

ANTIMICROBIAL PROPERTIES OF NANO-EMULSION FORMULATED FROM GARLIC, GINGER AND CINNAMON EXTRACTS AGAINST *Escherichia coli* AND *Salmonella typhi*

Sifat Antimikroba Formula Nanoemulsi Ekstrak Bawang Putih, Jahe, dan Kayu Manis Terhadap Escherichia coli dan Salmonella typhi

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ABSTRACT

Garlic, ginger and cinnamon had been reported for their antimicrobial activities, for instance against *Escherichia coli* and *Salmonella typhi*. The present study aimed to test antimicrobial activities of nanoemulsion of the mixture of garlic, ginger and cinnamon. The nanoemulsion was formulated from the mixture of garlic and ginger extracts and cinnamon essential oil at the ratio of 80:10:10 using a high pressure homogenizer at 300 bars for 5 cycles. The nanoemulsion powder was prepared using a spray dryer with the inlet and outlet temperatures of 160–170 °C and 70–80 °C, respectively, and maltodextrin as a filler. The nanoemulsion was tested against *E. coli* and *S. typhi*. The particle size of nanoemulsion and powdered formulas were characterized using a scanning electron microscope. The LC_{50} values were identified based on the Brine Shrimp Lethality Test. The results showed that quality of the raw materials confirmed the WHO requirements. The particle size of the emulsion ranged from 151 to 306 nm with the polydispersity index of 0.39–0.52. The 10% and 15% active compounds of the nanoemulsion inhibited *E. coli* and *S. typhi* with the LC_{50} values of 680.15–970.50 ppm and 607.17–903.31 ppm, respectively. The study suggests that the nanoemulsion of a mixture of garlic, ginger and cinnamon extracts could be developed as a food preservative.

[**Keywords:** Antimicrobe, cinnamon, garlic, ginger, nanoemulsion]

ABSTRAK

Bawang putih, jahe, dan kayu manis telah dilaporkan mempunyai aktivitas antimikroba, misalnya untuk *Escherichia coli* dan *Salmonella typhi*. Penelitian bertujuan menguji aktivitas antimikroba nanoemulsi campuran bawang putih, jahe, dan kayu manis. Formulasi nanoemulsi diperoleh dari campuran ekstrak bawang putih, jahe dan minyak atsiri kayu manis dengan komposisi 80:10:10 menggunakan homogenizer tekanan tinggi dengan tekanan 300 bar sebanyak 5 siklus. Untuk stabilitas produk, nanoemulsi juga dibuat menjadi serbuk dengan pengering semprot pada suhu inlet 160–170 °C dan suhu outlet 70–80 °C, menggunakan maltodekstrin sebagai bahan pengisi. Nanoemulsi diuji kemampuan daya hambatnya terhadap mikroba *E. coli* dan *S. typhi*. Ukuran partikel nanoemulsi dan serbuk dikarakterisasi menggunakan scanning electron microscope dan toksisitas produk (LC_{50}) diuji dengan Brine Shrimp Lethality Test. Hasil penelitian menunjukkan bahwa kualitas bahan memenuhi persyaratan WHO.

Ukuran partikel nanoemulsi berkisar antara 151 dan 306 nm dengan nilai indeks polidispersitas 0,39–0,52. Nanoemulsi dengan senyawa aktif 10% dan 15% dapat menghambat *E. coli* dan *S. typhi* dengan toksisitas (LC_{50}) untuk konsentrasi senyawa aktif 10% berkisar antara 680,15 dan 970,50 ppm dan untuk konsentrasi 15% senyawa aktif antara 607,17 dan 903,31 ppm. Penelitian ini menunjukkan bahwa nanoemulsi campuran ekstrak bawang putih, jahe, dan kayu manis dapat dikembangkan lebih lanjut sebagai pengawet pangan.

[**Kata kunci:** Antimikroba, bawang putih, jahe, kayu manis, nanoemulsi]

INTRODUCTION

The uses of natural compounds as food preservatives have been practiced because some plants possess antimicrobial activity to protect themselves from pathogenic infection. The chemical compounds of certain plants, such as polyphenols had been reported to have biological effects as antioxidant, anticarcinogen, antiinflammatory and antimicrobial activities (Papadopoulou et al. 2005). They stated that some phenolic compounds such as resveratrol, hydroxytyrosol, oleuropein, quercetin and a number of phenolic acids were able to inhibit the growth of various pathogenic microorganisms.

Natural preservatives were defined as chemical constituents extracted from natural sources which offer intrinsic ability to protect products against microbial growth (Nwabanne et al. 2014). The chemical compounds derived from plant in the form of secondary metabolites, such as essential oil, flavonoids, alkaloid and phenolic compounds had been widely tested as preservatives in processed foods, pharmaceuticals, biological medicine and natural therapy (de Souza et al. 2005). Their capability of inhibition on microbial growth, oxidation and enzymatic reactions occurring in the foodstuff had been reported by Singh et al. (2010).

Food preservatives can be categorized into two types based on their source of origin, that are synthetic and natural. The preservative activity of the compound depends on its concentration and type of test organism. The effectiveness of antimicrobial effect can be proven if they could inhibit more than synthetic preservatives (Tajkarimi et al. 2010).

Garlic, ginger and cinnamon are common ingredients of cooking in Indonesia. Those spices also possess a wide range of phenolic contents. Garlic has a phenolic content of 7.5 mg gallic acid equivalents (GAE) g⁻¹, ginger has 1.02 mg GAE g⁻¹, and cinnamon has 13.66 mg GAE g⁻¹ (Cui et al. 2016). The chemical compounds derived from garlic, cinnamon and ginger had been tested as antimicrobes. Garlic contained alliin, allicin, *allinase* enzyme and diallyl disulfide (Gebreyohannes and Gebreyohannes 2013). Allicin and other sulfur compounds are the major compounds responsible for the antimicrobial effect of garlic. Nonetheless, the main compound suggested to be responsible for garlic effect is volatile allyl methyl sulfide (AMS) as a lead compound of volatile garlic metabolites (Becker et al. 2012). Ginger has an antimicrobial activity against *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis* and showed widest zone of inhibition against *S. typhi* (Islam et al. 2014). The gingerol and shogaol are identified as more active compounds. The phenolic compounds found in ginger which have antimicrobial activities are eugenol, shogaol, zingeron, gingerdiol and gingerol (Singh et al. 2008). The main components found in cinnamon essential oil are cinnamaldehyde or non-phenolic allyl hydroxycinnamate which can inhibit *Pseudomonas fluorescens* (Shan et al. 2007). It had been reported that cinnamon oil had a broad range of antimicrobial activities against gram-positive and gram-negative bacteria.

The antibacterial abilities of essential oils lead to their wide application in many food and beverage products to prolong the shelf life. Consumers' growing demands for natural products rather than synthetic ones make the essential oils highly desirable due to their natural properties. However, the uses of active compounds are very limited due to their poor water solubility. The oil-in-water (O/W) emulsions or nanoemulsions provide good solution to improve the solubility of active compound. It had been reported that the nanoemulsification significantly increased the antioxidant, antibacterial and antibiofilm activities of essential oil. Moreover, the nanoemulsified essential oil had higher efficiency in inhibiting the bacteria in tofu than did the pure essential oil, indicating a better application prospect of essential oil nanoemulsions (Lou et al. 2017). Therefore, nanoemulsification may provide an excellent way to improve the activity and efficiency of lipo-soluble active

agents, so their application can be expanded in various aqueous foods.

Information on the toxicity of natural products is very important to avoid the danger of those products as a preservative. The toxicity of plants may be generated from chemical compounds contained in those plants. The brine shrimp lethality test has been applied as an alternative bioassay technique to screen the toxicity of plant extract (Moshi et al. 2010). There was a good correlation between lethal concentration that kill 50% of the exposed population (LC₅₀) using *A. salina* and the result of acute oral toxicity assay in mice (Arslanyolu and Zerrin 2006).

The previous research had produced the composition of biopreservative using a mixture of garlic and ginger extract, and cinnamon oil at a ratio of 80:10:10 as an effective dose for chicken carcass preservation (Winarti et al. 2016). The growth of *S. typhi* and *E. coli* were inhibited 16.3 and 17.7 mm, respectively by applying the compositions.

The purpose of the present study was to test the antimicrobial activity of biopreservative base on garlic, cinnamon and ginger in a form of nanoemulsion.

MATERIALS AND METHODS

Characterization of the Materials

Garlic (*Allium sativum*), ginger (*Zingiber officinale* Roxb.) and cinnamon (*Cinnamomum burmanii*) each was characterized for its quality, including moisture content, ash content, ash insoluble in acid, as well as water and alcohol soluble extractives based on WHO monographs (1999). Garlic, ginger and cinnamon were purchased from the market. Only materials that met the WHO standard were used in the experiment.

Preparation of Garlic and Ginger Extracts and Cinnamon Oil

Garlic was peeled and crushed using a blender by adding water at a ratio of 1:3, then filtered and the filtrate was used as formulation material to be tested. Ginger was washed using clean water, sliced 6–7 mm in thickness, and sun dried for 3 days. The dried ginger was ground using a hammer mill to pass a 40 mesh screen. Ginger powder was then macerated in technical alcohol at a ratio of 1:6, left overnight, then filtered. The filtrate was evaporated using an evaporator equipment at reduced pressure until crude extract was obtained. The dried cinnamon bark was

ground to pass a 50 mesh screen, then distilled with steam and water to extract the essential oil.

Nanoemulsion Formulation

Nanoemulsion was prepared in two-step processes where a macroemulsion was first prepared and then converted into nanoemulsion in the second step. The nanoemulsion was made using a higher pressure homogenizer (HPH). The composition of garlic:ginger:cinnamon at 80:10:10 ratio as an active compound was added 0.5% tween and the remaining composition was water, as a solvent. The emulsion was homogenized by ultraturrax for 3 minutes at a speed of 11,000 rpm and continued with HPH at a pressure of 300 bars for 5 cycles. The nanoemulsion formula was made for three replicates.

Encapsulation process for the nanoemulsion was done using a spray dryer (Merck LabPlant, type SD.05), at an inlet temperature of 160–170 °C and an outlet temperature of 70–80 °C using maltodextrin as a filler to make the powdered product (Desai and Park 2005). The total concentration of the filler was 20% in solution before dried. Encapsulation products were then analyzed for particle size, morphology, toxicity (LC_{50}) and stability during 7 days of storage at room temperature.

Particle Size Analysis

Particle size and size distribution of nanoemulsion were measured by using a particle size analyzer (Malvern Zetasizer). The particle size was measured in a dynamic light scattering (DLS) instrument, involving the diameter of the sphere that diffuses at the same speed as the particle being measured. Five drops of the sample were dissolved in 20 ml aquadest and then put into a disposable cuvette of ± 1 ml. The refractive index value was set according to the samples.

Stability Test

Nanoparticle stability was tested at various pH, ranging from 2 to 8 during the 7 days storage at room temperature (28 °C). The pH was adjusted using phosphate and chloride buffers. Phosphate buffer was made from the mixture of 0.89 g Na_2HPO_4 and 0.69 g NaH_2PO_4 to make 100 ml solution. Chloride buffer was prepared by mixing 0.2 M KCl and 0.2 M HCl into 200 ml. Nanoparticles were stored in plastic bottles of 30 ml. Nanoparticle stability was tested by measuring the content of total phenols released into the storage. Total phenol content was performed using the Folin-Ciocalteu with the

preparation of the extraction of phenolic compounds using methanol, continued with sonication for 20 minutes (Tsai et al. 2011).

Antimicrobial Assay

The method used for testing antimicrobial properties was the agar well diffusion assay (ASWD) following Ammor et al. (2006) and Budde et al. (2003). The targeted bacteria were *E. coli* and *S. typhi*. These bacteria were cultured on Plate Count Agar (PCA)/Merck medium. Culture solution was poured into a petri dish, let until cool and frozen. A hole of 5 mm diameter was made on the media, and then the nanoemulsion was poured into the hole, incubated at 36 °C for 48 hours. The inhibition zone was determined by measuring the diameter of clear zone in mm. The control treatment used a commercial preservative, namely sodium propionate.

Identification of Chemical Compound

Chemical compounds of the extracts were identified using a gas chromatography mass spectrometry (GCMS). The type of the equipment was Shimadzu QP 2010, Column type DB-MSI with capillary column and a length of 60 m and a diameter of 0.25 mm. Temperature of the column was programmed at 50–230/5 °C/min. and injector temperature was 225 °C. The powdered formula was macerated with ethanol overnight, then filtered to separate waste and filtrate. The filtrate was evaporated by reducing the pressure until produced crude extract. The extract was used for analysis using GCMS.

Toxicity Test

Toxicity tests used the method of Brine Shrimp Lethality Test (BSLT) (Meyer et al. 1982). BSLT is the methods of investigating bioactive compounds present in natural materials using shrimp larva where the toxic properties are based on the number of larva mortality. An extract is said to be toxic if it has a LC_{50} value of less than 1000 $\mu\text{g ml}^{-1}$.

RESULTS AND DISCUSSION

Characteristics of Raw Materials

The quality of raw materials affected the quality of product and its effectiveness. The raw material quality for the study met all the criteria requirements of WHO (Table 1). The alcohol-soluble extractive of garlic and cinnamon was much lower compared to the water-

soluble extractive, but it was much higher than required. The alcohol and water soluble extractives were an indication of the presence of secondary metabolite substances in plants.

Nanoemulsion Formula

Nanoemulsion has better stability to particle aggregation and good separation due to their small droplet sizes (Ahmed et al. 2012). The nanoemulsion derived from the mixture of garlic, ginger and cinnamon at a composition of 80:10:10 showed two different colors and particle sizes when using different homogenizers, namely ultraturrax and high pressure homogenizer (Figure 1).

Nanoemulsion made by ultraturrax showed brown color, whereas for HPH had milky white color. Particle sizes of both nanoemulsions were different ranging from 151 to 306 nm with PDI values of 0.39–0.52 (Table 2). Nanoemulsion derived from HPH had a particle size smaller than that of ultraturrax. This meant that HPH reduced the droplet size from micro-

to nano-scale ranges. Commonly, the nanoemulsion has droplets covering the size range of 100–500 nm (Sharma et al. 2010). However, the droplet size shown in Table 2 revealed that the encapsulated formula had a larger particle size due to maltodextrin addition as a filler. The use of heat in the spray drying process made the particles to agglomerate. Gulseren et al. (2012) reported that agglomeration of nanoparticles could be caused by heat in the spray drying process.

Antimicrobial Assay

The activity of individual extract was tested for *E. coli* and *S. typhi*. The result showed that extracts of garlic, ginger and cinnamon oils had a good inhibition against *E. coli* and *S. typhi* (Table 3). The inhibition values were more than 7 mm meaning that they had a potential as an antibacteria following the criteria of Prabuseenivasan et al. (2006). Differences in inhibition values showed by the compounds tested indicated that the active compounds or the secondary metabolites of garlic, ginger and cinnamon were

Table 1. Quality of ginger, cinnamon and garlic used in the study.

| Characteristic | Ginger | WHO Standard | Cinnamon | WHO Standard | Garlic | WHO Standard |
|-------------------------------|------------|--------------|------------|--------------|------------|--------------|
| Moisture, % | 12.0 ± 0.2 | - | 11.3 ± 0.2 | - | 9.8 ± 0.2 | - |
| Ash content, % | 4.0 ± 0.2 | Max. 6 | 3.6 ± 0.2 | Max. 6 | 3.3 ± 1.2 | Max. 6 |
| Ash insoluble in acids, % | 2.4 ± 0.3 | Max. 2 | 1.9 ± 0.2 | Max. 2 | 0.3 ± 0.0 | Max. 1 |
| Water soluble extractive, % | 8.2 ± 0.3 | Min. 10 | 63.2 ± 0.2 | Min. 10 | 80.8 ± 0.1 | Min. 5 |
| Alcohol soluble extractive, % | 16.1 ± 0.3 | Min. 4,5 | 6.7 ± 0.1 | Min. 14 | 6.7 ± 0.7 | Min. 4 |



a



b

Fig. 1. Nanoemulsion formula prepared using ultraturrax (a) and higher pressure homogenizer (HPH) (b); b1 = HPH 10% active compound replicate 1; b2 = HPH 10% active compound replicate 2; b3 = HPH 15% active compound replicate 1; b4 = HPH 15% active compound replicate 2.

Table 2. Particle size of nanoemulsion containing 10% and 15% active compounds.

| Homogenization method | Z-average (nm) | PDI |
|---------------------------------------|----------------|-------------|
| Ultra turax | | |
| 10 % active compound | 306 ± 15 | 0.52 ± 0.05 |
| 15% active compound | 227 ± 17 | 0.44 ± 0.03 |
| Higher pressure homogenizer | | |
| 10% active compound | 153 ± 3 | 0.41 ± 0.06 |
| 15% active compound | 151 ± 4 | 0.39 ± 0.03 |
| Encapsulated with 15% active compound | 588 | 0.886 |

different. According to Mikaili et al. (2013), garlic was effective against a number of gram-negative and gram-positive bacteria, including *Staphylococcus*, *Salmonella*, *Vibrio*, *Mycobacteria* and *Proteus* species. Allicin and other sulfur compounds were the major compounds for the antimicrobial effect of garlic. Ginger also showed antimicrobial and other biological activities due to gingerol and paradol, shogaols and zingerone (Giriraju and Yunus 2013). Natural substances derived from plants have an important role in host-pathogen relationships, because of a variety of phytochemical contents as active biological properties other than as antimicrobial and antioxidant (Gupta et al. 2014). The use of natural antimicrobial compounds as preservatives protected food from spoilage by inhibiting the growth of pathogenic microorganisms and increasing shelf life.

Nanoemulsion products containing 15% active compounds inhibited *E. coli* higher compared to that of *S. typhi* (Table 4). The encapsulated product also inhibited less than did the nanoemulsion product. It meant that the nanoemulsion encapsulated product can be used as food preservative to extend the shelf life. When all formulas were compared to the sodium propionate, the natural preservatives were more effective.

Alliin and allicin are the main bioactive compounds present in the aqueous extract of garlic or raw garlic homogenate. When garlic is chopped or crushed, allinase enzyme is activated and produces allicin from alliin (Bayan et al. 2014). Other compounds such as cinnamaldehyde, trans-cinnamaldehyde and eugenol were found in cinnamon oil, and shogaol-6, shogaol-8, gingerol-10, and gingerol-6 were found in ginger.

The toxicity test based on LC_{50} showed that all nanoemulsion and encapsulated products had the LC_{50} of less than 1000 ppm, suggesting that the

Table 3. Effectiveness of garlic extract, cinnamon oil and ginger extract against *Escherichia coli* and *Salmonella typhi*.

| Material | Inhibition (mm) | |
|----------------|-----------------|-----------------|
| | <i>E. coli</i> | <i>S. typhi</i> |
| Garlic extract | 20 ± 2 | 27 ± 1 |
| Cinnamon oil | 32 ± 1 | 36 ± 2 |
| Ginger extract | 6 ± 1 | 40 ± 1 |

products were effective for food preservation (Table 5). Ceylan and Fung (2004) reported that an extract of plants can be used for food preservative if they have LC_{50} ranging from 500 to 1000 ppm or 0.05 to 0.1%. Furthermore, natural antimicrobial compounds isolated from plants could be used singly or combined with other preservatives (Davidson 2006).

Images from SEM showed the outward appearances of the encapsulated product particles, including the shape, structure and pattern. The particles containing 10% and 15% active compounds gave similar form of a spherical shape with a few shriveled particle surface which indicated shrinking (Figure 2). Shrinking and wrinkle could be caused by high temperature during the spray drying process, as reported by Taufiq et al. (2015). Klinkesorn et al. (2006) reported that wrinkle on the surface of the particles was the result of mechanical pressure or heat caused by high temperature during the spray drying process. Particle size also affected the ability of powder flow and separation. Overall, diameter of the powder ranged from 2 to 10 mm.

Nanoemulsion Stability

The stability of nanoemulsion product was measured at different pH (3, 5 and 7) during 7 days of storage at room temperature (28 °C) for the phenolic content. Sample at pH 3 tended to be fairly stable than that at pH 5 and 7 (Figure 3). The phenolic compounds of the nanoemulsion were originated from garlic, ginger and cinnamon. Stability meant that the particle should not remain stationary while in a dispersed state. Stability was important because it indirectly affected the size and shape stability. It was reported that phenolic compounds, such as caffeic, chlorogenic and gallic acids were not stable under high pH (Friedman and Jürgens 2000).

Table 4. Bacterial inhibition activities of the nanoemulsion and encapsulated products at 10% and 15% active compounds against *Escherichia coli* and *Salmonella typhi*.

| Type of product | Inhibition (mm) | |
|----------------------|-----------------|-----------------|
| | <i>E. coli</i> | <i>S. typhi</i> |
| Nano emulsion | | |
| 10% active compound | 35 ± 2 | 17 ± 1 |
| 15% active compound | 45 ± 1 | 17 ± 1 |
| Encapsulated product | 20 ± 1 | 16 ± 2 |
| Sodium propionate | 9 | 13 |

Table 5. Toxicity values (LC₅₀) of the compounds.

| Product | LC ₅₀ (ppm) |
|---|------------------------|
| Nanoemulsion from ultraturax | |
| 10% active compound | 680.15 |
| 15% active compound | 607.17 |
| Nanoemulsion from higher pressure homogenizer | |
| 10% active compound | 970.50 |
| 15% active compound | 903.31 |
| Encapsulated product | 715.04 |

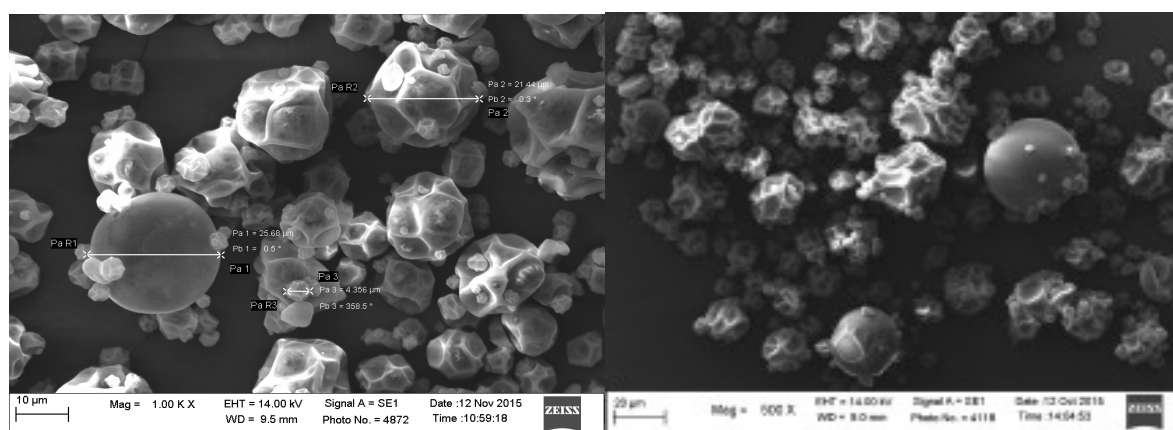


Fig. 2. Microstructure of encapsulated surface from total solid of 10% (a) and 15% (b) active compounds.

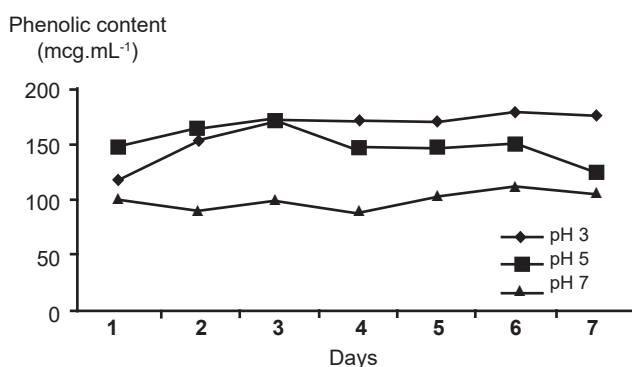


Fig. 3. Phenolic content of nanoemulsion at various pH.

CONCLUSION

Biopreservatives have been formulated from garlic, ginger and cinnamon extracts in the form of nanoemulsion using high pressure and high shear homogenization. Nanoemulsion produced using high pressure homogenizer showed better inhibition activities against *E. coli* and *S. typhi* than that prepared by high shear homogenizer. In the form of nanoemulsion, antimicrobial activities were higher than that in the form of spray dried powder (encapsulated products).

The formulated nanoemulsion and encapsulated products had LC₅₀ values of less than 1000 ppm with the chemical components of alliin, allicin, cinnamaldehyde, trans cinnamaldehyde, eugenol, shogaol-6, shogaol-8, gingerol-10 and gingerol 6. This study suggests that the formulated nanoemulsion of a mixture of garlic, ginger and cinnamon extracts could be developed as a food preservative.

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