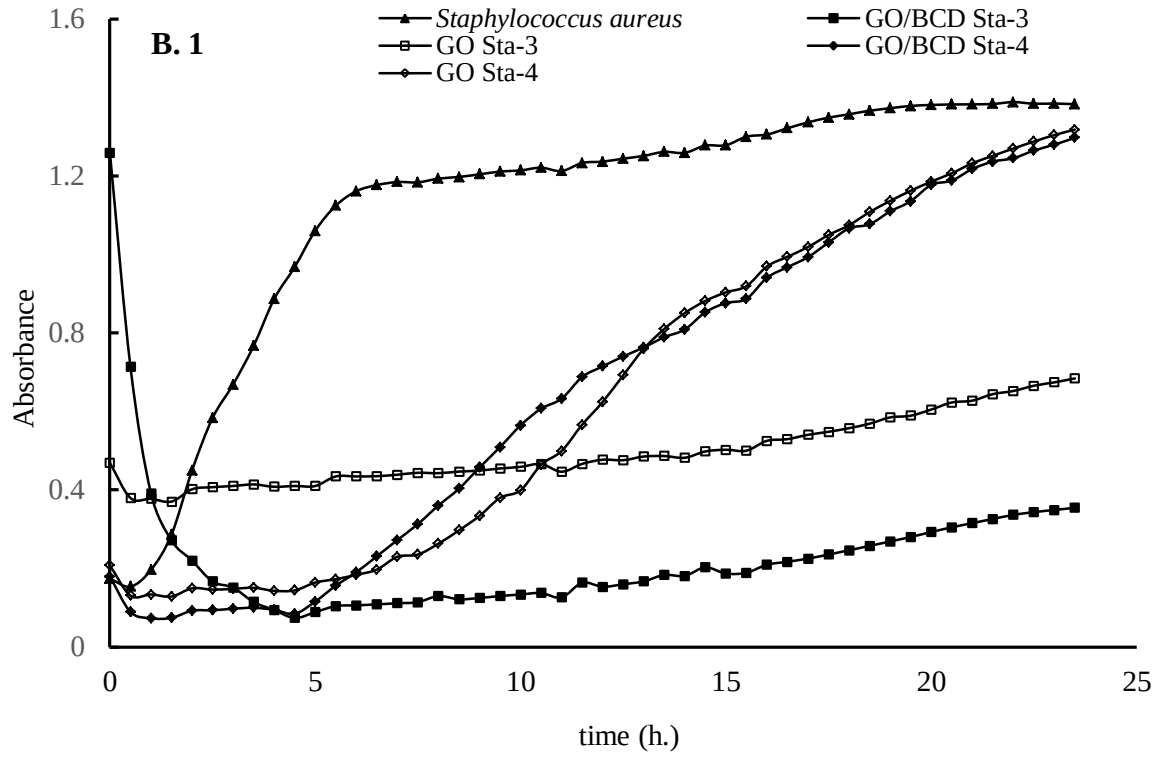
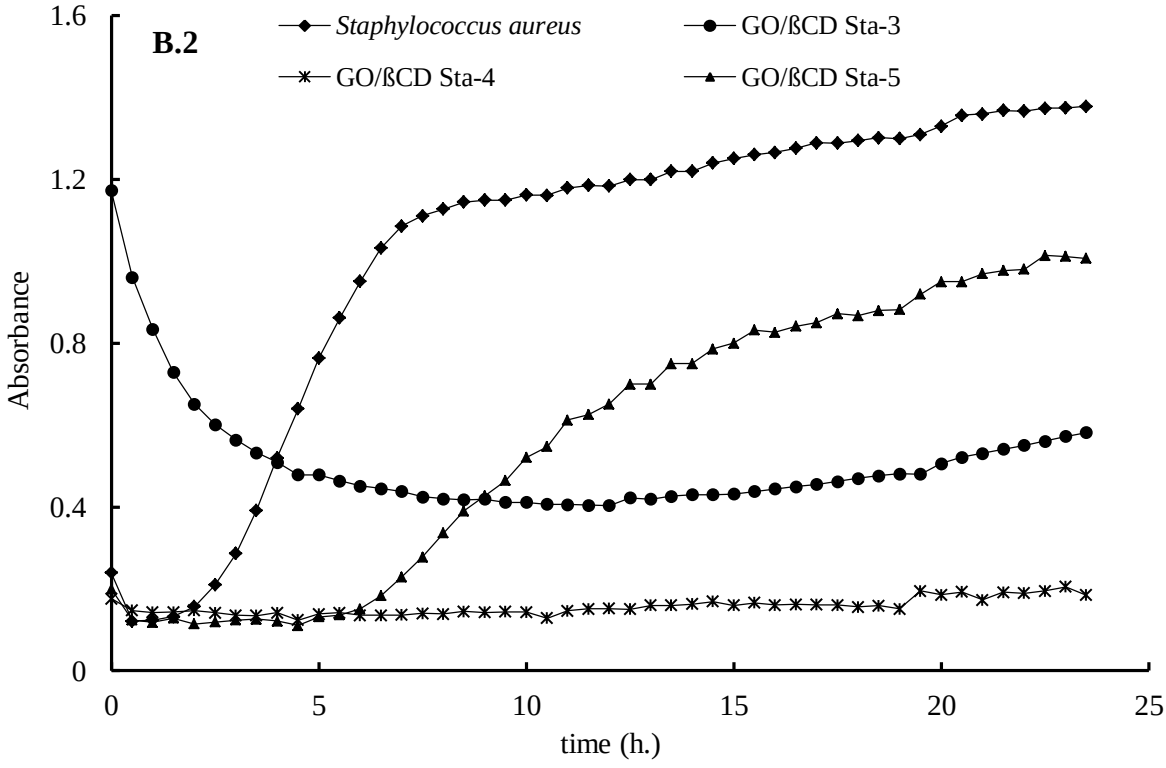


Figure 2. The growth of selected strains at the presence of garlic oil or oil/beta-cyclodextrin (with and without initial release): A.1, control; B.1 *S. aureus*; C.1 *B. cereus*; D.1 *E. coli*; E.1 *S. entrica* with initial release and A.2, control; B.2 *S. aureus*; C.2 *B. cereus*; D.2 *E. coli*; E.2 *S. entrica* without initial release.





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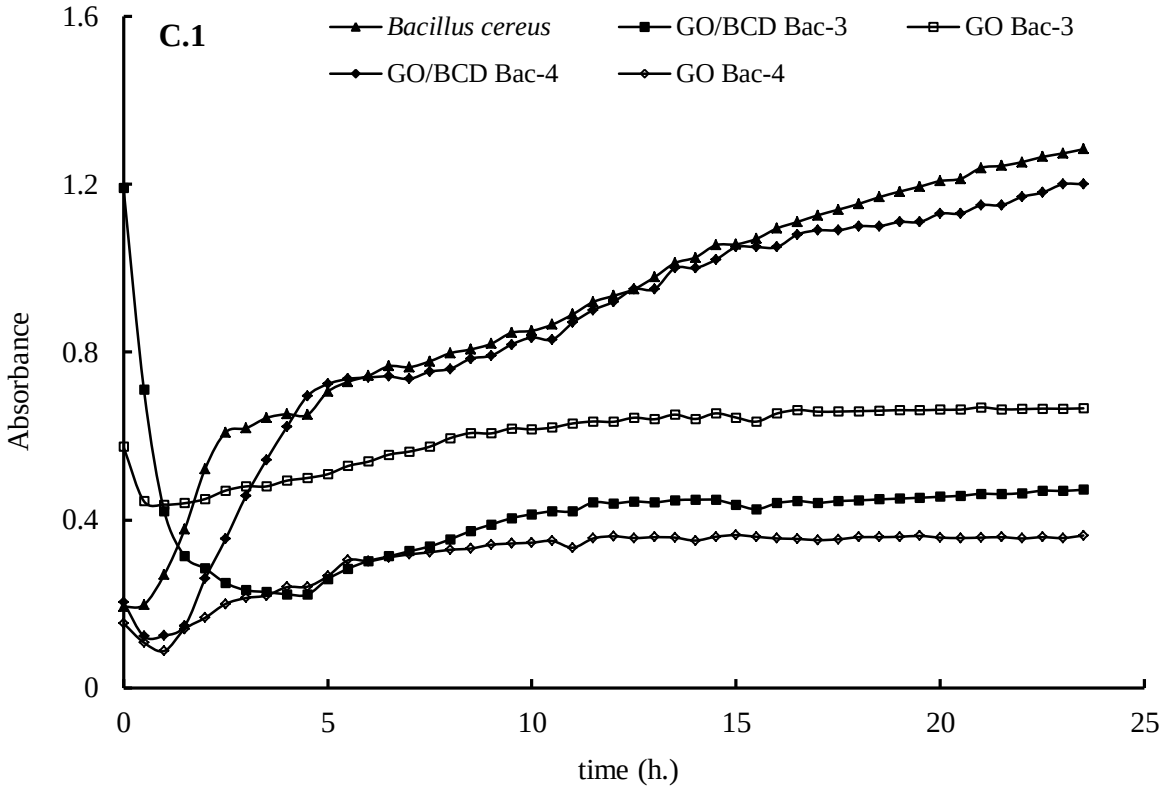


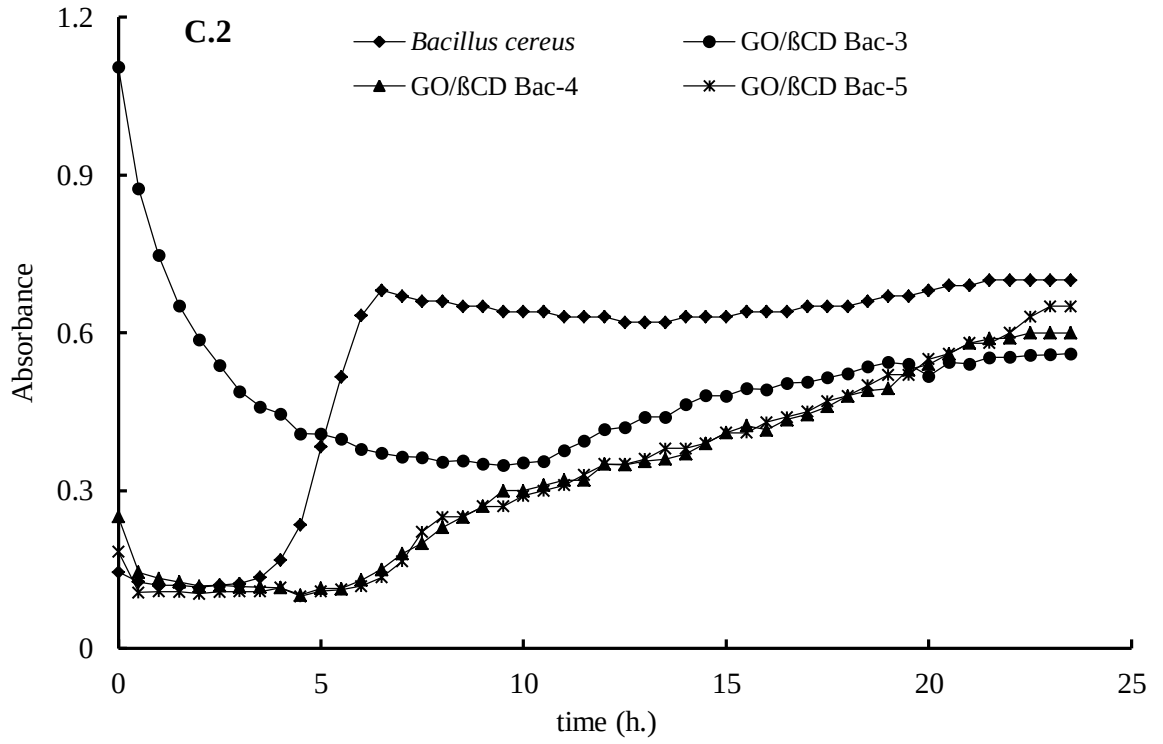
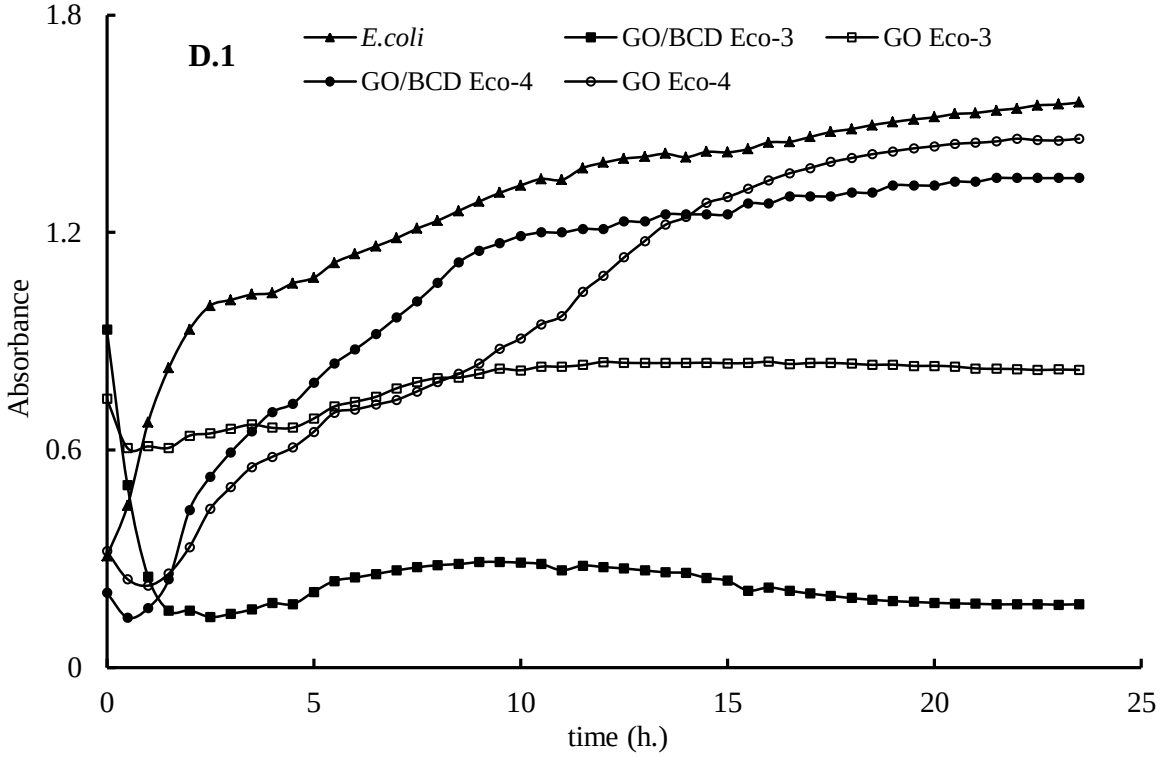
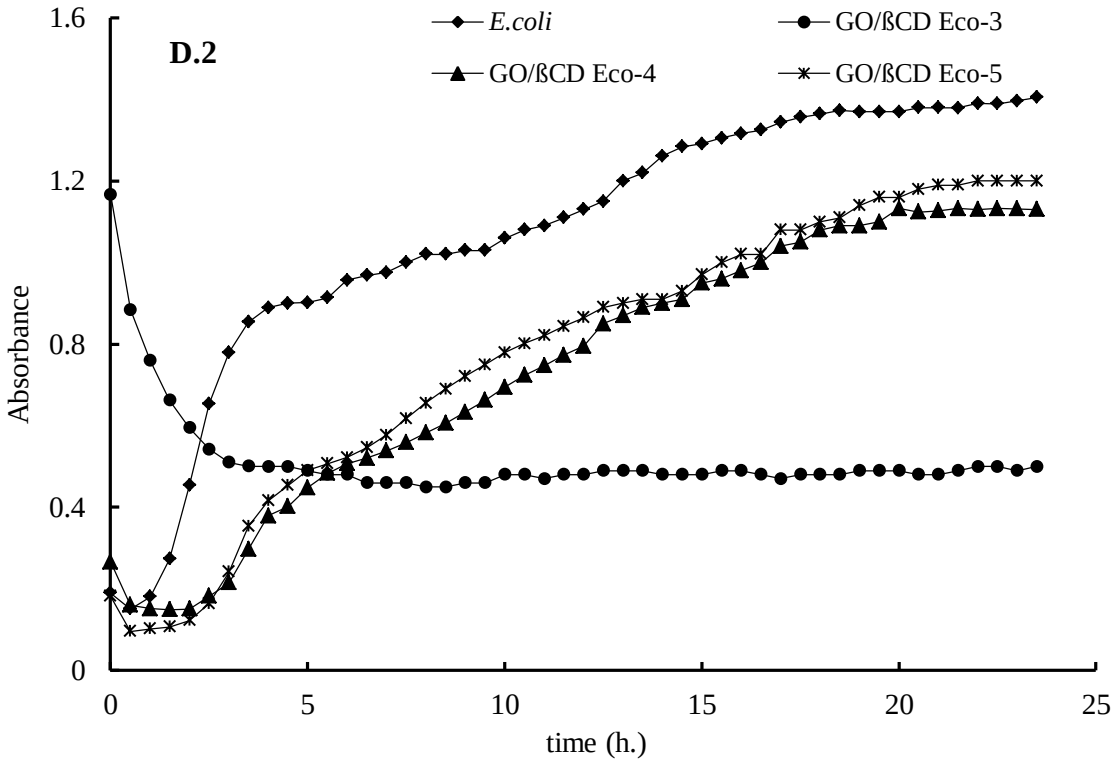


Figure 2.continued





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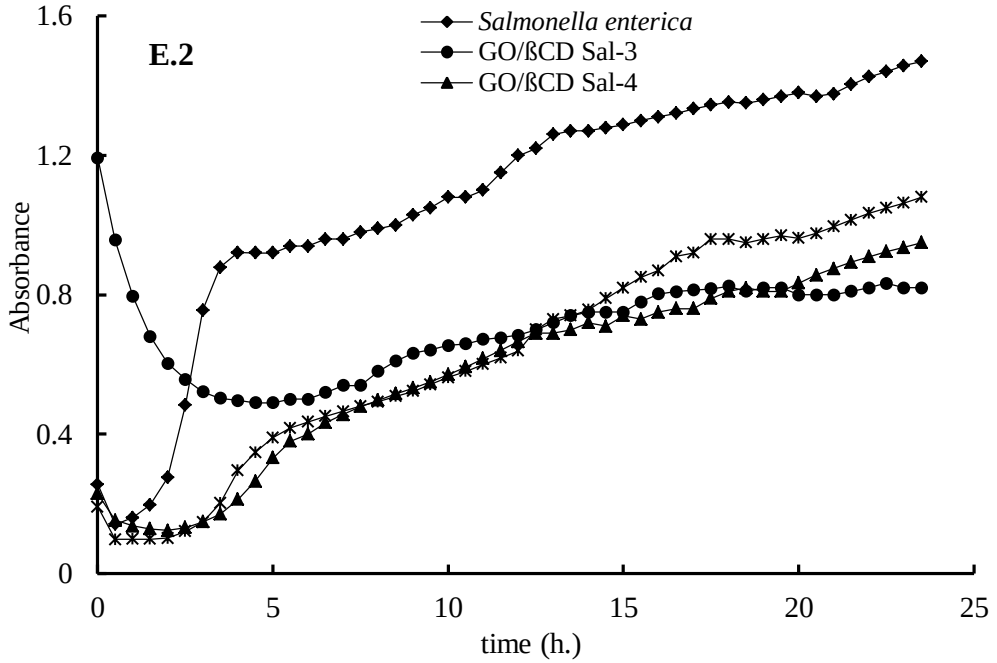

Figure 2. The growth of selected strains at the presence of garlic oil or oil/beta-cyclodextrin (with and without initial release): A.1, control; B.1 *S. aureus*; C.1 *B. cereus*; D.1 *E. coli*; E.1 *S. entrica* with initial release and A.2, control; B.2 *S. aureus*; C.2 *B. cereus*; D.2 *E. coli*; E.2 *S. entrica* without initial release.

The culture media and stabilizers (Tween 80 and DMSO) had low and constant absorbance, with no interfere on the samples' absorbance. As low concentrations, both GO and GO/beta-CD had no effects on the growth inhibition of microorganisms; only their first two and three concentrations are shown on Fig. 2. The quantitative results are shown in two ways, including the percent of area under the curve (absorbance 600× min), and growth inhibition (%).The growth curves of selected bacteria in the presence of GO and GO/beta-CD (with and without release) are also shown in Fig. 2. The effect of initial release of GO/beta-CD on the growth inhibition of all four bacteria was considerable. GO/beta-CD without initial release had milder slope of curves and longer period of MIC (the time 0 to minimum absorbance) comparing with GO/beta-CD with initial release. The period of MIC for *S. aureus*, *B. cereus*, *E. coli*, and *S. entrica* for GO/beta-CD without initial release was 12, 10, 9.5, and 6 hours, respectively. While this time for GO/beta-CD with initial release was 4.5, 4.5, 2.5, and 1.5, respectively. GO had the shortest period. Based on the period of MIC, the order was as following:
GO<GO/beta-CD with initial release<GO/beta-CD without initial release. After a rapid decrease during the first half an hour, the samples containing *S. aureus* and GO or GO/beta-CD with initial release (10^{-4}% w v^{-1}) had a constant absorbance, up to next 5 hours, and then an ascending absorbance may due to increasing growth of bacteria (Fig. 2.B.1). Finally, these samples had an absorbance the same as positive control after 24hours of incubation GO/beta-CD with initial release (10^{-4}% w v^{-1}) had no effect on the growth inhibition of *B. cereus*, the same growth curve as positive control (Fig. 2.C.1). After 5 h, GO at the concentration of 10^{-4}% w v^{-1} had a constant absorbance. GO/beta-CD at concentration of 10^{-3}% w v^{-1} had the most effective during the first two hours. Only GO/beta-CD with initial release at concentration of 10^{-3}% w v^{-1} was effective in the growth inhibition of *E. coli* (Fig. 2.D.1). The growth of *S. entrica* containing GO or GO/beta-CD with initial release after 5 hours was the same as positive control at concentration of 10^{-4}% w v^{-1}. Again, GO/beta-CD with initial release at concentration of 10^{-3}% w v^{-1} was the most effective in the growth inhibition of *E. coli* (Fig. 2.E.1).

The area under the curves: The effectiveness of GO and GO/beta-CD (with and without initial release) by calculating the area under the curve is shown in Fig. 3. The lower percent of the area under the curve assumed lower growth of the selected strain, and therefore, more effectiveness of treatments. GO/beta-CD with initial release at concentration of 10^{-3}% w v^{-1} had the best effect on the three selected strains, including *S. aureus*, *E. coli*, and *B. cereus*, which had also the lowest percent of area under the curve. The effectivenesses of GO and GO/beta-CD without initial release on *E. coli* were the same (no significant difference). The highest area under the curve (%) was observed for the sample containing *S. entrica* and GO/beta-CD without initial release (89.02±5.39 and 92.42±1.58 at concentrations of 10^{-3} and 10^{-4}% w v^{-1}, respectively). At concentration of 10^{-4}% w v^{-1}, the effect of GO and GO/beta-CD without initial release on *B. cereus* was the same. There was no significant difference between the samples containing GO and *E. coli* and *S. entrica*.

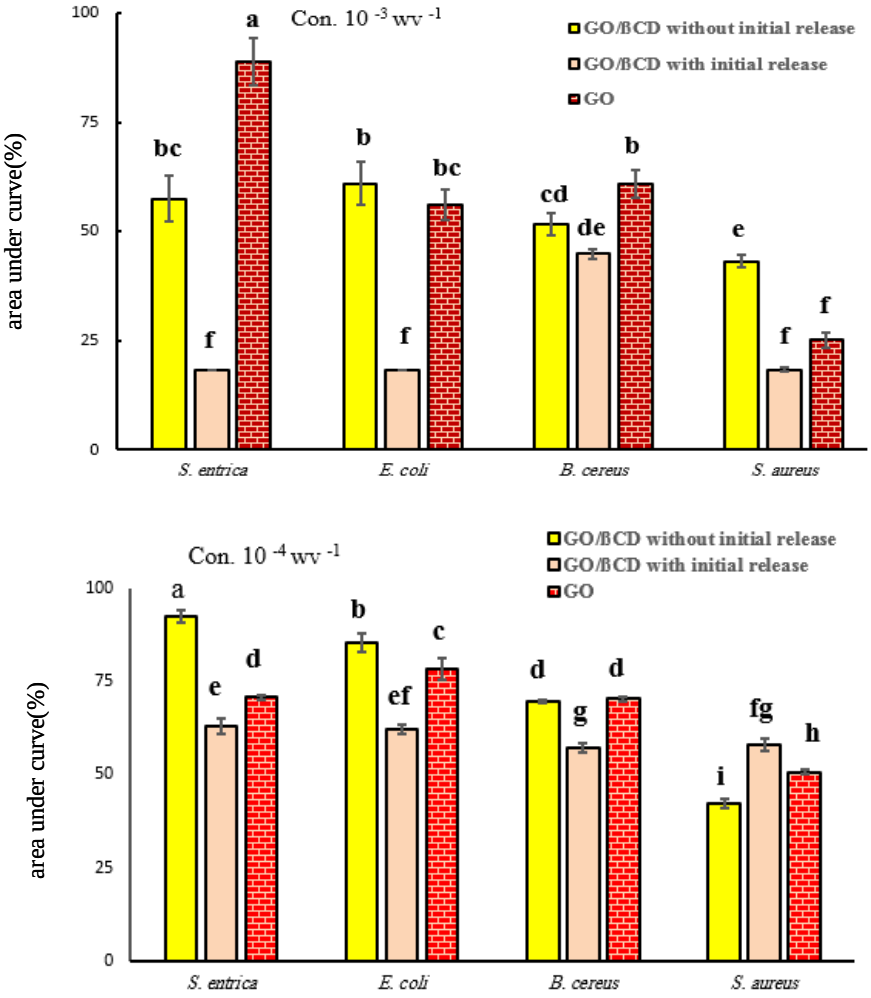

Figure 3. Area under the curve (%) of selected strains, the effectiveness of garlic oil or garlic oil/beta-cyclodextrin (with and without initial release). Values denoted by different letters on each column are significantly different (P≤ 0.05).

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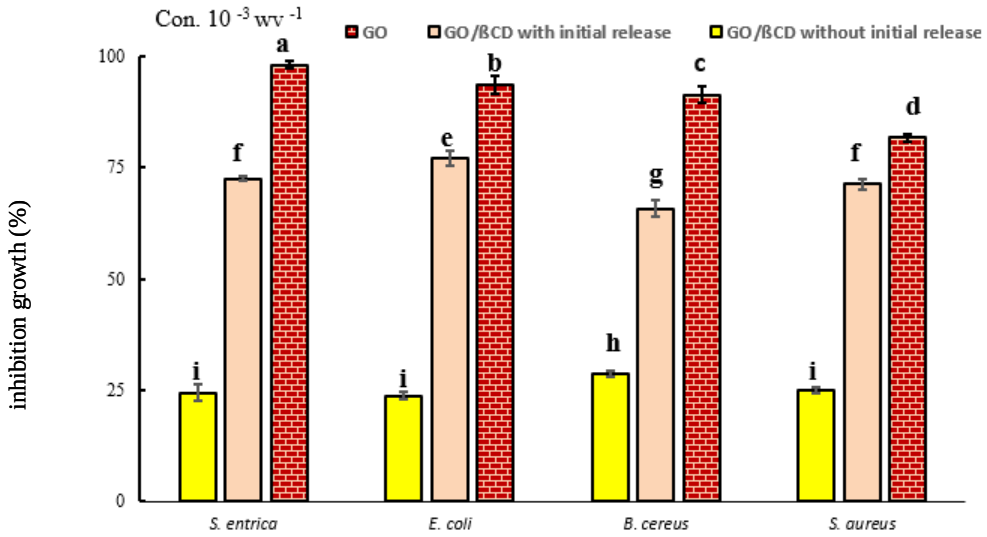

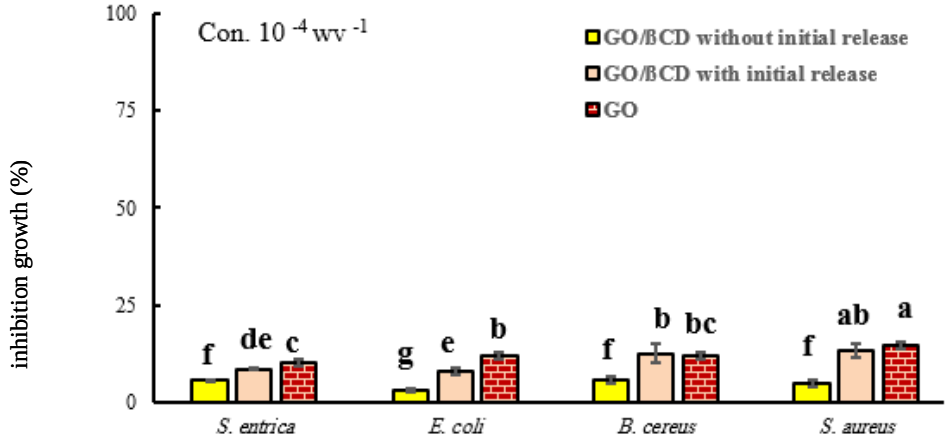

Figure 4. Growth inhibition (%) of selected strains, the effectiveness of garlic oil or garlic oil/beta-cyclodextrin (with and without initial release). Values denoted by different letters on each column are significantly different (P≤0.05).

The most effectiveness of GO, GO/beta-CD with and without initial release were observed for *S. aureus*, *B. cereus*, and again *S. aureus*, respectively. It seems that GO/beta-CD with initial release had the lowest percent of area under the curve and also the most effectiveness; GO and GO/beta-CD without initial release were in the second and third positions, respectively. As GO loading of GO/beta-CD was about 11%, and GO/ beta-CD had lower area under the curve than GO at the same concentration, it may not necessarily implicate the more effectiveness of GO/beta-CD. It may be due to precipitate of its white suspension, which was unavoidable.

3.2.3. Growth inhibition (%)

The effectiveness of GO and GO/beta-CD (with and without initial release) by calculating the growth inhibition (%) is shown in Fig. 4. At higher concentrations (10^{-3}% w v^{-1}), all treatments were also considerably more effective than the lower concentrations (10^{-4}% w v^{-1}). GO had the most and the least growth inhibition (%) on *S. entrica* (98.10%), and *S. aureus* (81.82%), respectively. At concentration of 10^{-3}% w v^{-1}, the least growth inhibition (%) belonged to the samples containing GO/beta-CD without initial release and *S. aureus*, *E. coli*, and *S. entrica*. GO/beta-CD with initial release had the same effect on *S. aureus* and *S. entrica* (with no significant difference). In all treatments, GO had the most growth inhibition, GO/beta-CD without initial release was the least effective, and GO/beta-CD with initial release was in the second position. It shows that the initial release of GO/beta-CD caused more inhibitory effect. At concentration of 10^{-4} %w v^{-1}, GO/beta-CD without initial release had the same growth inhibition effect on *S. aureus* and *B. cereus*, also *S. entrica* and *E. coli*. Effect of GO and GO/beta-CD with initial release on *B. cereus*, *S. entrica* and *S. aureus* showed no significant statistical difference (the same effectiveness).

GO had the most growth inhibition, and the shortest period of MIC; but based on the calculated area under the curves, GO/beta-CD with initial release caused the lowest area and the best effect, which can be because of the presence of white suspension, and its precipitation after a while, which is an unavoidable effect. Since, GO loading of GO/beta-CD (11%) should not be ignored, it may be concluded the near effect of GO and GO/beta-CD with initial release on growth curves of selected strains.

Ouwehand et al. studied the effects of 13 essential oils on potential pathogens, by calculating the area under their growth curves, and observed that Gram-positive strains were more sensitive. Among the Gram-negative pathogens, *E. coli* was the most sensitive to essential oils. Carvacrol, cinnamaldehyde and thymol had the best growth reducing properties [31]. Kumar and Berwal showed the sensitivity of *S. aureus*, *S. typhi*, *E. coli* and *L. monocytogenes* to fresh garlic. The best result belonged to *E. coli*, and inhibition occurred fast. *L. monocytogenes* was the most resistant pathogen to garlic [11]. The present study showed that the most effectiveness of GO was on *S. aureus* in all concentrations. The highest area under the curve (the least antibacterial effect) was observed for the sample containing *S. entrica* and GO/beta-CD without initial release. GO/beta-CD with initial release had the same effect on *S. aureus*, *E. coli* and *S. entrica*.

4. Conclusion

Our study on antioxidant activity and antibacterial properties of GO, GO/beta-CD with and without initial release showed the weak antioxidant activity of GO in DPPH assay, and effectiveness of GO and GO/beta-CD with initial release on the growth inhibition of four selected strains (*S. aureus*, *B. cereus*, *E. coli*, and *S. entrica*).

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6. Conflict of interest

The authors declare that they have no conflict of interest.

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