RESEARCH ARTICLE

Antioxidant, antibacterial, and antifungal properties of nanoemulsion of clove essential oil

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ABSTRACT

Objective(s): The application of synthetic antimicrobial and anti-fungal compounds is reducing in last decades in the food and nutrition fields owing to their various side effects and increasing interest of consumers to eat natural foodstuffs without artificial constituents. Clove (*Syzygium aromaticum*) has numerous medicinal property including analgesic, antiseptic, stimulants, carminative and natural antihelminitic. The present study was aimed to evaluate the antifungal, antioxidant and antibacterial properties of clove essential oil (CEO) under *in-vitro* condition.

Methods: Antioxidant property of nano emulsion of CEO was evaluated in terms of radical scavenging ability against DPPH free radical. Antibacterial and antifungal effects of nano emulsion of CEO were evaluated using agar disk diffusion assay.

Results: Antioxidant property of CEO was found to be 92.45% ± 5.49. Based on our findings, the food-borne pathogens were shown the highest sensitivity to the CEO (inhibition zone = 5.12-14.34 mm), followed by probiotic microorganism (inhibition zone = 2.57-4.44 mm), and fungi (inhibition zone = 2.13-3.19 mm), respectively.

Conclusions: The results of the present study indicated that nano emulsion of CEO has good antimicrobial and antioxidant property under *in-vitro* condition.

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INTRODUCTION

The application of synthetic antimicrobial and antifungal compounds is reducing in last decades in the foodstuff and nutrition fields owing to their various side effects and increasing interest of consumers to eat natural foodstuffs without artificial constituents [1]. Therefore, various natural constituents derived from medicinal plants including extract and essential oil (EO) have a critical role as plant products to enhance the palatability as well as flavor and odor properties of food products [2]. It has a lot of versatility and the different plant parts provide a pool of phytochemicals, which has a broad spectrum of biological activities [3]. Clove (*Syzygium aromaticum*) belongs to the *Myrtaceace* family and has numerous medicinal properties including analgesic, antiseptic, stimulants, carminative, and natural antihelminitic effects [4]. It comprises about 30–35 species originating in the Europe, North Africa, Asia Minor, and near East [5]. The EOs were obtained from buds, leaves, and stem of plant, which differ in their color, flavor, and chemical composition [6]. The US Food and Drug Administration (US FDA) has been recognized the EO as a food additive grade of four [7]. Previous studies indicated that the main chemical composition of clove EO (CEO) are eugenol, β-caryophyllene, and eugenol acetate [8, 9]. These compounds have the main essential effects of clove as anti-inflammatory, analgesic, antimicrobial, antiparasitic, antioxidant, antimutagenic, anti-

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convulsant, and anticarcinogenic agent [10, 11]. They are also known for their therapeutic potential to control vomiting, nausea, diarrhoea, flatulence, cough, stomach distension, relieve pain, and cause uterine contraction [12]. CEO has been found to show antifungal activity against pathogenic fungi including *Tricophyton rubrum, Tricophyton mentagrophytes var. interdigitale*, *Epidermophyton flocosum, Candida spp.*, and *Aspergillus spp.* [13-15]. The antibacterial activity of CEO alone and in combination with different antimicrobial compounds against common food-borne pathogens has been evaluated in a widespread of food products such as cucumber [4], rainbow trout fillets [16], ground beef [5], cooked pork sausages [11], and ground chicken meat [17].

Food-borne pathogens are causing a great number of diseases with remarkable effects on human health. The global burden of food-borne diseases is significant and it is estimated that, only in the United States of America (USA), each year roughly 48 million people in the USA gets sick, 128,000 are hospitalized, and 3,000 die from food-borne diseases [18]. *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Bacillus subtilis, Bacillus cereus*, and *Staphylococcus aureus* have been isolated frequently from various foodstuffs [19]. Probiotic microorganisms including *Lactobacillus* spp. and *Bifidobacterium* spp. are the main tools of bio-preservation in dairy and non-dairy products for controlling pathogens, improving food safety, and having the potential for favor consumer health [20]. Based on our knowledge, limited studies have been reported on antifungal, antioxidant, and antibacterial potential of nano emulsion of CEO under *in-vitro* condition and food models. Therefore, the present study was aimed to evaluate the antifungal, antioxidant, and antibacterial properties of nano emulsion of CEO under *in-vitro* condition.

MATERIALS AND METHODS

Materials

Clove buds were obtained from a local grocery in Kermanshah, Iran. Proximate composition of clove buds including moisture, ash, crude fat, crude protein, and crude fiber was determined based on the method reported by the Association of Official Analytical Chemists [21], as presented in Table 1. All chemicals, culture media, and materials were analytical grade, and obtained from Merck, Germany.

Extraction of clove essential oil

The dried plant was exposed to the hydro-distillation using a Clevenger-type apparatus for approximately 3 h [22]. The isolated CEO was dried over anhydrous sodium sulfate (Na_2SO_4), transferred to a sealed dark vial, and stored under refrigerated condition (4 ± 1°C) until further use.

Preparation of nano emulsion of clove essential oil

The nano emulsion of CEO was prepared based on the previously published method by Nenaah, [23].

DPPH free radical scavenging assay

Antioxidant property of nano emulsion of CEO was evaluated in terms of radical scavenging ability against DPPH free radical based on the method of Shahbazi, [6]. An amount of 100 ppm of nano emulsion of CEO and standard DPPH solution (0.1 mM) were prepared in methanol as a stock solution. After that, serial dilutions in range of 1 to 40 µg/ml was prepared from the stock solution. The mixtures were kept at room temperature for incubation in the dark for 30 min and absorbance was measured at 527 nm using distilled water as blank and reagent blank (methanol + DPPH) as a control. Ascorbic acid was used as a standard. All the treatments were performed in triplicates.

Bacterial and fungal strains

The bacterial and fungal strains including *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 11774), *L. monocytogenes* (ATCC 19118), *S. typhimurium* (ATCC 14028), *E. coli* O157:H7 (ATCC 10536), *L. acidophilus* (PTCC 1643), *L. reuteri* (PTCC 1655), *L. casei* (PTCC 1608), *L. rhamnosus* (PTCC 1637), *Aspergillus niger* (ATCC 1015), and *Candida albicans* (ATCC 3153) were obtained from the culture collection of the Iranian Research Organization for Science and Technology (IROST). Bacterial strains were cultured at 37 °C for 24 h in de Man, Rogosa &

Table 1. Proximate composition of clove buds

	Moisture	Ash	Crude fat	Crude protein	Crude fiber
Clove	35.52% ± 2.45	6.42% ± 0.45	3.26% ± 0.19	5.69% ± 0.42	11.48% ± 0.77

Sharpe (MRS) broth/Brain Heart Infusion broth (BHI), adjusted to a final density (8 log CFU/ml), and used as an inoculum dose. The fungi were activated on the Potato Dextrose agar (PDA).

Antibacterial and antifungal effects of nano emulsion of clove essential oil

For agar disk diffusion method, 15 ml molted BHI agar was cast into petri dishes with a diameter of 90 mm. Then, 100 µl of the each test bacterial suspension, containing 8 log CFU/ml, was spread on the BHI agar by surface method. The disks (diameter = 6 mm) containing 10 µl nano emulsion of CEO were placed on the surface of inoculated BHI agar. After incubation at 37 °C for 24 h, the diameter of the inhibition zones (mm) were measured. For antifungal examination of nano emulsion of CEO against *A. niger* conidia and *C. albicans* cells, PDA was used with similar method form antibacterial test.

Statistical analysis

A one-way ANOVA was used to carry out the statistical analysis of all data. Significance levels used were $P < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity of nano emulsion of clove essential oil

Antioxidant property of CEO was found to be 92.45% ± 5.49 (Table 2). Our finding was also exhibited as IC50 (i.e. sample concentration needed to scavenge fifty percent of the free radicals) index. Lower IC50 value showed higher antioxidant potential activity. The IC50 of CEO was calculated to be 32.55 µg/ml. Lower IC50 for CEO was reported by Adefegha, [24], who indicated that IC50 value of CEO obtained from Akure, Nigeria was 12.3 µg/ml. In the study of Gülçin et al., [25], the antioxidant activity of CEO at different concentrations of 20, 40 and 60 µg/ml in terms of DPPH radical scavenging activity was in the range of 92.5-94.9%. Lee and Shibamoto, [26] found that clove extract (*Syzygium aromaticum* (L.) Merr. et Perry) exhibited strong radical scavenging activity with IC50 value of 0.55 µg/ml in comparison with the standard tert-butylated hydroxytoluene (IC50 14.75 µg/ml) against DPPH. Previous studies reported that eugenol and eugenyl acetat were the main chemical composition of CEO [25-27]. The hydroxyl group available in eugenol on the aromatic ring is responsible for the antioxidant activities. The phenolic radical developed is stabilized by resonance with the double bonds of aromatic ring as the substitution of acetate and benzoate group destabilize the formation of free radical. This was further supported by the fact that phenolic compounds have ability of transferring electrons or hydrogen atoms by neutralizing free radicals resulted in blocking oxidative processes [3].

Antibacterial and antifungal effects of nano emulsion of clove essential oil

Antibacterial property of nano emulsion of CEO against food related pathogens (*S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium* and *E. coli* O157:H7), probiotic microorganisms (*L. acidophilus*, *L. reuteri*, *L. casei* and *L. rhamnosus*) and fungi (*A. niger* and *C. albicans*) are given in Tables 3-5, respectively. According to our findings, the food related pathogens were shown the highest sensitivity to the CEO (inhibition zone = 5.12-14.34 mm), followed by probiotic microorganisms (inhibition zone = 2.57-4.44 mm), and fungi (inhibition zone = 2.13-3.19 mm), respectively. There are reports in the literatures regarding the antibacterial activities of CEO and its major compounds especially β-caryophyllene and similar to that of eugenol [28-30]. The antibacterial

Table 2. Antioxidant property of nano emulsion of clove essential oil

	DPPH%	IC50 (µg/ml)
Essential oil	92.45% ± 5.49[a]	32.55 ± 6.98[a]
Ascorbic acid	93.76% ± 4.65[a]	11.55 ± 3.28[b]

Different lowercase letter in same column indicates significant differences ($P < 0.05$).

Table 3. Antibacterial effect of nano emulsion of clove essential oil against food-borne pathogenic bacteria

	Inhibition zone (mm)					
	S. aureus	*B. subtilis*	*B. cereus*	*L. monocytogenes*	*S. typhimurium*	*E. coli*
Essential oil	14.34 ± 0.09[a]	7.69 ± 0.07[b]	9.87 ± 0.01[b]	8.88 ± 0.05[b]	5.67 ± 0.06[c]	5.12 ± 0.03[c]

Different lowercase letter indicates significant differences ($P < 0.05$).

Table 4. Antibacterial effect of nano emulsion of clove essential oil on probiotic bacteria

	L. acidophilus	*L. reuteri*	*L. casei*	*L. rhamnosus*
Essential oil	4.44 ± 0.01	3.76 ± 0.02	3.11 ± 0.02	2.57 ± 0.03

Table 5. Antifungal property of nano emulsion of clove essential oil

	A. niger	*C. albicans*
Essential oil	2.13 ± 0.01	3.19 ± 0.02

mechanism of CEO was related to be due to the -OH groups located at the *meta* and *ortho* positions in main chemical composition of CEO , respectively, which are able to interact with the cytoplasmic membrane of bacterial cells [31]. It is believed that this phenomenon can disrupt the bacterial phospholipid membrane resulting in inhibition of electron transport, protein translocation, phosphorylation and other enzymatic activity and thus leading to cell death [32]. Hemalatha et al., [33] reported that the methanolic extract of clove had excellent antibacterial activity against *B. subtilis*, *S. aureus*, *Klebsiella pneumonia*, and *Vibrio cholera*. Cui et al., [34] found that CEO exhibited favorable antimicrobial activity for both *E. coli* and *S. aureus*. Moreover, previous studies indicated that CEO and its extract has ability to inhibit spore germination, radical growth and reduced dry weight of *Fusarium oxysporum* [35]. CEO has been found to be effective at 100 µl against *F. chlamdosporum*, *Heterodera oryzae*, and *Rhizoctonia bataticola* [36]. Antifungal properties of EOs are likely because of the presence of various terpenoids which are lipophilic in nature and are capable of cell membrane disruption causing cell death or inhibit the sporulation and germination of food spoilage fungi. different other mechanisms indicated for antifungal potential of EOs involved inhibition on ergosterol synthesis, inhibiting enzymes involved in cell wall synthesis, altering cell wall morphology, producing oxygen reactive species and changing cell membrane permeability [3].

CONCLUSION

The results of the present study indicated that nano emulsion of CEO has good antimicrobial and antioxidant property under *in-vitro* condition. Further study also is necessary for investigating of the effects of CEO on survival of *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, *E. coli* O157:H7, *L. acidophilus*, *L. reuteri*, *L. casei* and *L. rhamnosus* in different fresh and processed foodstuffs.

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CONFLICT OF INTEREST

The authors did not report no conflict of interest.

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